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Full Length Research Paper

Effect of Gamma Irradiation or Potassium on Some Primary and Secondary Metabolites of *Brassica rapa* *(L.)* Root Under Cadmium Stress

Shaimaa Abd El-Hameed Abo-Hamad, Khalil Mahfouz Ghareeb Saad -Allah, Essam El-Deen Mohammed Abo-Kassem

Botany Department, Faculty of Science, Tanta University, Tanta, Egypt

Abstract

The effect of cadmium chloride concentrations (25 50 75 and 100 mg/kg soil), seeds pre-irradiated by low doses of gamma rays (15 30 45 and 60 Gy), potassium chloride (60 mg/kg soil) and the combination of cadmium with gamma and cadmium with potassium on *Brassica rapa* primary and secondary metabolites at different growth stages were investigated. It was found that, combination of low doses of gamma or potassium with cadmium significantly increased the root soluble protein content at the various growth stages compared with the content of plants singly treated with cadmium. Proline, alkaloids and phenolic compounds were accumulated in roots in response to cadmium compared with the control and their accumulation was magnified by gamma irradiation. This suggested an important role for these compounds in plant response to cadmium pollution.

Keywords: *Brassica rapa,* cadmium chloride, gamma irradiation, potassium, proteins, proline, alkaloids and phenolics.

INTRODUCTION

Environmental pollution by metals became extensive as mining and industrial activities increased in the late 19th and early 20th century. The current worldwide mine production of Cu^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} is considerable (Pinto et al., 2004). Cadmium is a heavy metal with high toxicity and has an elimination half-life of 10-30 years (Jan et al., 1999). High concentrations of cadmium in soils represent a potential threat to human health because it is incorporated in the food chain mainly by plant uptake (Alvarez-Ayuso, 2008). Gallego et al. (2012) reported that cadmium can be accumulated in all plant parts affecting growth and development and the plasma membrane is the first living structure that is target of cadmium toxicity. Heavy metals had a strong inhibition effects on soluble protein. Cadmium inhibited soluble protein content faster and stronger than copper did. Among amino acids, proline responds most sensitively to stress conditions (Neumann et al., 1989). Proline accumulation in plants can serve as biomarker of heavy metal stress. Proline concentration was greatly increased in roots, stems and leaves of Cd-exposed plants. It may be suggested that this amino acid is involved in detoxification of heavy metals by its direct function or by way of its biosynthesis in chelating peptides (Wu et al., 2004).

The secondary metabolism is essential for the fitness of plants, many secondary metabolites play important function as chemical defence compounds against herbivores, microbes or competing plants (Wink, 1997). Among the secondary metabolites, alkaloids is one of the largest groups of chemical arsenals produced by plants. Many plants respond to cadmium stress by an increase of their total alkaloid content as recorded in *Papaver somniferum* (Lachman et al., 2006) and *Catharanthus roseus* (Zheng and Wu, 2004). Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenyl-propanoid pathways in plants (Randhir et al., 2004).

Dudjak et al. (2004) reported that treatment of barley plants with Cd^{2+} in a nutrition solution caused the increase in the total polyphenols in various parts of the plant.

Potassium is an important macronutrient and the most abundant cation in plant tissues (Jordan-Meille and Pellerin, 2008). It takes part in many essential processes in plants such as enhancement of photosynthetic rate, plant growth and yield under stress conditions (Umar and Moinuddin, 2002). Potassium has been shown to decrease the uptake of Cd^{2+} as observed in wheat (Zhao et al., 2003).

Many studies reported that low doses of gamma rays stimulated seed germination, plant growth and oil production (Zheljazkov et al., 1996 and Moussa, 2006). Farghal and Abd El-Hamid (1996a) noticed that low doses of gamma rays caused accelerated growth rate, progressive increment of protein content and amino acids.

