Journal on plant Sciences Researches, Vol.1, No.3, Autumn 2006

The effect of aquatic extract of canola (*Brassica napus* L.) on chlorophyll content, nitrate reductase, catalase and peroxidase activities enzymes of soybean (*Glycine max* L.) seedling in hydroponic culture

[*]Niakan, M., Tajari, T

Department of biology, Islamic Azad University. Gorgan-branch, Gorgan,Iran

Abstract

Canola contains allelochemicals that effect on metabolism of weeds and crop plants.The aim of this research was to study the effect of aquatic extract of canola (*Brassica napus* L. *cv* Hyola 401) on chlorophyll content in cotyledon, nitrate reductase, catalase and peroxidase activity in root, stem and cotyledon of soybean seedling (*Glycine max* L. *cv* Gorgan 3) in Hoagland culture. The seeds of canola (*Brassica napus* L.*cv* Hyola 401) was planted in pot and provided aquatic extract of total plant in 5 leaf stage.This extract was added to Hoagland culture and catalase,peroxidase and nitrate reductase activities in leaf, stem and root and chlorophyll a and b content in cotyledons in soybean seedling (*Glycine max* L.*cv* Gorgan3) after 9 days were evaluated. The results showed that chlorophylls (a and b) amounts in cotyledons and catalase and peroxidase in root and stem and cotyledon decreased in comparison with to control (Hoagland only) in while nitrate reductase activity reduced in cotyledon and in root and stem increasd.

Key words: Canola, Catalase, Chlorophyll, Extract, Growth, Hydroponic, Nitrate reductase, Peroxidase, Soybean

Introduction

Allelopathy is derived from the Greek words “allelo” and “pathy” meaning reciprocal sufferings of two organisms, also known as biochemical interaction, among plants, describes any direct or indirect (harmful or benefical) effect of a plant on another plant through the relases of chemical that escape into the environment (Rice, 1984). Allelochemicals are the small molecular weight compounds excreted from plants during the process of secondary metabolism. Allelochemicals may affect different physiological process in plants such as photosynthesis, respiration, cell division and nutrient uptake (Rice, 1984). It has been reported that allelochemicals affected the mineral uptake, pigment synthesis, photosynthesis, protein synthesis, electron transport involving cytochrom (Rice 1984., Rizvi & Rizvi, 1992). Allelochemicals first damage the cytolema and then send the stress information into the cell through the target point on the cytolema to affect the adsorption of incretions and ions (Khalid et al., 2002).

Many of species of Brassicaceae (as canola) contain a group of secondary metabolites called glucosinolates. The glucosinolates are allelochemicals that may be a sink for nutrients like nitrogen and sulphur and the products of hydrolysis may have important roles in the defence system the myrosinase mediated degradation of glucosinolates gives rise to an unstable thiohydroximate-O-sulphonate which on release of sulphate can result in the production of isothiocyanates, thiocyanates, nitriles and elementary sulphur. These compounds have alleopathic potential (Rice, 1984).

It has been reported reported that allelochemicalls effect on growth and photosynthesis by chlorophyll degredation (Ervin & Wetzel 2001, Zeng et al., 2001). Also allelochemicals inhibit activating enzyme such as peroxidasa, catalase and amylase (Williams & Hoagland, 1982).

[*]*e.mail: mnniakan@yahoo.com*

There is a little information about allelopathic effect of canola on growth, chlorophyll, antioxidant enzyme and nitrate reductase activity.The aim of this research was to study the effect of aquatic extract of canola (Hayola 401 cultivar) on chlorophyll a&b amounts, catalase, peroxidase and nitrate reductase activity in soybean seedling (*Glycine max* L. *cv* Gorgan 3) under hydroponic culture.

Methods & Materials

Canola planted and extracts preparation

Seed of canola (*Brassica nanus* L.cv Hayola 401) was planted in pots.Each pots include Silt-Clay soil tissue.After 65 days in 5 leaf phase total plants harvested and was dried in dark and griaded.5 g of this dried sample was added to 150 ml distilled water and was shaked to 12 hours.These mixture was passed of Wathman paper (number 2) and micropore filter (0.2 micron) (Narwal & Tauro, 1996). Of this extract (%100 concentrations) solution to %70 concentrations was prepared.

