ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Physiological and Molecular Response of Garlic (*Allium sativum* L.) to Cadmium Stress

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ABSTRACT

Cadmium (Cd) is an important industrial agent and environmental pollutant that is a major cause of plants disease. This study was conducted to investigate the impact of Cd on the physiological and molecular traits of garlic growth and active constituent. The garlic cloves were irrigated with different concentrations of Cd. The obtained results show that the measured pigments and total soluble sugars decreased with increased Cd concentration in growth medium. Meanwhile protein and proline content increases due the important role of proline as osmoregulation of plants, stabilization the protein synthesis machinery and an effective singlet oxygen quencher. Cd supports the activity of antioxidant enzymes (catalase, peroxidase, polyphenol oxidase and superoxide dismutase) as a defense system ROS detoxification during normal metabolism and particularly during stress. Alliinase gene expression in the green leaves of garlic plants was negative correlated with Cd stress. The maximum expression were observed at low concentration of Cd (5 and 10 mM). Higher doses of Cd cause a great reduction in allicin production in garlic leaves.

Keywords: Garlic, Cdmium, Pigments, Antioxidant enzymes, Protein, Alliinase transcripts

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ISSN: 0975-8585

INTRODUCTION

Garlic has been used medicinally in almost every known civilization. Recently, garlic is still being employed in folk medicine all over the world for its fungicidal, acaricidal, nematicidal, and bactericidal properties [1]. After chopping or crushing, the enzyme allinase converts alliin (S-allyl-l-cysteine sulfoxide) to allicin [2]. Allicin has been shown to act as an antioxidant by scavenging ROS and preventing lipid oxidation and production of pro-inflammatory messengers [3].

Cadmium is naturally present in soils at trace amounts, high levels of Cd is toxic in some soil environments and highly soluble in water [4]. Cd causes oxidative stress by inducing generation of ROS and disturbing the antioxidative systems in their scavenging [5]. Cd accumulation causes reductions in photosynthesis, alterations in the membranes function by lipid peroxidation, diminishes water and nutrient uptake, interfere with homeostatic pathways for essential metals, inhibition of enzyme activities and disruption of cell transport processes [6].

Proline was accumulated in shoots of *Brassica juncea*, *Triticum aestivum* and *Vigna radiata* in response to cadmium toxicity [7]. Total soluble proteins increased in cucumber by Cd due to de novo synthesis of stress proteins which constitute enzymes involved in glutathione and phytochelatin biosynthesis for Krebs cycle and antioxidants [8]. An increase or decrease in the activity of antioxidants is a direct indication of the adaptive response of plants to avoid the metal toxicity [9]. Higher plants are developing unique molecular mechanisms to cope with different stress factors [10]. Cd can affect protein kinase expression in Arabidopsis. Kilian et al [11] studied the gene expression of several abiotic stresses (heat, cold, heavy metals, drought, salinity, osmotic, UV-B, light and wounding) on the seedling of *A. thaliana.* Cd acts as intermediate signaling molecules to regulate the expression of genes [12].

The objectives of this study are to investigate some physiological parameters (pigments, antioxidant enzyme activities, allicin production) as well as molecular parameter (Allinase gene expression) of garlic plants exposed to Cd stress.

MATERIALS AND METHODS

Plant Material

The garlic cloves (*Allium sativum* L.) were obtained from the Agricultural Research Center, Giza, Egypt. It's genotypes, namely 'Balady', a locally adapted garlic cultivar widely grown in Egypt. It is an early cultivar with large number of relatively small clove per bulb.

Treatment and cultivation

Cloves of garlic were germinated in plastic pots filled with pot most soils irrigated with tap water in a illuminated place to 15 days. After 15 days of planting, the germinated seedlings classified into five groups as:
Irrigated with normal tap water (control).

Irragated with 5 mM CdCl2 .
Irragated with 10 mM CdCl2.
Irragated with 20 mM CdCl2.
Irragated with 50 mM CdCl2.

After 3days of irrigation with Cd solutions the following analysis were carried out on garlic leaf tissues:

Pigment contents

The photosynthetic pigments chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoides in fresh garlic leaves were determined using the spectrophotometric method that described by Hassanein et al. [13] (2009). The concentration of each pigment was calculated using the following equations:

Chl a = 10.3 E663 − 0.918 E644

Chl b = 19.7 E644 – 3.87 E663
Carotenoides= 4.2 E452 – (0.0264 chl a + 0.4260 chl b)

Finally, the pigment contents were expressed as ug g^{-1} fresh weight (FW) of leaves.

Estimation of total soluble sugars (TSS)

Total soluble sugars were extracted by overnight submersion of garlic leaves dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 6000 rpm. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water. TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol Spectrocololourimeter VEB Carl Zeiss [14].

