International Journal of Plant Production 3 (1), January 2009
ISSN: 1735-6814 (Print), 1735-8043 (Online)
This is a refereed journal and all articles are professionally screened and reviewed.

GUASNR
www.ijpp.info

Effects of Phenolic Extracts of Canola (*Brassica napuse* L.) on Germination and Physiological Responses of Soybean (*Glycin max* L.) Seedlings

G.R. Haddadchi*, Z. Gerivani

Department of Biology, College of Science, Gorgan University of Agricultural Sciences and Natural Resources Gorgan, Iran.
*Corresponding author. E-mail: haddadchie@yahoo.com

Received 01 March 2008; Accepted after revision 17 October 2008; Published online 15 January 2009

Abstract

Phenolic extracts of canola (*Brassica napuse* L.) contains substances that inhibited the germination and seedling growth of soybean (*Glycin max* L.). These compounds also affect activity of some enzymes. Hear, we investigated the allelopathic effects of various canola plant parts on soybean germination, seedling growth, and biochemical aspects. Phenolic extract of canola shoot (including leaf, stem, and flower) and root were made by methanol 70%, to determine their effects on germination, seedling growth, and fresh and dry weight of hypocotyl and radicle of 8-d old soybean seedlings, and their effects on activity of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), polyphenoloxidase (PPO), protein content of hypocotyl and radicle, and lipid peroxidation (MDA) of radicle. The results showed that there were no significant differences in CAT activity and protein content. Increasing the phenolic extract concentration of separated canola plant parts decreased soybean germination and seedling length and weight, radicle was more sensitive to extract source than hypocotyl. The POD and SOD activity of radicle and hypocotyl and lipid peroxidation of radicle were increased by concentration of both phenolic extracts compared with control. The PPO activity of radicle and hypocotyl was increased under root extracts, but shoot extracts had not significantly effects. The degree of toxicity of canola root part was more than shoot part.

Keywords: Allelopathy; *Brassica napus*; Catalase; Peroxidase; Phenolic extracts

Introduction

The science of allelopathy as defined by Molisch (Molish, 1937) is the inhibitory as well as stimulatory effects of one plant on another, including the microorganisms. Allelopathic effects are mediated through release of allelochemicals. Allelochemicals are usually called secondary plant products of the main metabolic pathway in plants. These may be water soluble substances that are exudation volatilization, and decomposition of plant residues (Narwal, 1996). Probable major biosynthetic pathways leading to production of allelopathic chemicals are the shikimic acid or acetate pathways (Rice, 1984). Multiple

physiological effects have commonly been observed from treatments with many allelochemical phenolics. These effects include decreases in plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turger pressure, and osmotic potential caused by phenolic compounds (Patterson, 1981; Barkosky and Einhellig, 2003; Harper and Balke, 1981; Glass, 1974).

Brassica spp, suppresses the weeds through their vigorous growth and release of allelocemicals, which may be used for weed control (Oleszek et al, 1996). Some *Brassica* species have harmful effects on crops including reduced seed germination and emergence of subsequent small-grain crops when grown in rotation (Bialy et al, 1990). The main components of phenolic substances in canola are sinapic acid and sinapin. The other phenolic acids and their esters, such as salicylic, o-coumaric, ferulic, syringic and cinnamic acid, are minor substitutes in canola (Zukalova, 1999). Plant treated with p-hydroxybenzoic acid (p-HBA) had significantly lower stomatal conductance, lower water, and less discrimination against ^{13}C. Interference with plant-water balance appears to be one mechanism of action of phenolic acids causing a reduction in plant growth (Barkosky and Einhellig, 2003). Phenolic compounds may be decreased seed germination, ion uptake, leaf expansion, chlorophyll content, photosynthesis and electron transport (Colpas et al, 2003; Glass, 1974; Patterson, 1981; Leu et al, 2002; Norman, 2004). The present research was conducted to evaluate the effects of phenolic extracts concentration of various canola parts on soybean seed germination and physiological responses.