Seed preparation

4 plats sterilles includes 10 seed of soybean (*Glycine max* L. *cv* Gorgan3) among of two Wathman papers (number 2) was placed in germinator in 24^{o}C and %70 humidity. Seeds daily were irrigation with distilled water. After 5 days seedling was transferred to special dishes includes hydroponic culture. In special dishes 70 ml solution (includes 20 ml Hoagland solution and 50 ml extract of total plant) as treatment and 70 ml Hogland solution as control was added in each of special dishes and 4 soybean seedling were placed in them.

Biochemical assay

Chlorophyll assay

Amounts of chlorophyll a and b in cotyledon of soybean seedling treated and control plants were evaluated by Bruisma (1963). At first cotyledon were weighted and abrade in 5ml acetone. Then in 3000rpm at 15 minute were centrifuged and supernatunts were separated and rate of their wave length absorbtion according to method of Bruisma (1963) in 645, 652 and 663 nm with spectrophotometers was used.

Peroxidase activity assay

Peroxidase activity in cotyledons stem and root in soybean seedling was assay to down form:

1-Solution extract

For preparation solution extract 1.2g tris and 2g ascorbate, 3.8borax, 2g EDTANa_2, 50g PEG were mixtured and distilled water was added to them (100 ml volume)

2-Enzyne extract

1g of sample (soybean cotyledon, stem and root) of treated plant and control was placed in 4 ml solution extract for 30 minute. The samples were keeped in 4^{o}C temperature for 24 hours. 2ml acetate buffer (0.2 M concentration, pH=5), 0.4 ml H_2O_2%3, 0.2ml benzedin was mixtured and added to 0.1 ml enzyme extract of samples and their absorption was readed in 530 nm by spectrophotometer (Koroi, 1989).

Catalase activity assay

1 ml of enzyme extract to 5 ml solution includes 3000 μM phosphate buffer (pH8), 100μM H_2O_2 were mixtured. For inhibition of enzyme activity 10 ml sulfuric acid %2 was added.This mixture was titration with potassium permangenate 0.01N. Enzyme activity was assay base on indissolved 1μM of H_2O_2 in minute (Chance & Maehly, 1995).

Nitrate reductase activity assay

Nitrate reductase activity measured according to Sym (1984) method. First samples (soybean cotyledon, stem and root) was weighted and used of solution encubate includes K_2No_3, propanol and phosphate buffer and Gris I and II agent. Sample absorbtion was readed in 520nm. For assay nitrate reductase activity of standard curve of nitrite in different concentrations was used.

Statistic calculation

Statistical analysis were conducted in a completed randomized design (CRD) with four replications and mean values were compared by Duncan test at $P<0.05$ and $P<0.01$.

Results and Discussion

Our results showed that soybean growth reduced in water extract of canola. It is reported that the beneficial effects of retained crop residues for erosion control has been well documented, however the yields and growth of crops can be reduced (Fettell and Gill, 1995; Malinda, 1995). Retained wheat residues have been shown to reduce yield and growth in several studies (Purvis, 1990).

Several factors may contribute to the poor growth of canola into wheat residues including (1) nitrogen immobilisation and (2) increased incidence of root disease. According to our results radicle and epicotyle length in soybean seedling in Hoagland solution and aquatic extract of canola decreased to comparision with control (Hoagland only) (table1).It is reported that growth of root to allelochemicals is more sensitive than shoot (Peng and Shao, 2001).

It has been reported that root at first absorbe allelochemicals of environment. Some of the hormones such as GA3 and IAA effects on seedling elongation. Inhibition of functional these hormones can reduce elongation of seedling. Allelochemicals such as flavonoides stopes polar transport of IAA and their function and cause growth reduction (Brunn et al, 1992). As it is reported allelochemicalls inhibit respiration and decrease of the ATP production those effects on germination and growth (Jimenez & Gliesman, 1987).