Estimation of proline

A leaf sample was extracted with phosphate buffer (pH 7.6) and was then centrifuged at 8000 g for 20 minutes. Proline was assayed according to the method described by Bates et al [15]. Two ml of extract, 2ml of acid ninhydrin and 2ml of glacial acetic acid were added and incubated for 15 min. in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

Protein extraction for SDS-PAGE

For SDS-PAGE, garlic leaf tissues of each sample were ground to powder under liquid nitrogen and melted in ice-cold extraction buffer (50 mM Tris-HCl, pH 8, 10 mM NaCl, 1% SDS, 5% 2-mercaptoethanol, 0.1 mM PMSF, 0.1 mM DTT), followed by centrifugation (10000 g) at 4 ^{o}C for 15 min. Protein content of the clear supernatants obtained after centrifugation were stored at -20 0C until used.

SDS-PAGE:

Proteins were separated by SDS-PAGE according to Laemmli [16] using protein vertical electrophoresis unit (Hoefer Scientific Instruments). Electrophoresis was started at 10 mA constant current until the tracking dye entered the separating gel and continued at 25 mA until the tracking dye reached the end of the gel. Protein subunit bands were stained with coomassie blue R-250. The protein marker from Sigma was used. The molecular weight of standard protein as follows: 7, 15, 20, 25, 35, 50, 70, 100, 140 and 240 kDa.

Assay of antioxidant enzyme activities

Enzymes extraction was carried following the method of Chen and Wang [17]. Garlic leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation (8000 g) at 4°C for 15 min. The supernatant was used immediately to determine the activities of enzymes.

Catalase (CAT) activity was determined spectrophotometrically by the decrease in absorbance at 240 nm [17]. The mixture (3ml) contained 1.9 ml phosphate buffer (50 mM, pH7.0), 100 µl enzyme extract, and 1 ml 0.3% H_2O_2. The reaction was initiated by adding enzyme extract. One unit of CAT activity was defined as the 0.01 reduction in absorbance at 240 nm per minute.

Peroxidase (POD) was measured according to Kumar and Khan [18] method. Activity of peroxidase was determined based on the appearance of brown colors resulting from guaiacol oxidation in the presence of hydrogen peroxide. Reaction mixture consisted of 50µL sample extract, 2.6 ml of 0.1M sodium phosphate buffer at pH 6.1 and 0.3ml of 1% guaiacol was added to the solution. A total of 0.3ml of 30% H_2O_2 was added prior to reaction. Changes in absorbance at 420 nm were followed for three minutes using a spectrophotometer. Peroxidase activity was calculated using the formula below and expressed as unit/mg protein:

Specific activity of peroxidase = Total activates of the sample/protein content of the sample.

Total activities = Abs. x dilution factor × 1000/volume of enzyme used in the assay.

Polyphenol oxidase (PPO) activity was determined using a spectrophotometric method based on an initial rate of increase in absorbance at 420 nm [19]. Phosphate buffer solution pH 7.0 (0.1M, 1.95 ml), 1 ml of 0.1 M pyrogallol as a substrate and 50 µl of the enzyme extract were pipetted into a test tube and mixed thoroughly. Changes in the absorbance of the solution at 420 nm were monitored for 1 min. Activity of PPO was expressed as the amount of purpurogallin (ug purpurogallin /mg protein/h) formed.

Superoxide dismutase (SOD) activity was measured according to the method of Beauchamp and Fridovich [20]. The reaction mixture (1.5 ml) contained 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 2.25 mM nitro-blue tetrazolium (NBT), 60 mM riboflavin and enzyme extract. After mixing, the contents in the covet were illuminated for 10 min. A tube with enzyme extract kept in the dark served as a blank, while the control tube which contained no enzyme extract was kept in the light. The absorbance was measured at 560 nm against a blank using a UV-vis spectrophotometer (Model Ultrospec 3000, Pharmacia Biotech). NBT reduction in the light was measured in the presence and absence of enzyme extract. SOD activity is presented as absorbance of control minus absorbance of sample, giving the total inhibition. One unit of activity is the amount of enzyme required for 50% reduction in color and was expressed in units of the enzyme 100g-1 FW h^{-1}.

RNA extraction and Real-time RT-PCR analysis

RNA was prepared from garlic leaves following the BCP (1-bromo-3-chlorpropane) protocol [21]. Preparation of first strand cDNA was performed as described by Chomczynski and Mackey [22]. Quantitative PCR were performed on an ABI PRISM, 7300 Sequence Detection System (Applied Biosystems, USA) following the manufacturer's instructions. Amplifications were performed in the presence of SYBR Green (SYBR® GreenER™ qPCR SuperMixes; Invitrogen). For the detection of Alliinase transcripts, primers were 5'-TGACCTCAACACATTCGGTTT -3' and 5'- CGTTTCAAACCCAGAGCAGT -3'. For the detection of ACTIN2 transcripts, primers were 5'-GGTAACATTGTGCTCAGTGGTGG-3' and 5'-GGTGCAACGACCTTAATCTTCAT-3'. The final primer concentration was 200 nM in the reaction mixture. Amplification conditions were 10 min of initial denaturation at 95 ˚C, followed by 40 cycles each of 15 s denaturation at 95 ˚C and 1 min combined annealing and extension at 60 ˚C.

