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Methanol Extract Potential of Field Bindweed (Convolvulus arvensis L.) for Wheat Growth Enhancement

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Abstract: This study aimed to investigate the allelopathic potential of field bindweed (Convolvulus arvensis L.) methanol extract at different concentrations (75, 150, 300 and 600 ppm) on the growth and some physiological processes of wheat (Triticum vulgare L. var. Sides 1). The pot experiment was processed during the winter season from November, 2008 to April, 2009, in the greenhouse of, Botany Department, Beni-Suef University. The irrigation of wheat grains with low methanol extract concentrations (75, 150 and 300 ppm) had stimulatory effects on lengths and dry weights of both root and shoot compared with the control. In the same way, the contents of chlorophyll, carbohydrate, protein and phenolic compounds were enhanced. Antioxidant enzymes (catalase, peroxidase, phenoloxidase and suberoxide dismutaze) activities were gradually stimulated while a gradual suppression in lipid peroxidation and H₂O₂ content was recorded reaching the highest values at 300 ppm. On the other hand, the treatment with the highest concentration (600 ppm) slightly inhibited all the measured parameters except lipid peroxidation and H₂O₂ contents. HPLC analyses of phenolic compounds in the field bindweed methanol extract revealed the abundance of p-coumaric and p-hydroxybenzoic acids, while both resorcinol and cinnamic acids were found in traces. The present results indicated the stimulatory potential of lower methanol extracts concentrations that could be used as a bio-fertilizer.

Key words: Convolvulus arvensis, Triticum vulgar L. sides, methanol extract, allelopathic potential

INTRODUCTION

Allelopathy is a natural and an environment-friendly technique which may prove to be a unique tool for weed control, increase crop yields, decreasing our reliance on both synthetic pesticides and improving the ecological environment (Lovelace et al., 2001; Minorsky, 2002; Fitter, 2003; Inderjit and Duke, 2003). Allelochemicals vary in chemical composition, concentration and localization in plant tissues. Moreover, they vary from plant to plant with changes in both biotic and a biotic conditions (Inderjit and Duke, 2003). These chemicals are largely classified as secondary metabolites produced as offshoots of primary metabolic pathways particularly the shikimic acid or acetate metabolic pathways (Croteau et al., 2000). Exposure of plants to allelochemicals affected their growth and development (Hegab et al., 2008; Niakan et al., 2008; Zhang et al., 2010) and morphological changes can be caused by a variety of more specific effects acting at the cellular or molecular level in the receiver plants (Zhang et al., 2010;

Singh et al., 2006; Peng et al., 2004a, b). Plant phenolic compounds are among the major allelochemicals implicated in allelopathy (Inderjit and Mallik, 2002; Chon et al., 2005). Plant phenolic compounds at low concentrations have been widely reported to be substances stimulatory to seed germination and plant growth (Hegab, 2005; Hegab et al., 2008; Hassan and Ghareib, 2009; Ghareib et al., 2010). Some phenolics like caffeic, ferulic and p-coumaric acids appear to be more active antioxidants, food flavour precursors and are considered to be an important part of the general defense mechanisms (Shahrzad and Bitsch 1996; Floridi et al., 2003). Many studies have revealed that all weeds present in crop fields are not harmful to crops (Oudhia et al., 1999). The stimulatory allelopathic effects of extracts and leachates of different weeds parts on germination and seedling vigour and final yield of agricultural crops have been reported (Oudhia and Tripathi, 1998; Ghareib et al., 2010). Also, the extracts of many dominant plants in Taiwan contain allelopathic compounds, including phenolic acids, alkaloids and flavonoids that can be used as biofertelizar, natural herbicides and fungicides, which are less disruptive of the global ecosystem than are synthetic agrochemicals (Chou, 1995; Hegab and Ghareib, 2009).

There are many examples of allelopathic activity between plants including weed-crop, crop-weed and crop-crop interactions. The allelopathic potential of common weeds for germination and seedling growth of several crop species were reported (Qasem and Hill, 1989; Pardals *et al.*, 1992). Previous results reported the detrimental effects of several weed species on the growth and yield of wheat (Inderjit and Dakshini, 1998; Singh *et al.*, 2003; Kong *et al.*, 2007). In the same manner some weed residue possesses allelopathic potential to increase the growth and yield of some crop (Chivinge, 1985; Hagin, 1989).

Field bindweed is a twining, perennial weed that reproduces by both seeds and adventitious shoots arising from a spreading root system. It becomes a problem when competes strongly with many crops such as wheat, corn an causes their yield decrease (Holm *et al.*, 1977; Weaver and Rilly, 1982). This study involved the investigation of the possible interference of field bindweed residue allelochemicals during growth of wheat (*Triticum vulgare* L. Sides 1).