Our results showed that amonts of Chla & b in cotyledon of soybean seedling in treated samples to comparison with control decreased (table1).Rice (1984) showed that allelochemicals such as phenolic acid inhibit biosynthesis of chlorophyll precursors.Decreasing of chlorophyll by allelochemicals result of inhibition of chlorophyll biosynthesis or induction of their degredation pathway..There are two pathways for chlorophyll degredation: 1) Chl dephytlation that by chlorophyllase was catalyzed and 2) Mg dechelation that by Mg-dechlatase was catalyzed (Matile et al, 1996). It is thought that allelochemicals such as phenolic acid induce activity of chlorophylase and Mg-chlatase (Yang et al, 2004).

Aquatic extract of canola caused to decreased peroxidase and catalase activity in cotyledon, stem and root of soybean seedling (fig 1,2).

It is reported that allelochemicals effects on some of the enzyme activity for example enzyme activity such as catalase, peroxidase (POD), polygalactoronase, super oxidedismutase (SOD) and amylase in presence of allelochemicals reduce (Williams & Hoagland, 1982., Rizivi & Rizivi, 1992). Reduction of SOD and POD activity caused the reactive oxygen species (ROS) accumulation in leaf that resulting to lipid peroxidation in membrane and their destruction (Bais et al., 2003) and DNA degradation (Appel, 1993).

Table 1. The effect of aquatic extract of canola (*Brassica napus* L. *cv*Hyola 401) in Hogland solution on length of root and epicotyl, amount of chl a & b, activity catalase and peroxidase in cotyledon, shoot and root of soybean (*Glycine max* L. *cv* Gorgan3) seedling in comparision with to control (Hoagland only) (X ± SE).

assays	organ	Control (Hoagland)	Treatment (Hoagland+ canola extract)
Length (cm)	root	5.12± 0.075 (a	2.77 ± 0.085 (b
Length (cm)	epicotyl	7.07 ± 0.04 (a	3.15 ± 0.01 (b
chl a (mg/g FW)	cotyledon	0.136 ± 0.001 (a	0.122 ± 0.001 (b
chl b (mg/g FW)	cotyledon	0.0637 ± 0.001 (a	0.0625 ± 0.0003 (b

Also ROS chloroplast and mitichondria desintegrate and photosynthesis and respirartion decrease and following ATP production in plant drop (Shiming, 2003).

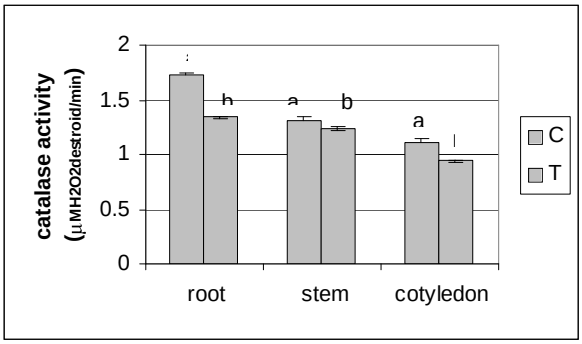

Fig 1. The effect of aquatic extract of canola (*Brassica napus* L. *cv* Hyola 401) in catalase activity ($\mu.MH_2O_2$ *destroid/min*) in cotyledon, shoot and root of soybean (*Glycine max* L. *cv* Gorgan3) seedling. C=control (Hoagland), T=treatment (Hoagland+extract) (X ± SE).

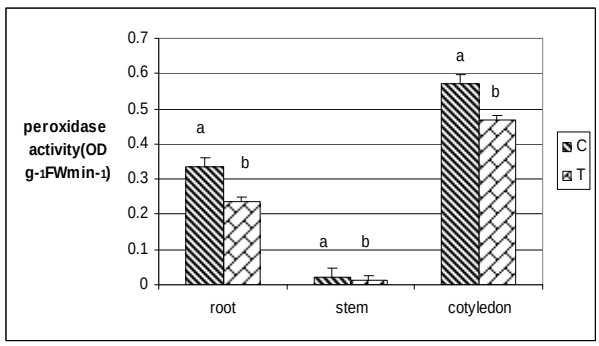

Fig 2. The effect of aquatic extract of canola (*Brassica napus* L. *cv* Hyola 401) in peroxidase activity ($OD\,g^{-1}.\,FW\,min$-) in cotyledon, shoot and root of soybean (*Glycine max* L. *cv* Gorgan3) seedling.