MATERIALS AND METHODS

Seeds of turnip (*Brassica rapa L.*) were obtained from the Crop Institute Agricultural Research Center, Giza, Egypt. Turnip seeds were divided into two sets, one of which was irradiated with gamma rays doses (15 30 45 and 60 Gy) emitted from cobalt 60 source. Irradiation process was performed at the National Center for Research and Radiation Technology, Nasr City, Cairo, Egypt. The other set was untreated with gamma rays.

The seeds (irradiated and non-irradiated) were surface sterilized with 5% Clorox for 8 minutes and then rinsed thoroughly many times in distilled water. The sterilized seeds was germinated in plastic pots of 45 cm diameter and 40 cm depth, each pot was filled with 25 kg clay-sandy soil (2:1 w/w). The seeds were divided into five groups; the first one was sown in soil supplemented with 0 25 50 75 and 100 mg $CdCl_2$/kg soil. The second group of seeds was sown in soil supplemented with 60 mg KCl/kg soil. The third group of seeds received different gamma irradiation doses (15 30 45 and 60 Gy) was sown in an untreated soil. The fourth group was as an interaction between $CdCl_2$ concentrations and gamma irradiation doses. The fifth one was an interaction between $CdCl_2$ concentrations and KCl. Each treatment was represented by 4 pots and 20 seeds were sown in each one. The seeds were irrigated with tap water once every 3 days until the formation of the seedling, after that the plants were irrigated with tap water to 80% of its filed capacity whenever needed throughout the growth season. The seedlings in each pot were thinned into 10 similar seedlings after the full germination have been occurred. The plants were left to grow under the normal growth conditions in the plant garden of Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.

Roots of each treatment were randomly selected and harvested after 30 days (the early vegetative stage), 65 days (late vegetative stage) and 155 days (flowering stage). The harvested roots were washed thoroughly with tap water to get rid of any soil particles, and then they were washed with distilled water. Roots were dried in an air-forced oven at 60°C to a constant weight. The dried materials were ground using an electric mixer and the fine powders were kept in paper bags for the following analysis.

Estimation of total soluble proteins

The total soluble proteins content was estimated quantitatively in the borate buffer extract using the method described by Bradford (1976). The protein content was calculated as mg/gd.wt using a prepared calibration curve by Bovine Serum Albumin protein.

Determination of free proline

The free proline was determined in dry tissues according to Bates (1973). The proline content was calculated as mg/gd.wt using a prepared calibration curve by proline.

Estimation of total alkaloids

Total alkaloids were measured quantitatively according to the method described by Harbone (1973). Alkaloids content was calculated and expressed as mg/g dry weight of the plant samples.

Estimation of total phenolic compounds

Total phenolic content in *Brassica rapa* root was estimated quantitatively using the method described by Jindal and Singh (1975). A standard curve was prepared by using different concentrations of gallic acid and used for the determination of total phenolic compounds content (mg/gd.wt).

Statistical analysis

The obtained results were statistically analysed using the two ways analysis of variance (ANOVA) to determine the degree of significance (P) for the variations between the treatments and F test was calculated for treatments and their interactions. All of the statistical methods were according to the method described by Bishop (1983).

RESULTS AND DISCUSSION

The root soluble protein contents of *Brassica rapa* under the effect of different concentrations of $CdCl_2$, gamma irradiation doses, KCl and their combinations differed in the plant during the growth stages (Figure 1). The plant root protein content was decreased under $CdCl_2$ treatments at early vegetative and late vegetative stages,