MATERIALS AND METHODS

Sampling of plant material: The shoots of mature field bindweed plant were collected from different cultivated fields around Beni_Seuf governorate during fruit development stage on May, 2008. Shoots of the collected weeds were dried for 4 weeks under room temperature. The dried shoots were ground and stoked in plastic sacs in dark condition at room temperature until use. Grains of wheat were kindly obtained from the Crop Department, Sides Research Center, Ministry of Agriculture, Beni_Suef, Egypt.

Preparation of the extract solutions: Weed plant residues (100 g) were mixed with methanol for 24 h in the ratio of 1:10 w/v (residue: methanol). The extract was dried using rotary evaporator at reduced pressure and 45°C to obtain 14.5 g. The extract was dissolved using tap water to prepare the test solutions 75, 150, 300 and 600 ppm.

Greenhouse pot experiment: The greenhouse pot experiment was conducted for the assessment of the possible allelopathic effect of allelochemicals on some physiological activities associated with the growth of wheat crop. The pot experiment was carried out under natural conditions during the winter season from

November, 2008 to April, 2009, in the greenhouse of Beni-Suef University, Botany Department. A plastic pot (25 cm diameter x 30 cm depth), containing 4 kg of a mixture of clay-sandy soil (4:1, w/w), supplemented with all macro- and micro-nutrients needed by plants to grow normally. Each treatment was replicated twelve times in a completely randomized experimental design. Each pot was planted with fifty healthy wheat grains at 3 cm depth. After emergence, the seedlings were thinned to 10 healthy seedlings per pot. Pots were maintained in a greenhouse under natural conditions of light with a 10 h photoperiod, 20/15 C°±3- day/night temperatures and 55- 60% relative humidity. During the experiment, Plants were irrigated daily with tap water for control and with water extract for treatments. Growth criteria and photosynthetic pigments content of the representative wheat plant were studied after 20 days from the first day of sowing.

Growth criteria analyses: The lengths of shoots and roots were measured and their fresh weights were recorded. The samples were dried to constant weight in an oven at 60°C to determine the dry weight.

Biochemical analyses: Photosynthetic pigments were extracted and determined (Fadeel, 1962) and phenolic content was extracted (Jindal and Singh, 1975) and estimated by Folin-Ciocalteau phenol reaction (AOAC, 1990). Total soluble sugars were extracted according to the method of (Upmeyer and Koller, 1973) and determined by Nelson's test (Clarke and Switzer, 1977). Free amino acids in the TCA extract were determined as amino-N (Russell 1944) and total soluble protein content was determined quantitatively according to Bradford (1976a, b) method.

Anti-oxidant enzymes bioassay

Extraction: Fresh seedlings of wheat were extracted with 2.5 mL of 67 mM cold phosphate buffer (pH 7.0) as described by Shann and Blum (1987). The homogenates were centrifuged at 10000 rpm for 15 min at 4°C. The clear supernatant was used as a raw extract material for enzymatic assay.

Activity bioassay: Catalase (CAT) (EC 1.11.1.6) was assayed by measuring the initial rate of disappearance of $\rm H_2O_2$ (Kato and Shimizu, 1987). The decrease in $\rm H_2O_2$ was followed as a decline in the absorbance at 240 nm and the activity was calculated using the extinction coefficient (40 mM⁻¹ cm⁻¹ at 240 nm). The activity was expressed in units of μ M of destroyed $\rm H_2O_2$ min⁻¹ g⁻¹ fresh weight. Peroxidase (EC 1.11.1.7) activity was measured according to Kar and Mishra (1976). The enzyme activity was

expressed as the change in the optical density of pyrogallol min⁻¹ g⁻¹ fresh weight. Polyphenoloxidase (POL) (EC 1.10.3.1) activity was measured according to Kar and Mishra (1976). Enzyme activity was expressed as the change in the optical density of pyrogallol min⁻¹ g⁻¹ fresh weight. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined according to the method of (Beyer and Fridovich, 1987). One unit of SOD activity was defined as the amount of enzyme required to cause inhibition of the photo-reduction of NBT by 50% (U mg⁻¹ FW).

Determination of H₂O₂ content Lipid Peroxidation (LP):

H₂O₂ content was colorimetrically measured as described by Velikova *et al.* (2000). H₂O₂ level was calculated using the extinction coefficient 0.28 μmol⁻¹ cm⁻¹.

Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for determining the extent of lipid peroxidation. According to Preuss *et al.* (1998), lipid peroxidation was estimated by measuring the concentration of thiobarbituric acid reactive substances (TBARS) and expressed as n mol malondialdehyde (MDA) (nmol g⁻¹ FW) using the extinction coefficient (156 mM⁻¹ cm⁻¹).