Materials and methods

Plant sampling and preparation of phenolic extracts

Samples of canola were harvested at a flowering stage from a field of Experimental Farm, Gorgan University in March 2004. The plant samples separated into root and shoot including stem, leaf and flower. The both samples were oven dried at 60˚C for 72 h, ground to pass a1mm screen and then stored in a refrigerator at 4˚C until used (Chan et al, 2002).The 10 g of dried samples from root and shoot were extracted by soaking in 100 ml methanol/water (70/30) at 25 ˚C for 24 h. The extracts were filtered through four layers of cheesecloth to remove the fiber debris, and soaking again for 1h and were successively extracted four times. Methanolic extracts filtered again through whatman no.1 paper (Pasqualini et al, 2003) and the methanol evaporated at 45˚C under vaccum pump using a rotary evaporator. The aqueous phase obtained was used as a stock extract (Narwal and Tauro, 1996).

Total phenol of stock extracts per samples was determined by a modification of Prussian blue assay of Price and Butler (1977). Quantitative results were expressed with reference to gallic acid (Table 1).

Table 1. Total phenol of Root and Shoot extracts of canola.

Diluted Extract (v/v)	Total phenol (g/L)	
	Root extract	Shoot extract
10%	0.020	0.036
20%	0.041	0.071
30%	0.061	0.107

Seed bioassay

Stock extracts were made and diluted appropriately with distilled water to give the final concentration of 10, 20, and 30% (v/v).Total phenol of these extracts were evaluated (Table.1) and 15ml of diluted extract were piptted to the filter paper placed in each 15 cm diameter petridish and distilled water was used as a control. Twenty five seeds of soybean were sterilized with NaOCl 2% for 2 min and were placed between sheets and maintained in germinator under 25 ±1˚C for 8 days. Daily counts of germinated seed were made. After germination test and measuring the radicle and hypocotyl length, the seedlings were separated into hypocotyl and radicle parts for measuring fresh and dry weight and assay of polyphenoloxidase (PPO), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity and LP.

Enzyme extraction

Freezing hypocotyl and radicle (0.2g) were extracted with 4 ml of 50 mM phosphate buffer (pH 7.0) as described by Ng et al, (2003). The extract was centrifuged at 15000 g for 20 min at 4˚C and supernatant was used to determine the activity of PPO, SOD, CAT, and POD.

The protein concentration of the supernatant was determined according to the Bradford (1976) method using bovine serum albumin as standard.

PPO activity

The activity of polyphenoloxidase was determined by the method of Kar and Mishra, (1976) with some modification. The 3 ml reaction mixture contained 25 mM phosphate buffer (pH 6.8), 0.1 mM pyrogallol, 0.1 ml enzyme extract and blank without pyrogallol.

The absorbance of the purpurogallin formed was recorded at 420 nm, and activity was calculated using the extinction coefficient [2.47 $mM^{-1}cm^{-1}$] for purpurogallin.

POD activity

Peroxidase activity was assayed essentially according to the method of Prochazkova et al. (2001). The 3ml reaction mixture contained 50mM sodium-acetate buffer (pH 5), 20 mM guaiacol, 40mM H_2O_2, 0.05 ml enzyme extract diluted 6times. Absorbance due to the formation of tetra-guaiacol was recorded at 470 nm and enzyme activity was calculated as per its extinction coefficient of 26.6 $mM^{-1}cm^{-1}$.

CAT activity

Catalase activity was determined by the method of Goel et al. (2003) with some modifications. The 3 ml reaction mixture contained 50mM phosphate buffer (pH 7), 15 mM H_2O_2, 0.1 ml enzyme extract. The decrease in H_2O_2 was followed as the decline in absorbance at 240nm, and activity was calculated using the extinction coefficient [40 $mM^{-1}cm^{-1}$] for H_2O_2.

SOD activity

The superoxide dismutase activity was assayed by the method of Beauchamp and Fridovich (1971). The reaction mixture contained 1 ml of 50 mM phosphate buffer (pH 8), 0.1mM EDTA, 0.025 mM NBT, 0.1 mM xanthin, 33 µl enzyme extract. The reaction was started by addition of xanthin oxidase. A suitable concentration of xanthin oxidase was diluted, and 33 µl of it was added into the cuvette to initiate the reaction. A liner curve with a slop of 0.01absorbance per min in time scan was obtained by adjusting the concentration of xanthin oxidase. One unit of SOD activity was defined as the amount of SOD which produced one half of the maximum competition against NBT in the specified system, absorbance was measured at 560 nm.