Our results showed that nitrate reductase (NR) activity in stem, cotyledon and root in soybean seedling in presence of aquatic extract of canola change. In cotyledon, enzyme activity in

Hoagland solution with canola extract decreased while in root and stem increased (fig3).

It is reported that stress effect on NR activity. Salinity stress increased NR activity in root of *Zea mays* L. while decreased their activity in leaf of *Anacordium occidentale* L. (Katalin et al., 2000).

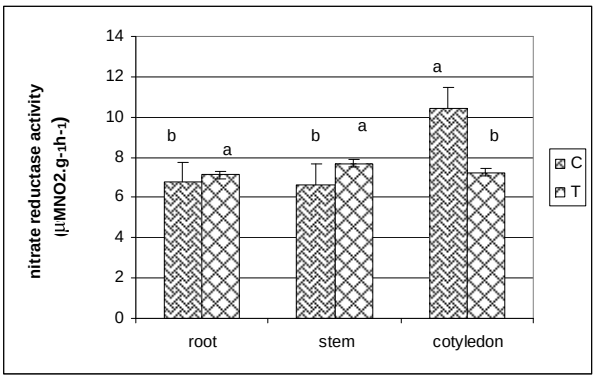

Fig 3. The effect of aquatic extract of canola (*Brassica napus* L. *cv* Hyola 401) on nitrate reductase activity ($\mu MNo^{-}{}_{2}g^{-1}.h^{-1}$) in cotyledon, shoot and root of soybean (*Glycine max* L. *cv* Gorgan3) seedling.

References

Appel, H. M. (1993). Phenolics in ecological interactions: The importance of oxidation. Chem. Ecol. 19: 1521-1552.

Bais, H.P., Vepechedu. R., Gilroy. S., Callaway. R.M. and vivanco. J.M. (2003) Allelopathy: From molecules and genes to species interactions. Science 301: 1377-1380.

Bruisma, j. (1963).The quantitative analysis of chlorophyll a & b in plant extract .Photochem. Photobiol, 12: 241.249.

Brunn, S.A., Muday, G.K. and Haworth, P. (1992). Auxin transport and the interaction of phytotropins. Plant Physiol. 98: 101-107.

Chance, B., and Maehley, A. (1955). Assay of catalases and peroxidase, Methods in Enzymology, 2, 764-775.

Ervin, G.N. and Wetzel, R.G. (2000). Allelochemical autotoxicity in the emergent wetland macrophyte *Juncus effusus* (Juncaceae) Am. J. Bot. 87: 853-860.

Fettell, N.A. and H. S. Gill 1995. "Long-term effects of tillage, stubble, and nitrogen management on properties of a red-brown earth." Australian Journal of Experimental Agriculture 35: 923-928.

Jimenez-orornio, J.J. and Gliessman, S.R. (1987). In allelochemicals role in agriculture and foresty. American Chemical Society, Washington, Dc. pp: 262-274.

Katalin, N. Omarov, R.T., Evdei, L. and Herman lips, S. (2000). Distribution of the Mo-enzymes. aldehyde oxidase, xanthine dehydrogenase and nitrate reductase in maize (*Zea maize* L.) roots as affected by nitrogen and Salinity. Plan Sci. 155: 45-58.

Khalid, Sh., Ahmad, T. and Shad, R.A. (2002). Use of allelopathy in agriculture. Asian Journal of Sciences. 3: 292-297.

Koroi, S.A.A. (1989). Gele electrophores tishe and spectrophoto metrscho unter uchungen zomeinfiuss der tem pelature auf straktur der amylase and peroxidose isoenzyme. Physiol.Veg, 20: 15-23.