Identification of free phenolic compounds by HPLC: The standard free phenol compounds used for HPLC analysis (P-Hydroxybenzoic, coumaric, Caffeic. p-Chlorogenic, Salicylic, Catechol, Vanillic, o-coumaric, Shikimic, Gallic, Syringic, Sinapinic, pyrogallic, Protocatechuic acid and trans-Cinnamic acids as well as Resorcinol, Scopoletin, coumarin, vanillin, Apigenin). The shoot residue was extract with methanol and acidified with dilute phosphoric acid to pH 2.5 and then partitioned two times with an equal volume of diethyl ether. The combined diethyl ether layers were evaporated to dryness and the resulting residue was dissolved in dissolved in HPLC grade MeOH to give 1000 ppm a 20 µL was injected into HPLC.

Statistical analysis: The experimental design was completely randomized with three replications. The results of greenhouse pot experiment were analyzed with one-way analysis of variance and the mean values were separated at p<0.01 and p<0.05. The statistical analysis was done using the SPSS®/PC computer software package version 11.1., 2001.

RESULTS

Growth criteria: Under green house experiment, results showed that the wheat grain treatment with lower

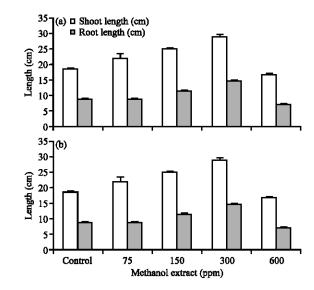


Fig. 1: Effect of different concentrations of methanol extract (ppm) of field bindweed shoots on the shoot and root growth criteria of treated wheat plant after 20 days of sowing. (a) Shoot and root length and (b) shoot and root dry weight

concentrations of methanol extract field bindweed shoots exhibited a marked stimulation of both shoot and root parameters of wheat with respect to their controls (Fig. 1a, b). The increase of the applied concentrations caused stimulation in length of shoot and root recording maximum values at 300 ppm (57 and 65.5%, respectively). Also, the rate of stimulation of dry weights were clear at the application of 150 ppm. At 300 ppm, the dry weights of shoots and roots of wheat after 20 days of sowing showed high significant increases up to 86.5 and 118.5% respectively, as compared with respective controls. In contrast, at the highest concentration (600 ppm), non significant decreases in shoot and root growth criteria were recorded.

Fruit properties: Compared to the control, the spike length, fresh weight and dry weight were highly significantly increased in all treatments with test solutions (Fig. 2). Moreover, the highest improvement was recorded when grains treated with 300 ppm (72, 73 and 76%, respectively with respect to control). Oppositely, the highest applied concentration (600 ppm) induced a slight inhibitory effect in fruit growth criteria compared with control.

Photosynthetic pigment content: The different content of pigments of wheat leaves relatively to the control treatment was progressively increased with the increase

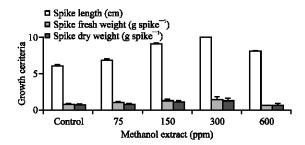


Fig. 2: Effect of different concentrations of methanol extract (ppm) of field bindweed shoots on the fruit properties of the treated wheat plant after 20 days of sowing

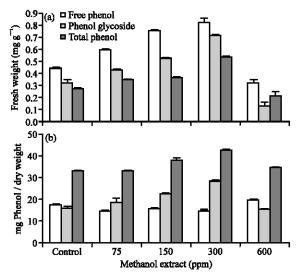


Fig. 3: Effect of different concentrations of methanol extract (ppm) of field bindweed shoots on chlorophyll (mg g⁻¹ fresh weight) and phenolic (mg g⁻¹ dry weight) contents of the treated wheat plant after 20 days of sowing. (a) chlorophyll contents and (b) phenolic contents

of the concentrations of the treatment solutions, while they decreased at 600 ppm (Fig. 3a, b). The most stimulating concentration was 300 ppm that it significantly increased pigment contents by about 86.4, 121.8 and 96.3% for Chlorophyll a, Chlorophyll b and cartenoids, respectively.

Phenolic content: The free phenolic content of wheat was slightly increased throughout the experiment accompanied by highly increase in phenolic glycoside. The highest values of free phenol inhibition and phenolic glycoside contents stimulation were obtained when the grains treated with 300 ppm and they accounted by 17.4 and 78.7%, respectively (Fig. 3). Meanwhile, at 600 ppm, the reduction in phenolic glycoside and the enhancement

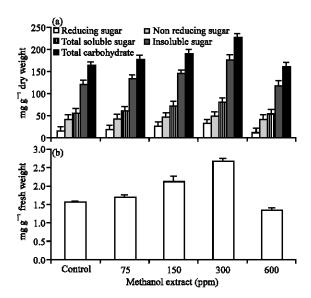


Fig. 4: Effect of different concentrations of methanol extract (ppm) of field bindweed shoots on carbohydrate (mg g⁻¹ dry weight) and soluble protein (mg g⁻¹ fresh weight) contents of the treated wheat plant after 20 days of sowing. (a) Carbohydrate contents and (b) total soluble protein

in free phenols contents were reported. Moreover, the phenolic glycoside/phenolic aglycone ratio was increased with the increasing the methanol extract of shoot residue of field bindweed.