Lipid peroxidation (LP)

The level of lipid peroxidation was measured in terms of TBARS content (Prochazkova et al, 2001). Radicle sample (0.1g) was homogenized in 2 ml 0.1 % trichloroacetic acid (TCA).The homogenate was centrifuged at 15000×g for 15 min. To 1 ml aliquot of the supernatant, 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95˚C for 30 min and than cooled in an ice bath. After centrifugation at 10000×g for 10 min the absorbance of the supernatant were recorded at 440, 532 and 600 nm. Malondialdehyde equivalents were calculated in the following manner ($nmol \cdot ml^{-1}$) (Du and Bramlage, 1992):

$[[(A_{532} - A_{600}) - [(A_{440} - A_{600})$ (MA of sucrose at 532nm/MA of sucrose at 440nm)]]/157000]$\times 10^6$

The MA (molar absorbance) of 1-10 mM sucrose at 532 nm and 440 nm was calculated to be 8.4 and 147, respectively, giving a ratio of 0.0571.

Statistical analysis

Germination and seedling growth bioassay were conducted in a complete randomized design with three replications. Means were compared by Duncan's multiple range tests at $P \leq 0.05$.

Results and discussion

Seed bioassay

Phenolic extracts from *B.napus* plant shoots (including leaves, flowers, and stem) and root showed inhibitory effects on seed germination and seedling growth of Soybean (Table 2 and 3). At the highest extract concentration, both of phenolic extracts significantly reduced seed germination. Phenolic extracts of root treatment was the more inhibitory effect than shoot treatment. In reaction to the growth evaluation of seedlings, hypocotyls and radicles length was affected by phenolic extracts of shoot and root treatments, the root extracts caused the greater reduction hypocotyls and radicles length. Radicle length was relatively more sensitive to autotoxic allelochemicals than hypocotyls length. The degree of inhibition increased with the extract concentration. The result found in this study is congruency with data of Balenory et al, (2000) who found that some phenolic compounds significantly reduced hypocotyl and radicle length when compared with the control. They

showed that ferulic and p-coumaric acids influence canola seed germination. Turk and Tawaha (2003) found that aqueous extracts of black mustard (*Brassica nigra*) caused the reduction in germination, hypocotyl and radicle length of *Avena fatua*. They reported that the most inhibitory effect of allelopathic plants was produced by leaf extracts, and all of these extracts had more pronounced effects on radicle growth than on hypocotyl growth. Colpas et al (2003) showed that some phenolic compounds such as ferulic acid, coumarin and naringenin, inhibited the soybean seed germination. Furthermore inhibition by p-coumaric acid on soybean seed germination was observed by Schuab et al, (2001). They reported that there were no significant differences in terms of cumulative seed germination in the present of the compound studied, although a delay was observed in the germinative process. In relation to the growth evaluation of seedling a great reduction in the length of radicles was verified, also a decrease of fresh and dry matter, mainly of radicles was observed. NG, P.L.L. et al, (2003) has demonstrated that cinnamic (CIN), caffeic (CAF), p-coumaric (p-CA), benzoic (BEN) and p-hydroxybenzoic acid (p-HB) reduced seedling fresh weight and seedling length and root length but did not affected germination percentage of canola.

Table 2. Effects of canola phenolic extracts on soybean seed germination percentage (G %), radicle length (RL), fresh weight (FW) and dry weight (DW) of radicle.

Total phenol (g/L)	G %	RL (cm)	FW (g)	DW (g)
None	82.67^{a}	6.80^{a}	0.130^{a}	0.005^{a}
		Root extract		
0.02	78.66^{a}	4.08^{b}	0.052^{b}	0.005^{a}
0.041	78.66^{a}	2.33^{c}	0.044^{bc}	0.002^{b}
0.0107	65.33^{a}	1.53^{d}	0.40^{c}	0.003^{b}
		Shoot extract		
0.036	86.66^{a}	5.83^{b}	0.084^{b}	0.004^{a}
0.071	77.33^{a}	3.39^{c}	0.064^{c}	0.005^{a}
0.107	60.00^{b}	2.04^{d}	0.069^{bc}	0.003^{a}

Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan's Multiple Range Test (DMAT).