HPLC analysis of Allicin production in garlic

Allicin was quantified by HPLC analysis of methanol extracts; 100 mg fresh garlic leaves were directly frozen in liquid nitrogen and homogenized to a fine powder using mortar and pestles and extracted in 500 µl of methanol: water (9:1, v/v). Extracts and cell debris were separated by centrifugation (13000 g) for 20 min at 4 ºC. Cell extracts were concentrated in vacuum, and residues were taken up in methanol: water (9:1, v/v). HPLC analysis was performed using a Thermo Scientific Surveyor Plus TM HPLC System (Thermo Scientific Co, USA) as described by Fujisawa et al. [23]. The system was completed with PDA Plus detector set at 350 nm. Metabolites and parent compound were separated on Hypersil gold C18 (10µm, 100X, 4.6 mm) columns (Surveyor, Thermo scientific co, USA) using acetonitrile: water (1:1, v/v) as mobile phase with flow rate of 1 ml/min at 25 °C and injection volume of 20 µl. Metabolites were identified by comparison with reference compound, allicin (Sigma Aldrich, Germany) peak at a retention time of 130 seconds. The amount of allicin produced in the leaf samples was quantified based on a standard curve of serial dilutions of allicin.

Statistical analysis

All data are reported as mean ± standard deviation for the three independent samples (n=3). Analysis of variance and significant differences among means were tested by one-way ANOVA using the COSTAT computer package [24].

RESULTS AND DISCUSSION

Effect of Cd on pigment contents of garlic.

ISSN: 0975-8585

There is a gradual decrease in the measured pigments with increasing of Cd concentration in growth medium (Fig.1). Therefore, it can be recorded that the decrease in pigment fractions in Cd treatment seedlings are due to the destructive effects on DNA and protein synthesis in garlic. The reduction of Chl a and Chl b in the present study, could be attributed to inhibition of chlorophyll biosynthesis or enhancement of their degradation. Cd induced reduction of photosynthetic pigment, disturbances in chloroplast metabolism and reducing the activity of enzymes as δ-amionolevulinic acid dehydratase by impairing δ-amiono levulinic acid (ALA) utilizations and protochlorophyllide reductase [25]. On the other hand, total carotenoids and Carot./Chl a+Chl b of garlic leaves, in the present study, were markedly increased under Cd treatment. De Pascale *et al* [26]. reported that carotenoids was react with lipid peroxidation products to terminate chain reactions and or directly reacted with singlet oxygen.

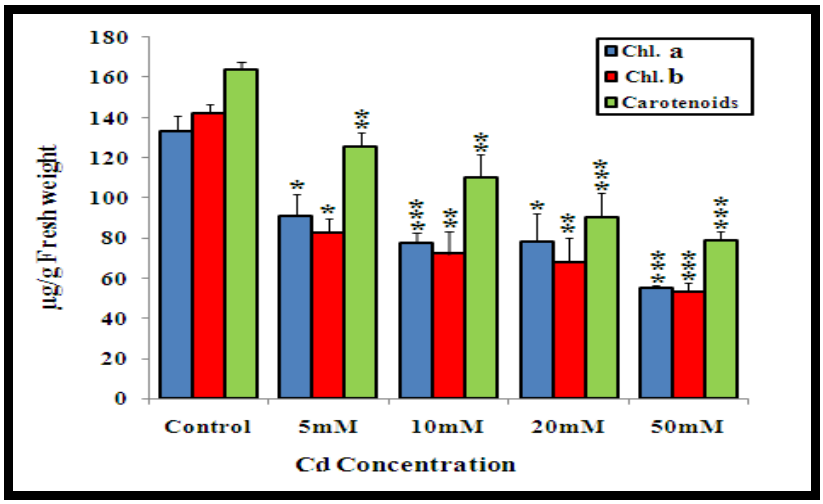

Fig. 1: Effect of cadmium on Chl a, Chl b, and carotenoid contents ((µg/g F W) of garlic leaves. (Data are means of three independent measurements. Vertical bars represent standard error)

Effect of Cd on total soluble sugars (TSS) of garlic.

There is a gradual decrease in the total soluble sugars in garlic seedling leaves with increasing Cd concentration (Fig. 2) in growth medium. The reduction of TSS by Cd treatment may be attributed to decreases of carbon assimilation [27]. Bhardwaj *et al* [28] stated that soluble carbohydrates content in plants decreased with increasing Cd, due to the formation of complexes of carbohydrate and/or to higher metabolic activities and hydrolyzing enzyme activity.

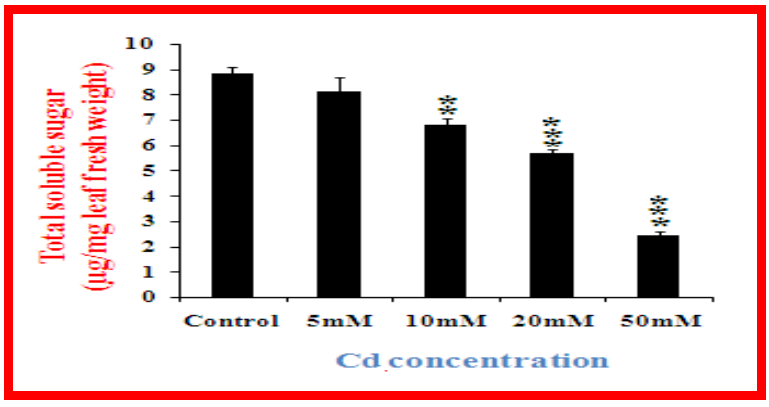

Fig. 2: Effect of cadmium on total soluble sugars content (Data are means of three independent measurements. Vertical bars represent standard error).