Carbohydrate content: At the growth stage, total soluble (both reducing and non-reducing) and insoluble sugar contents of wheat were improved at the application of all treatments which reach 43 and 47.7%, respectively at (300 ppm) as compared with their respective controls (Fig. 4a). This response was retarded by the highest level of applied concentrations.

Protein content: In this concern, the treatment with the methanol concentration of weed shoots extracts at 300 ppm highly significantly improved the content of protein in shoots by about 72.7% with respect to control (Fig. 4b). In the same time, the increasing the methanol extract concentrations of filed bindweed to 600 ppm suppressed the protein contents.

The activity of anti-oxidant enzymes: It is clear from (Fig. 5) that the low ethanol extract concentrations (75, 150 and 300 ppm) had stimulatory effects on the antioxidant activities. On the other hand, the highest concentration (600 ppm) had slight inhibitory effect on all the measured enzymes activity. In response to allelochemicals treatment

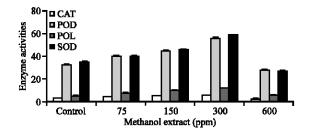


Fig. 5: Effect of different concentrations of methanol extract (ppm) of field bindweed shoots on activities of catalase (CAT) (μM H₂O₂ destroyed min⁻¹ g⁻¹ FW), Peroxidase (POD) (A420, μM Purpurogallin min⁻¹ g⁻¹ FW), polyphenoloxidase (POL) (A420, μM Purpurogallin min⁻¹ g⁻¹ FW), superoxide dismutase (SOD) (U g⁻¹ FW)

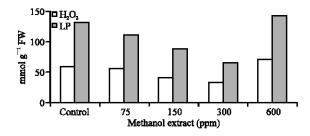


Fig. 6: Effect of different concentrations of methanol extract (ppm) of field bindweed shoots on lipid peroxidation (LP) (nmol MDA $\rm g^{-1}\,FW$) and $\rm H_2O_2$ (nmol $\rm g^{-1}\,FW$) content

of the weed extract, catalase activity produced a stimulatory pattern at 75, 150 and 300 ppm. Noticeabley, the catalase activity was markedly higher than control by mixing the test grains with 300 ppm of weed ethanol extract (87.7%), whereas at 600 ppm, it was diminished to 32.2% compared with control. Also the peroxidase enzyme activity was stimulated allelochemical treatments at the same concentrations (75, 150 and 300 ppm), recording the maximum stimulation (70.4%) at 300 ppm. In contrast, the treatment with 600 ppm exerted moderate inhibition (14.5%). Polyphenoloxidase and suberoxide dismutaze enzymes activity exhibited similar change patterns with higher degree of stimulation at lower concentrations and also was inhibited by the highest applied concentration of allelochemicals. Maximum Stimulation at 300 ppm, recorded 70.9 and 141% for POL and SOD, Respectively. Oppositely, the highest concentration (600 ppm) caused a moderate degree of inhibition of enzyme activity for POL (13.1%) and SOD (22.3%).

Table 1: Quantitative determination of HPLC analysis on some phenolic compounds present in methanol extract of shoot residue of field bindweed

	Retention time (min)		
Standard phenolic			Concentration
compounds	Standard	Sample	(μg g ⁻¹ dry weight)
Pyrogallic acid	9.2480	9.3140	12.420
Protocatechuic acid	12.736	12.437	5.063
Resorcinol	13.739	14.042	3.52
Chlorogenic acid	16.331	16.741	7.52
p-Hydroxybenzoic acid	16.832	16.707	47.82
Caffeic acid	18.016	18.223	7.14
Syringic acid	18.379	18.632	22.50
Ferulic acid	24.853	24.954	14.87
p-coumaric acid	29.333	29.410	54.50
Salicylic acid	30.389	30.445	13.87
Cinnamic acid	36.149	36.281	4.87

Lipid peroxidation and H_2O_2 content: Low levels of H_2O_2 and malondialdehyde (MDA) were recorded at treatments with 75, 150, 300, 600 ppm as compared with control and decreased to minimum values for H_2O_2 and MDA (44 and 51%, respectively) with 600 ppm treatment (Fig. 6). The reduction in H_2O_2 and MDA contents were followed by a slight stimulation up to 18 and 7% respectively, at the highest concentration (600 ppm).

Identification of phenolic composition in field bindweed by

HPLC: Table 1 shows the retention times and chromatogram of some representatives of a mixture of twenty five standard phenolic compounds detected at 254 nm. The quantities analyses of plant phenolics using HPLC were based on the comparison of the retention time of a mixture of standard phenolics with those in plant samples. The most abundant free phenolic compounds were in shoot residue of field bindweed were *o*-coumaric and p-Hydroxybenzoic acids (28 and 25%, respectively) while both Resorcinol and Cinnamic acid were found in lesser amounts (1.8 and 2.5%) respectively.