Table 3. Effects of canola phenolic extracts on soybean hypocotyl length (HL), fresh weight (FW) and dry weight (DW) of hypocotyl.

Total phenol (g/L)	HL (cm)	FW (g)	DW (g)
None	9.47^{a}	0.300^{a}	0.020^{a}
		Root extract	
0.02	7.37^{b}	0.140^{b}	0.017^{ab}
0.041	5.17^{c}	0.150^{b}	0.014^{b}
0.0107	4.02^{d}	0.094^{c}	0.006^{c}
		Shoot extract	
0.036	7.67^{b}	0.227^{ab}	0.023^{a}
0.071	5.12^{c}	0.185^{b}	0.020^{a}
0.107	4.24^{d}	0.177^{b}	0.020^{a}

Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan's Multiple Range Test (DMAT).

Biochemical assay

The results showed that there was no significant differences catalase activity. Total SOD activity of radicle increased with both extracts (Figure 1.1). SOD activity of hypocotyl increased with of shoot extracts and high concentrations of root extract compared to the

controls (Figure 1.2). The POD activity of radicle (Figure 2.1) and hypocotyl (Figure 2.2) increased with high concentrations of shoot extracts and low concentration of root extracts. Of the PPO activity, only under root extracts had significant differences. Root extracts at all concentrations increased PPO activity of radicle (Figure 3.1).The PPO activity of hypocotyl under high concentration of root extracts was increased (Figure 3.2). Increasing of PPO activity was associated by decreasing of radicle length. The lipid peroxidation (MDA) of radicle increased with extracts concentrations compared to the controls (Figure 5). In this study increasing and/or decreasing of lipid peroxidation associated by POD activity. Malondialdehyde is formed through auto oxidation and enzymatic degradation of polyunsaturated fatty acids in cells (Hodges et al., 1998). Thus development of seedlings and enzyme activity, both affected on lipid peroxidation.

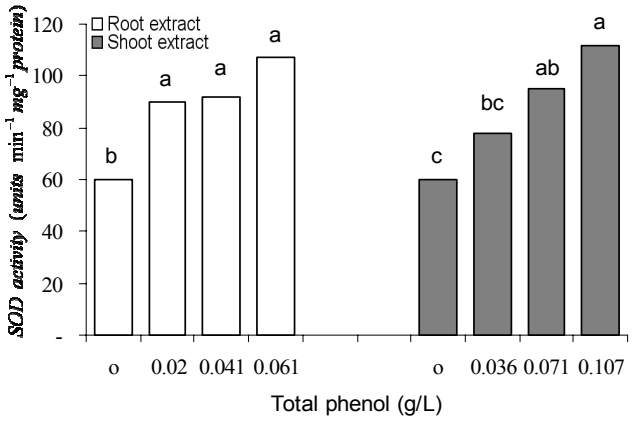

Figure 1.1. Effects of canola phenolic extracts on SOD activity of soybean radicle. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

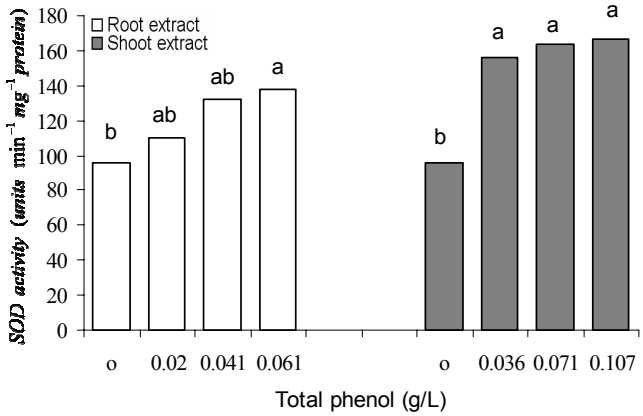

Figure 1.2. Effects of canola phenolic extracts on SOD activity of soybean hypocotyl. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

Difference caused by shoot and root extracts may be due to difference in toxicity of the compounds, in uptake or in the ability of seedlings to detoxify allelochemicals.