Malinda, D. K. (1995). Factors in conservation farming that reduce erosion. Australian Journal of Experimental Agriculture 35: 969-978.

Matile, P. Hortensteiner, S., Thomas, H. and Krautler, B. (1996). Chlorophyllase in the chloroplast envelope. Planta. 201: 96-99.

Narwal, S.S and Tauro, P. (1996). Suggested methodology for allelopathy: field observations and Methodology. P. 255-260.

Purvis, C. E. (1990). Differential response of wheat to retained crop stubbles. I. Effect of stubble type and degree of decomposition". Australian Journal of Agricultural Research 41: 225-242.

PengS-L and Shao. H. (2001). Reaserch significance and foreground of allelopathy.Chin J Appl Ecol, 12:780-786.

Rice, E. L. (1984). Allelopathy. 2nd ed. Academic press, Orland. pp: 226-291

Rizivi, S. J. H. and Rizivi. V. (1992). Expoitation of allelochemiocals in improving crop productivity. Cand hall, London. p: 443-473

Shiming, L. (2003). Allelopathy in South china agroecosystems. Institute of Tropical and Subtropical Ecology. P: 40-54

Sym, G.L. (1984). Optimisation of the *invivo* assay conditions for nitrate reductase in barly. J. Sci. Food. Agri, 35: 725-730.

Williams, R.D. and Hoagland, E. (1982). The effects of naturally Occurring phenolic compounds on seed germination.Weed Sci. 30: 206.

Yang, C.M., Chang, I.F., Lin, S.J. and C.H. (2004). Effects of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedling: II. stimulation of consumption-Orientation. Bot. Bull. Acad. 45: 112-125.

Zeng, R. S., Luo, S. M., Shi, Y.H., Shi, M.B. and Tu, C.Y. (2001). Physiological and biochemical mechanism of allelopathy of Secalonic acid F of higher plants. Agronomy Journal. 93: 72-79.

بررسی اثر عصاره آبی کلزا بر میزان کلروفیل، فعالیت آنزیم‌های نیترات ردوکتاز، کاتالاز و پراکسیداز در دانه‌رست سویا تحت شرایط هیدروپونیک

مریم نیاکان، معصومه تجری

گروه زیست‌شناسی دانشگاه آزاد اسلامی واحد گرگان

چکیده

گیاه کلزا حاوی ترکیبات آللوشیمیایی می‌باشد که این ترکیبات متابولیسم علف‌های هرز و گیاهان زراعی را تحت تاثیر قرار می‌دهد. هدف از این مطالعه بررسی اثر عصاره آبی کلزا بر میزان کلروفیل و نیز فعالیت آنزیم‌های نیترات ردوکتاز کاتالاز و پراکسیداز در ریشه، ساقه و لپه دانه‌رست سویا تحت شرایط هیدروپونیک می‌باشد. در این راستا بذرهای گیاه کلزا رقم هایولا ٤٠١ تحت شرایط گلدانی کشت و از کل گیاه در مرحله ۵ برگی عصاره آبی تهیه شد. این عصاره به محیط کشت هوگلند افزوده و پس از ۹ روز میزان کلروفیل a و b در لپه و فعالیت آنزیم‌های نیترات ردوکتاز، کاتالاز و پراکسیداز در سه بخش ریشه، ساقه و لپه دانه‌رست سویا رقم گرگان ۳ مورد ارزیابی قرار گرفت. نتایج نشان داد که مقدار کلروفیل a وb در لپه‌ها و نیز فعالیت آنزیم‌های کاتالازو پراکسیداز در ریشه، ساقه و لپه‌دانه رست‌های سویا تحت تیمار در مقایسه با شاهد (هوگلند) کاهش یافت، در حالی که فعالیت نیترات ردوکتاز در لپه کاهش و در ریشه و ساقه این گیاهان افزایش یافت.

واژه‌های کلیدی: پراکسیداز، سویا، کاتالاز، کلروفیل، کلزا، نیترات ردوکتاز، هیدروپونیک