ISSN: 0975-8585

Effect of Cd on proline content of garlic.

There is a gradual increase in the proline content in garlic seedling leaves with increasing Cd doses in growth medium (Fig. 3). Dhir *et al* [7] found that proline accumulation in shoots of *Brassica juncea*, *Triticum aestivum* and *Vigna radiata* in response to cadmium toxicity. Proline plays an important role in osmoregulation of plant cells, protection of enzymes, stabilization of the protein synthesis machinery, regulation of cytosolic acidity as well as an effective singlet oxygen quencher[29].They suggested that increased resistance to oxidative stress is due to some indirect metabolic or physiological consequences of the accumulation of proline. Meanwhile Schwitzguébel *et al* [30] reported that proline protects plants by scavenging ROS ($O2^{-\cdot}$ and $OH^{\cdot}$), so proline plays the role of antioxidant compounds in direct quenching of free radical reactions. Results in this study revealed that an increase in proline levels in all treatments reinforces the hypothesis that this amino acid detoxification ROS produced by Cd stress.

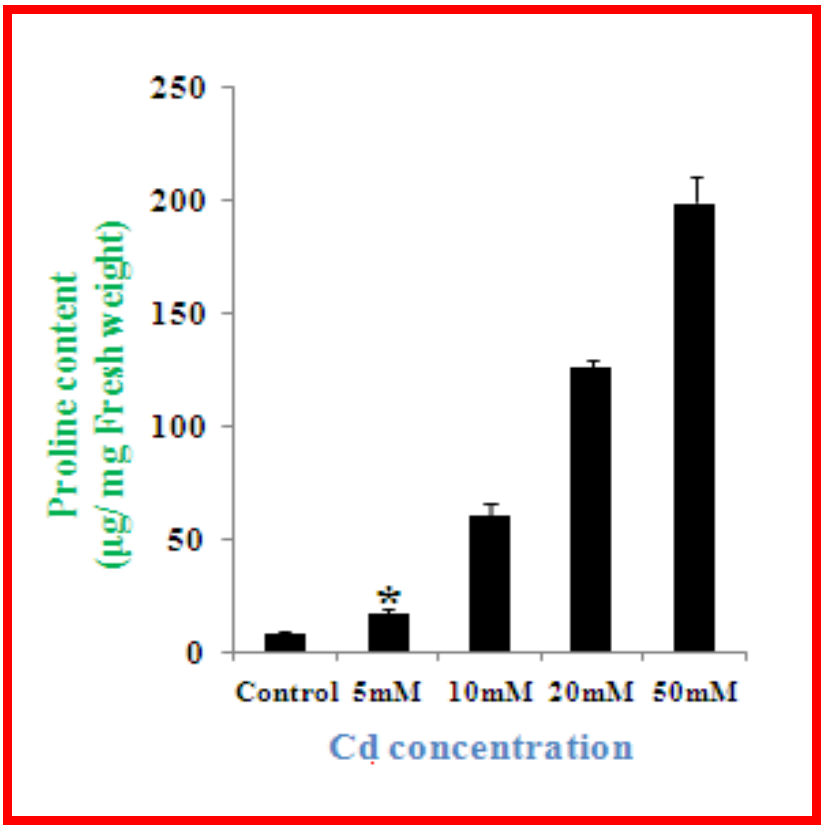

Fig. 3: Effect of cadmium on proline content (Each data point represents at least three independent measurements. Vertical bars represent standard error).

SDS-PAGE analysis of garlic protein under Cd stress

There is a visual fluctuation on the total protein expression of the plant grown in different Cd concentrations (Fig. 4). The intensity of protein bands of 30 kDa and 19 kDa was obviously increased with the higher concentration of Cd, regarding to control. The maximum intensity was visually detected at 50 mM Cd, comparing to control. A biotic stress has a significant effect on photosynthesis, so the plants may adjust photosynthesis via gene regulation to adapt to stress and some proteins involved in Calvin cycle and electron transport [31]. The pattern of protein in response to Cd stress could be related to garlic seedlings developing from cloves not seeds. For example, the protein content in leaves increased about 20% within 6 h of exposure to Cd, such increase may partly be due to the transportation of nitrogen or soluble proteins from cloves to shoot. It was reported that *Allium cepa* plants developing from bulbs were more tolerant to heavy metals than those developing from seeds [32].

In leaves of Thellun giella exposure to stress conditions increased the accumulation of two small subunits proteins, whereas the RuBisCO large subunit decreased [33]. Accumulation of tricarboxylic acid (TCA)

cycle enzymes could also suggest an efficient recycling of amino acids as energy source and their subsequent recruitment as substrates in other cellular pathways under stress exposure [34]. Moreover El-Khatib1 et al. [35] revealed that Cd exposure induced the synthesis of a considerable number of stress proteins of different molecular mass and density of protein bands appears to be affected by Cd concentration and time of exposure.