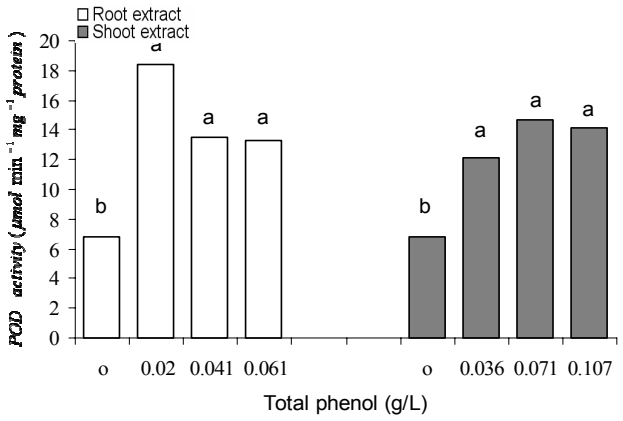

Figure 2.1. Effects of canola phenolic extracts on POD activity of soybean radicle. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

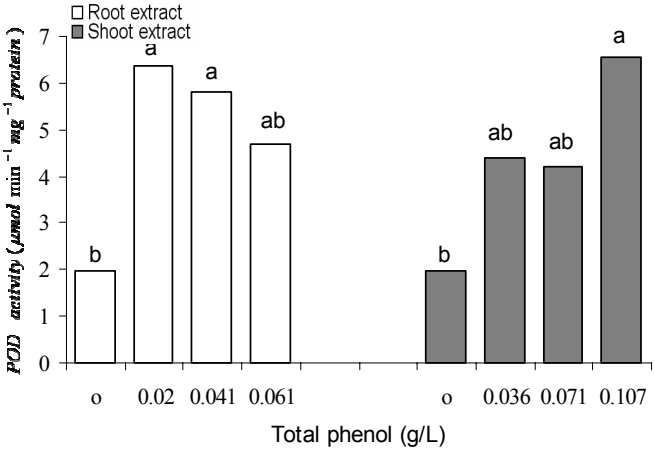

Figure 2.2. Effects of canola phenolic extracts on POD activity of soybean hypocotyl. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

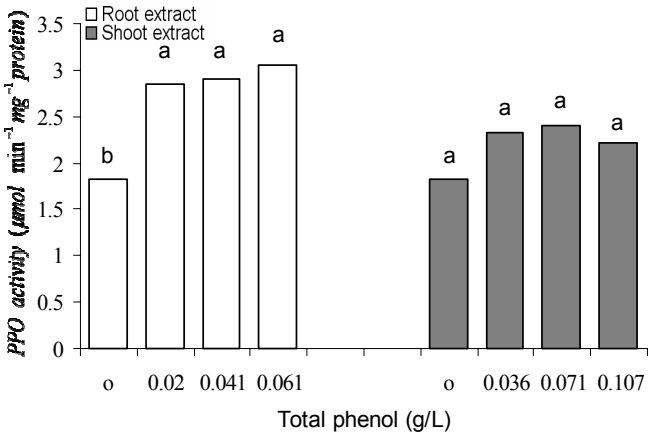

Figure 3.1. Effects of canola phenolic extracts on PPO activity of soybean radicle. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

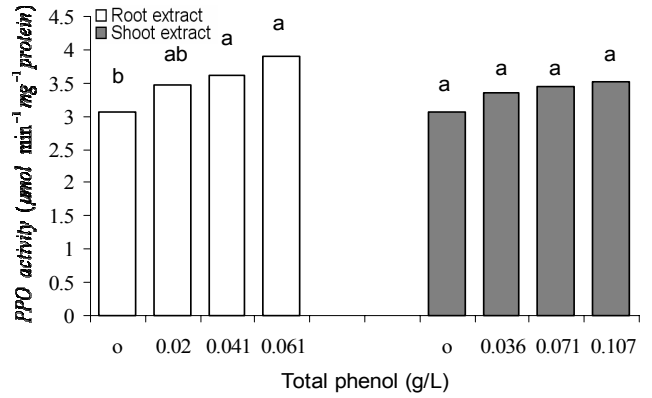

Figure 3.2. Effects of canola phenolic extracts on PPO activity of soybean hypocotyl. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