DISCUSSION

Some of the phenolic acids similar to that identified in *C. arvensis* (Table 1), were reported to play a stimulatory role in growth of some crop plants (Chung *et al.*, 2002; Hegab *et al.*, 2008). At low doses, phenolic compounds such as, caffeic, ferulic, Protocatechuic acid and vanillic acids have been reported to stimulate the growth, anti-oxidant enzymes activity, protein and chlorophyll contents of some crops (Baziramakenga *et al.*, 1997; Hegab, 2005; Hegab *et al.*, 2008; Hegab and Ghareib, 2009). On the other hand, at high concentration, plant phenolic compounds suppressed germination, carbohydrates and protein

contents as well as antioxidant enzymes activities while increased lipid peroxidation (Hegab, 2005; Hegab *et al.*, 2008).

The grains treatments with different concentrations of weed shoots methanol extracts caused enhancement for wheat growth at all application rates except the highest one (600 ppm). In this concern (Oudhia et al., 1997, 1999) reported that the inhibitory effect was a function of the concentrations and the greatest inhibition observed under the high concentration application. Also, Harmful allelopathic effects of these weeds on germination and seedling vigour of many agricultural crops have been reported (Narwal, 1994; Oudhia, 1999). Based on the present results, the accumulation of total phenolic compounds, mainly glycoside, in the shoot of wheat plant may be related to the stimulation of phenolics production and their glycosylation to adapt themselves against the external conditions. At this connection, the sugar units bind to free aglycones at the late stage of biosynthesis forming phenolic glycoside, which become non-toxic, more water-soluble and easily transportable to nonphotosynthetic tissue (Hrazdina and Wanger, 1985). Moreover, the increase of phenolic glycoside content over the aglycone form in the shoot of 20 days-old wheat plant indicates the ability of plants to protect themselves against the external conditions (Kleiner et al., 1999; Shvets et al., 1996).

Several studies revealed that phenolic acids can interfere with the production of chlorophylls. Many allelochemicals were reported to interfere with the chlorophyll production due to their interference with porphyrin containing compound (Kanchan Jayachandra, 1980). Yang et al. (2002) studied the action of p-coumaric acids on the biosynthesis of porphyrin precursors and concluded that the interfere of Mgchelatase affect the level of chlorophyll accumulation in rice seedlings. Additionally, Inderjit and Dakshini (1995) found that water soluble phenolics from Pluchea lanceolata influenced the chlorophyll content of the leaves of their test plants under green house conditions. It was clear that the allelochemical of field bindweed residue induced stimulatory effect on total soluble, insoluble sugar and total carbohydrate, as compared with their respective controls. This stimulation could accelerate the enzyme system involved in the insoluble sugar accumulation. This result in agreement with that of Tripathi et al. (1998) who stated that total sugar content in soybean leaves increased as a result of treatment with 5%concentration of Acacia leaf extract. At the highest residual concentration value, the reduction in chlorophyll contents was affected and consequently reduced the photosynthetic efficiency. It has been reported by Devi and Prasad (1996) that ferulic acid decreased the net rates of CO₂-assimilation, electron transport and photophosphorylation in maize.

The protein content was stimulated with application of different levels of allelochemicals and this stimulation correlated with stimulation in nucleic acid content. As p-hydroxybenzoic and p-coumaric acids increased incorporation of 35 S-methionine into protein (Baziramakenga et al., 1997). This may be attributed to the interference of allelochemicals with the cytoplasmic ribosomes and production of RNA, which in turn stimulates protein synthesis. The reduction in soluble protein, at the highest residual concentration, may be attributed to the effect of allelochemicals on DNA replication or translation by intercalation or with nucleic acids by ionic bonding with their negatively charged phosphate groups. On the other side the protein content of treated wheat plants was decreased by the highest application of allelochemicals this was may be due to the accumulation of phenolic aglycones that interfere with the cytoplasmic ribosomes and production of RNA, which in turn inhibited protein synthesis. In this respect, Bolwell et al. (1988) demonstrated that cinnamic acid derivatives depressed translational activity of polysomal mRNAase of bean cells that reduced the protein synthesis. Field bindweed induced both stimulatory and inhibitory effects on the antioxidant activity system of wheat. A stimulatory pattern at low concentrations and an inhibitory pattern at high concentrations were reported. This increase could be attributed to the ability of plants to improve the scavenging system (Vaidyanathan et al., 2003). The marked increases in anti-oxidant enzymes (CAT, POR and SOD) have also been observed in other studies on modes of action of allelochemical such as, phenols (Yu et al., 2003). Also, Hegab (2005), Ghareib et al. (2010) and Niakan and Saberi (2009) revealed an increase in activities of antioxidative enzymes (CAT, POD, POL and SOD) in target plants treated with phenolic compounds at low levels and he indicated their role in pea plants survival. In the same way,) showed that antioxidant enzymes (such as CAT, POD and POL) activity in root and shoot of Phalaris were enhanced by exposure to allelochemicals. On the other hand, at the higher concentrations, the treatments with the methanol extracts suppressed the activities of antioxidant enzymes. At higher concentration (600 ppm), the activity of certain defense enzymes was retarded and that could be explained by either inhibition of their synthesis or their inactivation and down regulation (Dixit et al., 2001). Upon the increase in the applied concentrations, H₂O₂ content was decreased at low concentrations followed by a slight stimulation at the highest