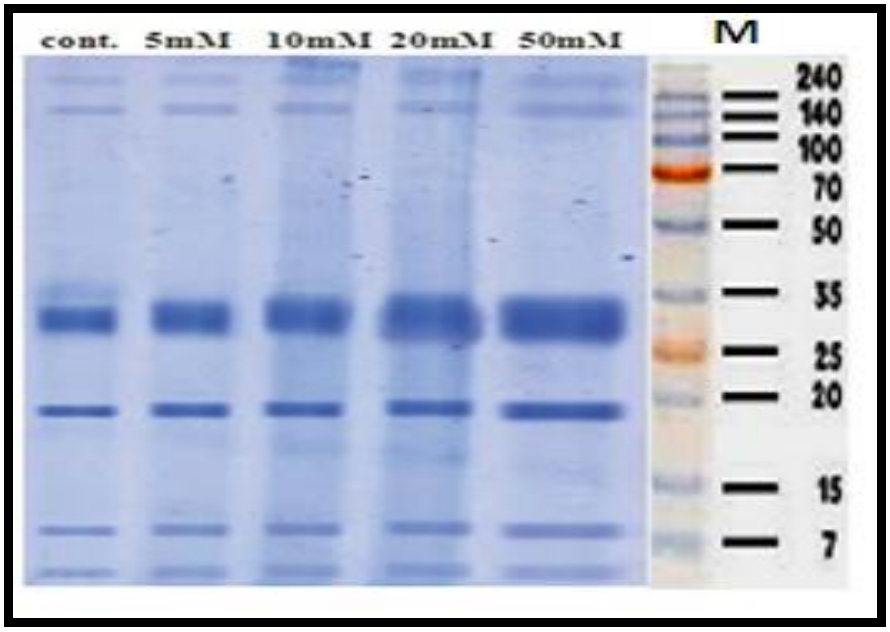

Fig.4: SDS-PAGE analysis of garlic leaves protein under Cd stress.

Effect of Cd on antioxidant enzymes of garlic

The cloves of garlic exposed to Cd support the activity of antioxidant enzymes (CAT, POD, PPO and SOD). It is obvious that the activity of antioxidant enzymes gradually increased with increasing Cd doses (Fig.5). Cd cause oxidative stress via increasing the formation of ROS like ($O2^{-\cdot}$), (1O2), ($OH^{\cdot}$) and H_2O_2 in plant cells. ROS interact with a number of other molecules and metabolites such as DNA, pigments, proteins, lipids and other essential cellular molecules [36]. Antioxidant defense system is essential for ROS detoxification during normal metabolism and particularly during stress. Many others record that the activities of SOD, CAT, and POD increased in plants by heavy metals [37].

SOD activity was induced in tomato seedlings after prolonged Cd treatment [38]. Moreover, a significant increase of SOD activity was shown in wheat leaves under exposure to Cd [39]. The obtained results revealed the increase of SOD activity under Cd stress. This increase in SOD has better protection against oxidant damage [40]. In garlic (*Allium sativum*) plants, SOD increased under short Cd treatment but decreased after long-term exposure [41].Long-term exposure to high Cd concentrations produced the down-regulation of Mn-SOD and CuZn-SOD transcripts, which is correlated with the reduction of their activities observed previously [42]. The tolerance of garlic leaves to Cd stress may be at least partially associated with increased SOD activity and other antioxidative enzymes [43].

CAT activity coordinated with SOD activity play a central protective role in the O-2 and H_2O_2 scavenging process. CAT has a significant role in plant defense systems against oxidative damage in peroxsomes and glyoxisomes where it converts H_2O_2 to H_2O and O_2 [44]. The activity of catalase increased in seedlings grown at moderately toxic Cd (100 µM) level whereas a highly toxic Cd (500 µM) led to a marked inhibition in catalase activity[45].The peroxides play protective effect due to removal of H_2O_2, peroxides, and especially lipid hydrogen peroxides. As a compensatory mechanism, in response to Cd, CAT transcription is upregulated [42].

POD is among the major enzymes that scavenge H_2O_2 in chloroplasts. Previous studies in other plants have reported increase, decreases and no changes in POD activity in response to heavy metal exposure [46].

ISSN: 0975-8585

POD activities at 5 and 10 mM of Cd increased more than control and then dropped, but were still higher than control. The increase in peroxidase activity was greater than SOD under Cd treatment [45]. POD plays important roles in eliminating the harmful ROS, by catalyzing reactions where a phenolic substrate is oxidized while the ROS are reduced to a much less harmful form. Besides their role in removal of ROS; POD participating in lignin biosynthesis that build up a physical barrier against toxic heavy metals [47] by catalyzing the oxidation of cinnamyl alcohols, which is the final catalytic step for the creation of lignin[48].The decrease of the activity of antioxidant enzymes (CAT, POD, PPO and SOD) after 3 days of treatment. Cd, binding to sulfydryl groups of structural proteins and enzymes, leads to misfolding, inhibition of activity and/or interference with redox-enzymatic regulation [47]. Another important toxicity mechanism is the chemical similarity between Cd2+ and functionally active ions situated in active sites of enzymes and signaling components.