Zeng et al (2001) reported that secalonic acid F isolated of *Aspergillus japonicus*, significantly reduced the activities of SOD and POD at a concentration of 0.3 mM. Harvoth et al (2002) showed that some of phenolic compounds were found to significantly increase chilling tolerance when added hydroponically to young maize seedlings. They reported that catalase activity was hardly inhibited by salicylic acid (SA), it has been suggested that the inhibition of catalase by SA plays a role in mediating stress responses. In soybean, Doblinski (2003) has demonstrated that p-CA or p-HD (at 0.5 and 1 mM) and Ozan (1996) reported that some plant phenolic increased soluble POD activity and cell wall (CW)-bound POD activity. At cellular membrane level, phenolic compounds oxidation lead to the

production of quinines, which are toxic compounds responsible for the generation of reactive oxygen species (ROS).

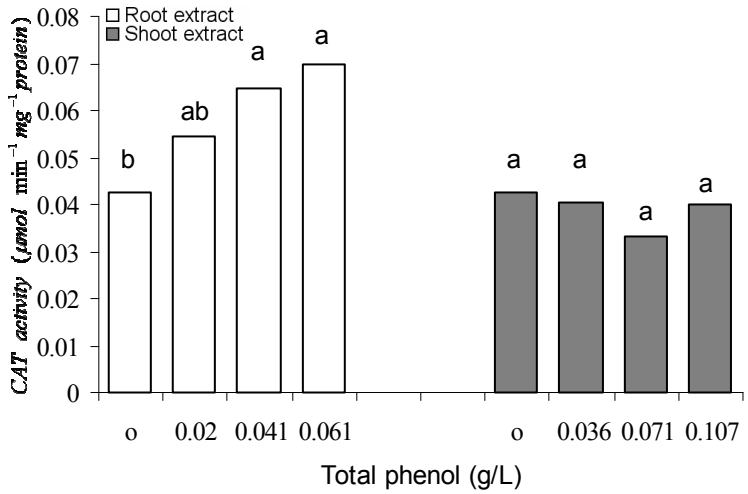

Figure 4.1. Effects of canola phenolic extracts on CAT activity of soybean radicle. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

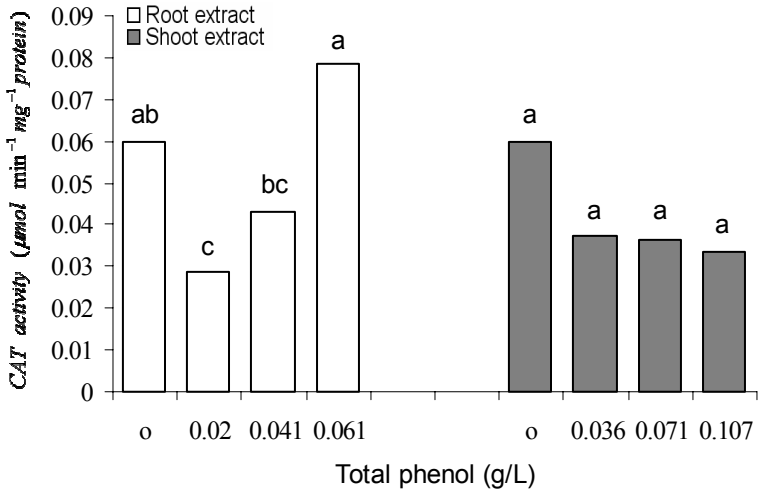

Figure 4.2. Effects of canola phenolic extracts on CAT activity of soybean hypocotyl. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