concentration causing oxidative stresses. Testa (1995) reported that the toxicity of many quinones and phenols can largely be attributed to the formation of semiquinone radicals that donate electrons to molecular oxygen, forming superoxide anions. Zeng et al. (2001) indicated a significant reduction of SOD and POD activities which may cause a mass accumulation of active O2 in plant leaves. The low levels of concentration caused inhibition in accumulated MDA and these results agreed with Hegab et al. (2005) and Ghareib et al. (2010) who stated that phenolic acids at low concentrations decreased the MDA content and prevent lipid proxidation. By increasing concentrations, the accumulation of MDA showed slight increase, in the same manner, Ghareib et al. (2010) indicated that the treatment of tomato plants with phenolic extract at high level caused an increase of LP.

CONCLUSIONS

The data recorded that the treatment with lower concentrations of field bindweed methanol extract (25, 75 and 300 ppm) was the causative factor responsible for the improvement of seedling growth, some biochemical contents and the activities of anti-oxidant enzymes of wheat crop. The allelopathic potential of methanol extract encouraged us to recommend its as an effective environmentally friendly bio-fertilizer for wheat growth.

REFERENCES

- AOAC, 1990. Official Method of Analysis of the Association of Official Analytical Chemists. 15th Edn., AOAC, Washington, DC.
- Baziramakenga, R., G.D. Levoux, R.R. Simard and P. Nadeau, 1997. Allelopathic effects of phenolic acids on nucleic acid and protein levels in soybean seedlings. Can. J. Bot., 75: 445-450.
- Beyer Jr., W.F. and I. Fridovich, 1987. Assaying for superoxid dismutase activity: Some large consequences of minor changes in conditions. Anal. Biochem., 161: 559-566.
- Bolwell, G.P., M. Mavandad, D.J. Miller, K.J. Edwards, W. Schuch and R.A. Dixon, 1988. Inhibition of mRNA levels and activities by *trans*-cinnamic acid in elicitor-induced bean cells. Phytochemistry, 25: 2109-2117.
- Bradford, M.M., 1976a. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

- Bradford, M.M., 1976b. Allelopathic effects of purple nutsedge (*Cyperus rotundus*) on the growth and development of cotton (*Gossypium hirsutum*), maize (*Zea mays*) and soybeans (*Glycine max*). Zimbabwe. Agric. J., 82: 151-152.
- Chou, C.H., 1995. Allelopathy and sustainable agriculture. ACS Symp. Ser., 582: 211-223.
- Chon, S.U., H.G. Jang, D.K. Kim, Y.M. Kim, H.O. Boo and Y.J. Kim, 2005. Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. Sci. Hort., 106: 309-317.
- Chung, I.M., K.H. Kim, J.K. Ahn, S.C. Chun, C.S. Kim, J.T. Kim and S.H. Kim, 2002. Screening of allelochemicals on barnyardgrass (*Echinochloa crus-galli*) and identification of potentially allelopathic compounds from rice (*Oryza sativa*) variety hull extracts. Crop Prot., 21: 913-920.
- Clarke, Jr. J.M. and R.C. Switzer, 1977. Experimental Biochemistry. 2nd Edn., W.H. Freedman and Co., San Francisco, pp. 335.
- Croteau, R., T.M. Kutchan and N.G. Lewis, 2000. Natural Products (Secondary Metabolites). In: Biochemistry and Molecular Biology of Plants, Buchanan, B., W. Gruissem and R. Jones (Eds.). American Socity of Plant Biologests (Formerly Physiologists), Rockville, Maryland, USA., pp. 1250-1318.
- Devi, S.R. and M.N.V. Prasad, 1996. Ferulic acid mediated changes in oxidative enzymes of maize seedlings: Implications in growth. Bio. Plant., 38: 387-395.
- Dixit, V., V. Pandey and R. Shyam, 2001. Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). J. Exp. Bot., 52: 1101-1109.
- Fadeel, A.A., 1962. Location and properties of chloroplast and pigment determination in roots. Physiol. Plant., 15: 130-147.
- Fitter, A., 2003. Making allelopathy respectable. Science, 301: 1337-1338.
- Floridi, S., L. Motanari, O. Marconi and P. Fantozzi, 2003. Determination of free phenolic acids in wort and beer by colorimetric array detection. J. Agric. Food Chem., 51: 1549-1554.
- Ghareib, H.R., M.S. Abdelhamed and O.H. Ibrahim, 2010. Antioxidative effects of the acetone fraction and vanillic acid from *Chenopodium murale* on tomato plants. Weed Biol. Manage., 10: 64-72.
- Hagin, R.D., 1989. Isolation and identification of 5hydroxyindole-3-acetic acid and 5hydroxytryptophan, major allelopathic aglycones in quackgrass (*Agropyron repens* L. Beauv.). J. Agric. Food Chem., 37: 1143-1149.