Polyphenol oxidase increased in the present study parallel with its role as oxygen species scavengers under stress [49]. PPO decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants [50].The mechanism of Cd detoxification is the formation and trapping of Cd –Ca crystals by phenol oxidases [51]. Therefore as long as the stress is not too strong for the plant's defense capacity, the main response to heavy metals is increase in the activity of some of antioxidant enzymes (CAT, POD, PPO and SOD).

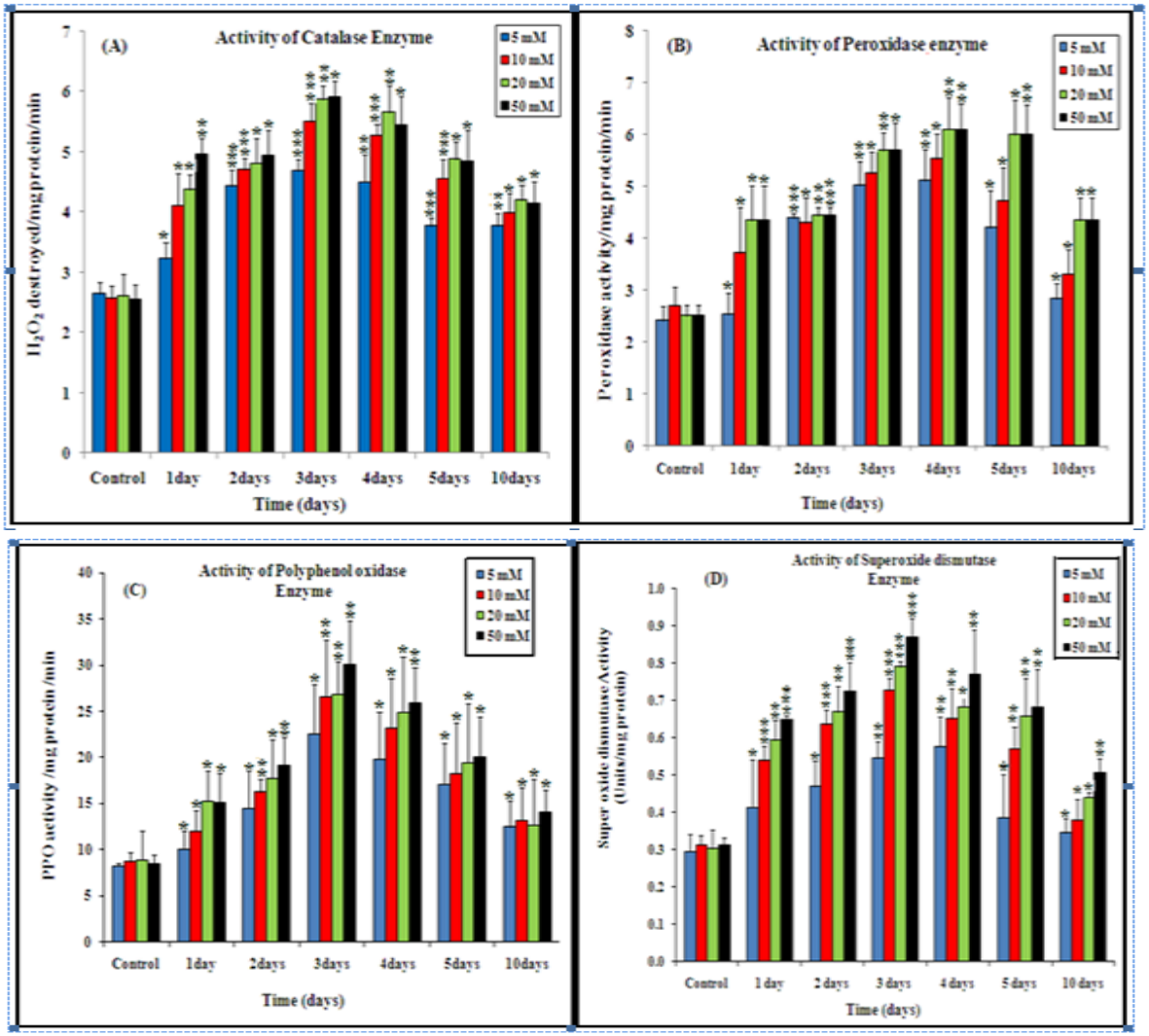

Fig.5: Effect of cadmium on the activity of antioxidant enzymes (Data are means of three independent measurements. Vertical bars represent standard error).

ISSN: 0975-8585

Effect of Cd on some molecular analysis of garlic.

Synthesis of first strand cDNA:

Abiotic stresses may cause molecular damage to plant cells either directly or indirectly through the formation of AOS. On molecular level plant response to Cd stress by the synthesis of stress-related proteins and signaling molecules [52].