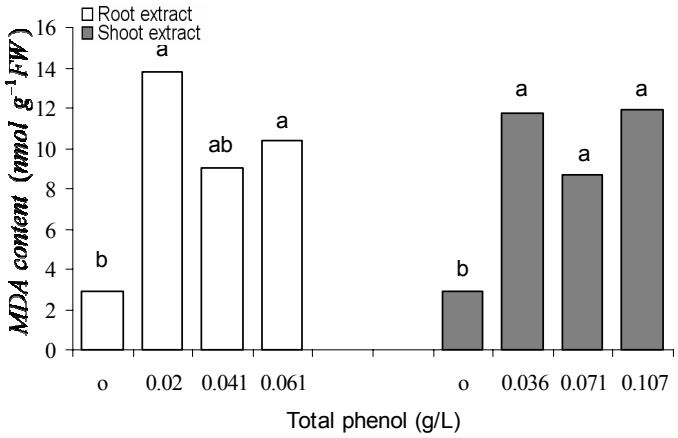

Figure 5. Effects of canola phenolic extracts on MDA content of soybean radicle. Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test (DMAT).

These free radicals are extremely dangerous to cells because they cause enzyme inactivation, membrane lipid peroxidation and decreased in the absorption by the roots (NG et al 2003). Furthermore, the increase of POD and PPO activities accompanied by the reduction of root growth strengthens the hypothesis of phenolic acids synthesis by the phenylpropanoid pathway incorporation in lignin, increase in the cell wall rigidity and growth reduction (NG et al 2003). There was a close association between POD and PPO (Nkang, 2001). To control of ROS and to protect cells under stress conditions, plants contain several enzymes scavenging ROS (SOD, CAT, POD, and glutathione peroxidase). Enhanced formation of ROS under stress conditions induces both protective responses and cellular damage. The scavenging of $O_2^{\cdot}$ is achieved through on upstream enzyme, SOD, which catalyses the dismutation of superoxid to H_2O_2. SOD genes have been shown to be sensitive to environmental stress. The intracellular level of H_2O_2 is regulated by a wide range of enzymes, the most important being catalase and peroxidases (Blokhina, 2003). Further studies are required, to determination the phenolic compounds of canola in reproductive stage and their effects on other antioxidant enzyme such as glutathione reductase, and electrophorethic patterns of proteins and enzymes, and anatomical study of soybean seedlings.

References

Baleroni, C.R.S., Ferrarese, M.L.L., Braccini, A.L., Scapim, C.A., Ferrares-Filho, O., 2000. Effects of ferulic and p-coumaric acids on canola (*Brassica napus* L. cv. Hyola 401) seed germination. Seed Sci. & Technol. 28: 333-340.

Barkosky, R.R., Einhellig, F.A., 2003. Allelopathic interference of plant-water relationships by para-hydroxybenzoic acid. Bot. Bull. Acad. Sin. 44:53-58.

Blokhina, O., Virolainen, E., Fagerstedt, V., 2003. Antioxidants, oxsidative damage and oxygen deprivation stress: a review. Annals of Botany. 91: 179-194.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

Beauchamp, C., Fridovich, I., 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 44: 276-287.

Colpas, F.T., Ono, E.O., Rodrigues, J.D., and Passos, J.R., 2003. Effects of some phenolic compounds on soybean seed germination and on seed-borne fungi. Brazilian Archives of Biology and Technology. 46(2):155-161

Doblinski, P.M.F., Ferrarese, M.L.L., Huber, D.A., Scapim, C.A., Braccini, A.L., Ferrarese-Filho, O., 2003. Peroxidase and lipid peroxidation of soybean roots in response to p-Coumaric and p-Hydroxybenzoic acids. Brazilian Archive of Biology and Technology. 46(2):193-198.

Du, Z., Bramlage, W.J., 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J. Agric. Food chem. 40:1566-1570.

Glass, A.D.M., 1974. Influence of phenolic acids on ion uptake. Plant physiol. 54:855-858.

Goel, A., Goel, A.K., Sheoran, I.S., 2003. Changes in oxidative stress enzymes during artificial agein in cotton (*Gossypium hirsutum* L.) seeds. J. Plant. Physiol. 160: 1093-1100.