- Hassan, S.M. and H.R. Ghareib, 2009. Bioactivity of Ulva lactuca L. acetone extract on germination and growth of lettuce and tomato plants. Afr. J. Biotech., 8: 3832-3838.
- Hegab, M.M. and H.R. Ghareib, 2009. Potential of Swiss chard (*Beta vulgaris* L. var. cicla) autotoxicity for weed control. Allelopathy J., 24: 301-308.
- Hegab, M.M., 2005. Assessment of the allelopathic effect of some phenolic compounds on some physiological processes of pea plant (*Pisum sativum*). Ph.D. Thesis, Faculty of Science, Beni-Sueif University, Egypt.
- Hegab, M.M., S.E.A. Khodary, O. Hammouda and H.R. Ghareib, 2008. Autotoxicity of chard and its allelopathic potentiality on germination and some metabolic activities associated with growth of wheat seedlings. Afr. J. Biotech., 7: 884-892.
- Holm, L.G., D.L. Plucknett, J.V. Pancho and J.P. Herbenger, 1977. The World's Worst Weeds. University Press of Hawaii, Hawaii, USA., pp. 609.
- Hrazdina, G. and G.J. Wanger, 1985. Compartmentation of Plant Phenolic Compounds: Site of Synthesis and Accumulation. In: The Biochemistry of Plant Phenolics, Van Sumere, C.F. and P.J. Lea (Eds.). Clarendon Press, Oxford, pp. 119-134.
- Inderjit, D. and K.M.M. Dakshini, 1995. On laboratory bioassay in allelopathy. Bot. Rev., 61: 28-44.
- Inderjit, D. and K.M.M. Dakshini, 1998. Allelopathic interferences of chickweed, Stellaria media, with seedling growth of wheat (*Triticum aestivum*). Can. J. Bot., 76: 1317-1321.
- Inderjit, D. and A.U. Mallik, 2002. Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems. Birkhäuser Publishing Ltd., Basal.
- Inderjit, K.L. and S.O. Duke, 2003. Ecophysiological aspects of allelopathy. Planta, 217: 529-539.
- Jindal, K.K. and R.N. Singh, 1975. Phenolic content in male and female *Carica papaya*: A possible physiological marker sex identification of vegetative seedlings. Physiol. Plant., 33: 104-107.
- Kanchan, S.D. and Jayachandra, 1980. Allelopathic effects of *Parthenium hysterophorus* L. Part II: Leaching of inhibitors from aerial vegetative parts. Plant Soil, 55: 61-66.
- Kar, M. and D. Mishra, 1976. Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiol, 57: 315-319.
- Kato, M. and S. Shimizu, 1987. Chlorophyll metabolism in higher plants. VII: Chlorophyll degradation in senescing Tobacco leaves: Phenolic-dependent peroxidative degradation. Can. J. Bot., 65: 729-735.

- Kleiner, K.W., K.F. Raffa and R.E. Dickson, 1999. Partitioning of ¹⁴C-labeled photosynthate to allelochemicals and primary metabolites in source and sink leaves of aspen: Evidence for secondary metabolite turnover. Oecologia, 119: 408-418.
- Kong, C.H., P. Wang and X.H. Xu, 2007. Allelopathic interference of *Ambrosia trifida* with wheat (*Triticum aestivum*). Agric. Ecosyst. Environ., 119: 416-420.
- Lovelace, M.L., R.E. Talbert, R.H. Dilday, E.F. Scherder and N.W. Buehring, 2001. Use of allelopathic rice with reduced herbicide rates for control of barnyardgrass (*Echinochloa crus-galli*). Ark. Aes. Res. Ser., 485: 75-79.
- Minorsky, P.V., 2002. Allelopathy and grain crop production. Plant Physiol., 130: 1745-1746.
- Narwal, S.S., 1994. Allelopathy in Crop Production. Scientific Publisher, Jodhpur, India, pp. 288.
- Niakan M., M. Tajari and M. Ghorbanli, 2008. Effects of salinity on allelopathic potential of canola (*Brassica napus* L.). Allelopath J., 21: 329-338.
- Niakan, M. and K. Saberi, 2009. Effects of *Eucalyptus* allelopathy on growth characters and antioxidant enzymes activity in phalaris weed. Asian J. Plant Sci., 8: 440-446.
- Oudhia, P. and R.S. Tripathi, 1998. Allelopathic potential of *Datura stramonium* L. Crop Res., 16: 37-40.
- Oudhia, P., 1999. Studies on allelopathy and medicinal weeds in chickpea fields. Int. Chickpea Pigeonpea Newslett., 6: 29-33.
- Oudhia, P., N. Pandey and R.S. Tripathi, 1999. Allelopathic effects of obnoxious weeds on germination and seedling vigour of hybrid rice. Int. Rice Res. Notes, 24: 36-36.
- Oudhia, P., S.S. Kolhe and R.S. Tripathi, 1997. Allelopathic effect of *Blumea lacera* L. on wheat. Indian J. Weed Sci., 29: 4-7.
- Pardals Jr, J.R., Y. Kono, A. Yamauchi and M. Iijima, 1992. Seminal root growth in sorgum (*Sorghum bicolor*) under allelopathic influences from residues of taro (*Colocasia esculenta*). Ann. Bot., 69: 493-496.
- Peng, S.L., J. Wen and Q.F. Guo, 2004a. Cell wall peroxidases in cucumber roots induced by phenolic allelochemicals. Allelopathy J., 13: 29-36.
- Peng, S.L., J. Wen and Q.F. Guo, 2004b. Mechanism and active variety of allelochemical. Acta Bot. Sinica, 46: 757-766.
- Preuss, H.G., S.T. Jarrel, R. Scheckenbach, S. Lieberman and R.A. Anderson, 1998. Comparative effects of chromium, vanadium and gymnema sylvestre on sugar-induced blood pressure elevations in SHR. J. Am. Collage Nutr., 17: 116-123.