Total RNA isolated from garlic plants used for the synthesis of cDNA (Fig.6). First strand cDNA synthesis was performed following the protocol [21]. The synthesized cDNA quality was tested by performing a PCR test using ACTIN2 Real-PCR primers. All the synthesized cDNA samples show positive signals after PCR analysis. These cDNA samples were used for RT-PCR analysis for Allinase expression in garlic plant which exposed to Cd stress. The full-length of AsFPS was 1263 bp in length which including 5', 3' untranslated regions, a poly A tail and a 1023 bp open reading frame [53]. The open reading frame of AsFPS encodes a protein of 341 amino acids with a predicted molecular mass of 39.61 kDa [54]. Most transcriptional element genes involved in plant stress responses have not only completely different expression profiles, but also some overlapping expression profiles, showing the complexity, specificity and crosstalk of plant gene regulatory network system. The cDNA sequence demonstrated a high degree of identity with a coat protein gene of a garlic latent Carla virus [55].

It has recently been demonstrated that the expression of BjCdR15, a bZIP protein identified in B. juncea, is induced after short Cd treatment [56]. This transcription factors (TFs) controls the expression of several metal transporters, is involved in long distance root-to-shoot Cd transportation and its overexpression in *A. thaliana* and tobacco plants enhances Cd tolerance and accumulation in the shoot [56]. MYB43, MYB48 and MYB124 proteins are specifically induced by Cd in roots [57]. The modulation of TF belonging to different groups may indicate the complexity of the response of plants to Cd, from the signal perception to the intracellular transduction cascade triggering the activation of genes responsible for Cd uptake, transport and detoxification.

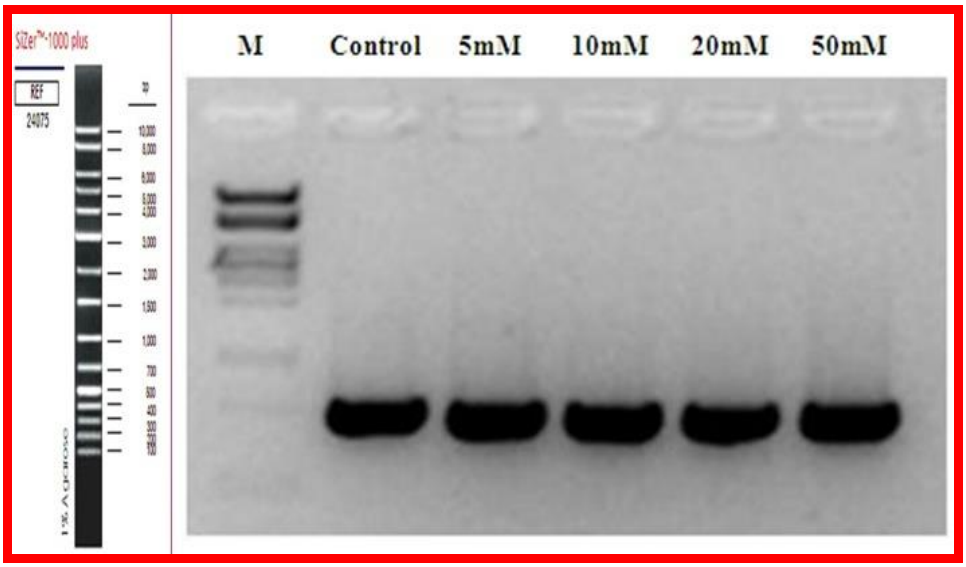

Fig.6: PCR for cDNA test (using (RT Allinase primers) from green parts of garlic plants which exposed to different concentrations of Cd. The products of the PCR were separated on a 2 % (w/v) agarose gel in 1x TAE buffer containing ethidium bromide for 1h at 12V/cm. lane1 is DNA lader 50pbs-Lane1:Positive Results of untreated garlic plant leaves Lane3 -6: Positive Results of garlic plant leaves that were allowed to grow under cadmium stress.

Alliinase gene expression by RT-PCR in garlic plants

Expression of alliinase gene was detected in the green leaves of garlic that exposed to Cd. Alliinase is the basic enzyme involved in allicin biosynthetic pathway in garlic plants. Alliinase mRNA transcript accumulation was measured by real-time RT-PCR. The amount of alliinase signal in the different RNA

preparations was standardized for the abundance of the transcript from the house keeping gene, ACTIN2. Variable amounts of alliinase signals in the different RNA preparations isolated from the garlic plants were observed under Cd stress. Cadmium affects the expression of ERF proteins that belong to the APETALA2 (AP2)/ethylene-responsive-element-binding protein (EREBP) family. Members of these transcription factors (TFs) can bind to several pathogenesis-related promoters and dehydration-responsive elements (DRE motif) [58]. It has been shown that ERF1 and ERF2 genes are induced after 2 h of Cd-treatment in *A. thaliana* roots [57]. Moreover, it has been reported that DREB2A is induced by Cd: DREB2A specifically interacts with the DRE motif in the promoter region of the rd29A and activates its transcription in Cd-exposed plants.