Harper, J.R., Balk, N.E., 1981. Characterization of the inhibition of K^+absorption in oat roots by salicylic acid. Plant Physiol. 68:1349-1353.

Hodges, D.M., Delog, J.M., Forey, C.F., Pronge, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 207: 604-611.

Horvath, E., Janda, T., Szalai, G., Paladi, E., 2002. In vitro salicylic acid inhibition of catalase activity in maize:differences between the isozymes and a possible role in the induction of chilling tolerance. Plant Science.163:1129-1135.

Kar, M., Mishra, D., 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. 57:315-319.

Leu, E., Krieger-Liszkay, A., Goussias, C., Gross, E.M., 2002. Polyphenolic allelochemicals from the aquatic angiosperm *Myrophyllum spicatum* inhibit photo system II. Plant Physiol. 130: 2011-2018.

Molish, H. 1937. Der einfluss einer pflanze auf die andere-allelopathic. Jena:Fisher.

Norman, C., Howell, K.A., Millar, A.H., Whelan, J.M., Day, D.A., 2004. Salicylic acid is an uncoupler and inhibitor of mitochondrial electron transport. 134: 492-501.

Narwal, S.S., 1996. Suggested methodology for allelopathy laboratory bioassays. Allelopathy: Field Observations and Methodology. pp. 255-266.

NG, P.L.L., Ferrarese, M.L.L., Huber, D.A., Ravagnani, A.L.S., and Ferrarese-Filho, O., 2003. Canola (*Brassica napus* L.) seed germination influenced by cinnamic and benzoic acids and derivatives: effects on peroxidase. Seed Sci. and Technol. 31: 39-46.

Nkang, A., 2001. Effects of cyanid pre-treatment on activities of polyphenoloxidase and peroxidase in seeds of *Guilfoylia monostylis*. Seed Sci. & Technol. 29: 557-565.

Norman, C., Howell, K.A., Millar, H., Whelan, J.M., Day, D.A., 2004. Salicylic acids is an uncoupler and inhibitor of mitochondrial electron transport. Plant Physiol.134:429-501.

Oleszek, W., Ascard, J., Johansson, H., 1996. Brassicacae as alternative plants for weed control in sustainable agriculture. Scientific Publihsers, Jodhpur, India. Pp: 3-22.

Ozan, A., Nair, M.G., Safir, G.R., 1996. In vitro effects of plant phenolics on root peroxidase activity of *Trifolium repens*. Allelopathy Journal. 3(1): 59-64.

Pasqualini, V., Robles, C., Garzino, S., Greef, S., Bousquet-Meloll, A., Bonin, G., 2003. phenolic compounds content in *Pinus halepensis* Mill. needles: a bioindicator of air pollution. Chemosphere. 52: 239-248.

Patterson, D.T., 1981. Effects of Allelopathic chemicals on growth and physiological responses of soybean (*Glycin max*). Weed Science. 29(1):53-59.

Price, M.P., and Butler, L.G., 1977. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J. Agric. Food Chem. 25: 1268-1273.

Prochazkova, D., Sairam, R.K., Srivastava, G.C., Singh, D.V., 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Sci. 161: 765-771.

Rice, E.L., 1984. Allelopathy, 2nd edition. Academic press, NewYork.

Schuab, S.R.P., Braccini, A.L., Ferrares-Filho, O.F., Scapim, C.A., Braccini, M.C.L., 2001. Physiological seed quality evaluation and seedling lipid and protein content of soybean (*Glycin max* L.) in the presence of p-coumaric acid. Seed Sci. & Technol. 29:151-162.

Turk, M.A., Tawaha, A.M., 2003. Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.), 22: 673-677.

Zeng, R.S., Luo, S.M., Shi, Y.H., Shi, M.B., Tu, C.Y., 2001. Physiological and biochemical mechanism of allelopathy of secalonic acid F on higher plants. Agron. J. 93:72-79.

Zucalova, H., VA-AK, J., 1999. Natural antioxidants in winter rape (*Brassica napus* L.). Proceeding of 10th international rapeseed congress, Canberra, Australia. The Regional Institute: online community publishing.