- Qasem, J.R. and T.A. Hill, 1989. Possible role of allelopathy in the competition between tomato, Senecio vulgaris L. and Chenopodium album L. Weed Res., 29: 349-356.
- Russell, J.A., 1944. Note on the colorimetric determination of amino nitrogen. J. Bio. Chem., 156: 467-468.
- Shahrzad, S. and I. Bitsch, 1996. Determination of some pharmacologically active phenolic acids in juice by high-performance liquid chromatography. J. Chromatogr., 741: 223-231.
- Shann, J.R. and U. Blum, 1987. The uptake of ferulic and p-hydroxybenzoic acids by *Cucumis sativus*. Phytochemistry, 26: 2959-2964.
- Shvets, S.A., P.K. Kintia and M.A. Naibi, 1996. Steroidal glycosides from *Petunia hybrida* L. seeds and their biological activity. Adv. Exp. Med. Biol., 404: 251-262.
- Singh, H.P., D.R. Batish, S. Kaur and R.K. Kohli, 2003. Phytotoxic interference of *Ageratum conyzoides* with wheat (*Triticum aestivum*). J. Agron. Crop Sci., 189: 341-346.
- Singh, H.P., D.R. Batish, S. Kaur, K. Arora and R.K. Kohli, 2006. α-Pinene inhibits growth and induces oxidative stress in roots. Ann. Bot., 98: 1261-1269.
- Testa, B., 1995. The Metabolism of Drugs and Other Xenobiotics. Academic Press, New York, pp. 475.
- Tripathi, S., A. Tripathi, D.C. Kori and S. Tiwari, 1998. Effect of tree leaves aqueous extracts on germination and seedling growth of soybean. Allelopathy J., 5: 75-82.
- Upmeyer, D.J. and H.R. Koller, 1973. Diurnal trends in net photosynthetic rate and carbohydrate levels of soybean leaves. Plant Physiol., 51: 871-874.

- Vaidyanathan, H., P. Sivakumar, R. Chakrabarsty and G. Thomas, 2003. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.)differential response in salt-tolerant and sensitive varieties. Plant Sci., 165: 1411-1418.
- Velikova, V., I. Yordavow and A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid raintreated bean plants: Protective role of exogenous polyamines. Plant Sci., 151: 59-66.
- Weaver, S.E. and W.R. Rilly, 1982. The biology of Canadian weeds. 53-Convolvulus arvensis L. Can. Plant Sci., 62: 461-472.
- Yang, C.M., C.N. Lee and C.H. Chou, 2002. Effects of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings: I. Inhibition of supply-orientation. Bot. Bull. Acad. Sin., 43: 299-304.
- Yu, J.Q., S.F. Ye, M.F. Zhang and W.H. Hu, 2003. Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. Biochem. Syst. Ecol., 31: 129-139.
- Zeng, R.S., S.M. Luo, Y.H. Shi and C.Y. Tu, 2001. Physiological and biochemical mechanism of allelopathy of secalonic acid on higher plants. Agron. J., 93: 72-79.
- Zhang, Y., M. Gu, K. Shi, Y.H. Zhou and J.Q. Yu, 2010. Effects of aqueous root extracts and hydrophobic root exudates of cucumber (*Cucumis sativus* L.) on nuclei DNA content and expression of cell cyclerelated genes in cucumber radicles. Plant Soil, 327: 455-463.