RNA was prepared from green garlic leaves following the BCP (1-bromo-3-chlorpropane) protocol [22]. The final primer concentration was 200 nM in the reaction mixture. It was obvious that Cd has inhibitory effect on Alliinase gene expression (Fig.7).The higher levels of Alliinase expression were observed at low concentration of Cd (5 and 10 mM). The presence of two free —SH groups, belonging to Cys220 and Cys350, in each subunit of allinase, make it more sensitive to Cd [59]. Quantitative mRNA in situ hybridization (QISH) in Thlaspi caerulescens shows that transporter gene expression changes during Cd/Zn hyper-accumulations [60]. Cadmium induced changes in cellular expression for ZNT1, ZNT5 and MTP1 could also be part of plants acclimatization to Cd toxicity. ZNT1 may function in micronutrient nutrition while ZNT5 may be involved in metal storage associated with hyper-accumulation [60].

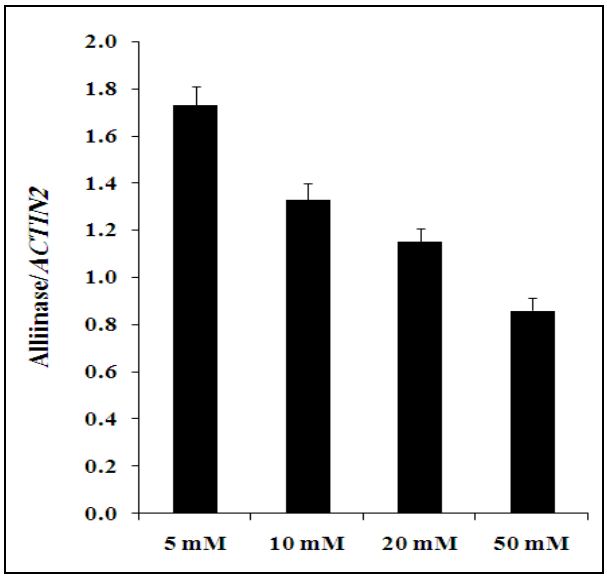

Fig.7: PCR analysis of the Alliinase gene expression in garlic leaves exposed to Cd stress. Each value is the relative accumulation of the respective RNA compared with ACTIN2 levels measured in the preparation. Each data point is based on at least three independent RNA preparations. Vertical bars show standard error.

Effect of Cd on allicin production in garlic

The production of allicin was estimated (HPLC analysis) in garlic that irrigated with cadmium solution. Fig.(8) shows the clear differences in allicin production in the leaves of garlic seedlings that exposed to Cd stress.The reduction of allicin content in the treated samples may be due to low activity of GTP that was inhabited by Cd. These results together with the RT-PCR results indicate the clear correlation between Alliinase gene expression and decrease of allicin production in garlic plants. There are two different pathways to produce alliin, the precursor to Allicin. One uses the antioxidant glutathione (GSH) and an alyll group from an unknown source in the plant [61], the other also needs this alyll group, but instead of GSH it uses the amino acid serine. As it is a well-known fact that glutathione content is affected by increasing stress, due to its antioxidant [62] and detoxicative [63] properties, we can assume that glutathione is used to protect the plant and so is no longer available for allicin production. These factors show that glutathion is here rather used for detoxification, than for the production of allicin. That is why the only possible way for the plant to synthetize enough allicin under heavy metal stress and guarantee best defence properties. The results we found out for allicin content in garlic were also found by Bideshki and Arvin [64] in the sense that they found similar absolute allicin concentrations as we did for our control plants.

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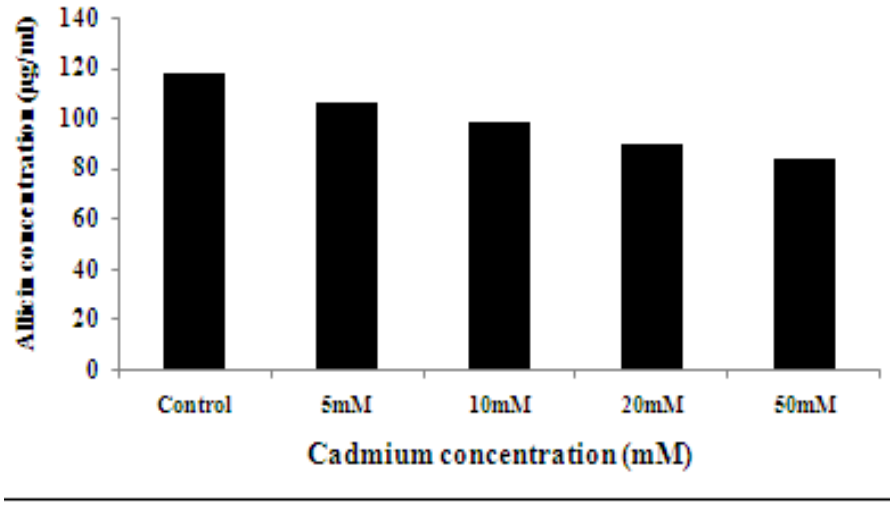

Fig.8: HPLC analysis of allicin production in garlic plants that exposed to Cd stress.

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