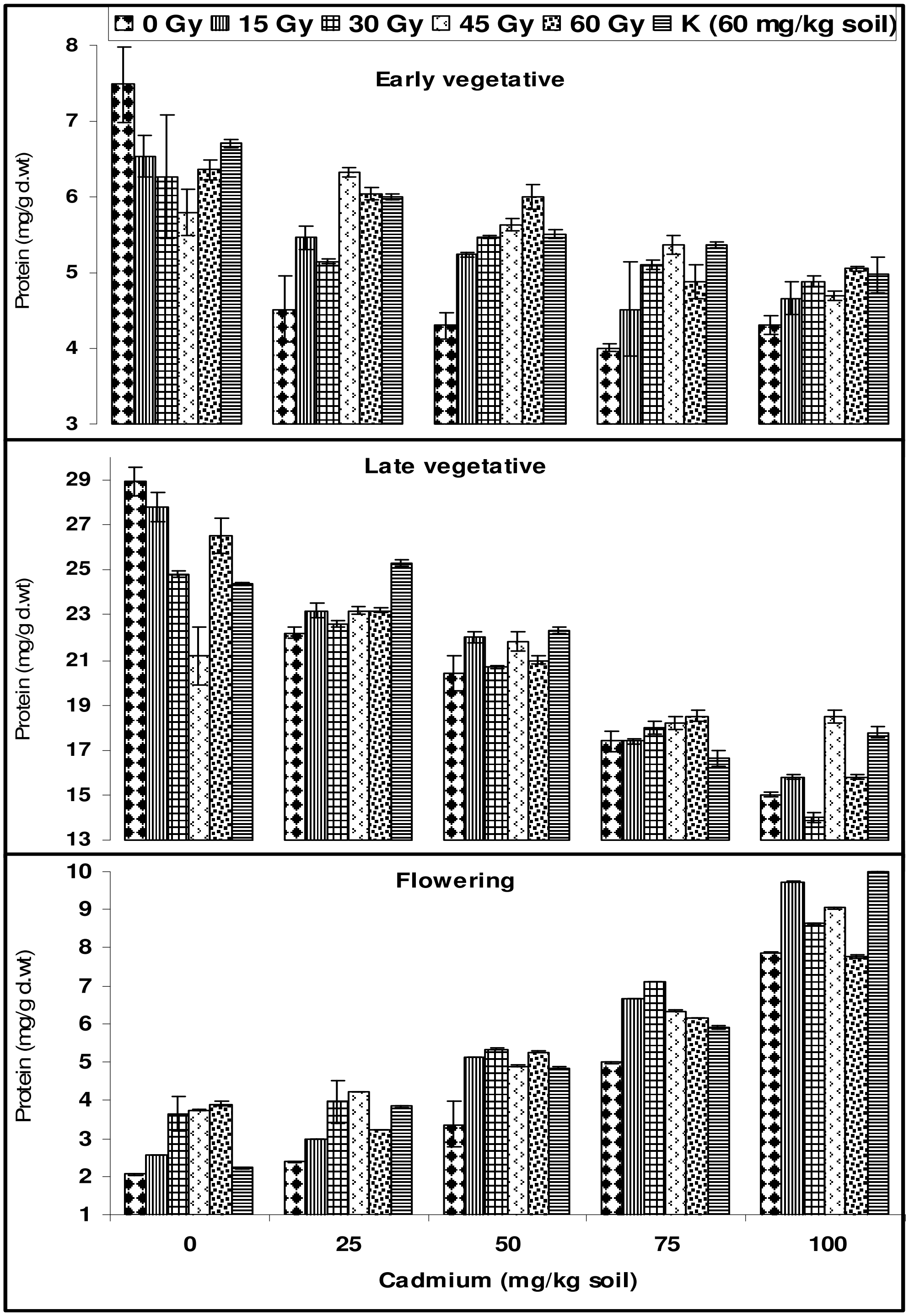

Figure 1. The interactive effect of gamma irradiation and potassium application on the root total soluble protein content (mg/g d.wt) of cadmium stressed *Brassica rapa* (L.) plant at the different growth stages.

but it was increased at the flowering stage. Boussama et al. (1999) showed that Cd caused a decline in total protein content with a progressive increase of the protease activity in the tissue. It is also likely that the heavy metals induced fragmentation of proteins due to toxic effects of reactive oxygen species led to reduced protein content (Davies et al., 1987). On the other hand, many authors reported the increase of total protein content in response to cadmium. One possible mechanism for alleviating Cd toxicity might be the

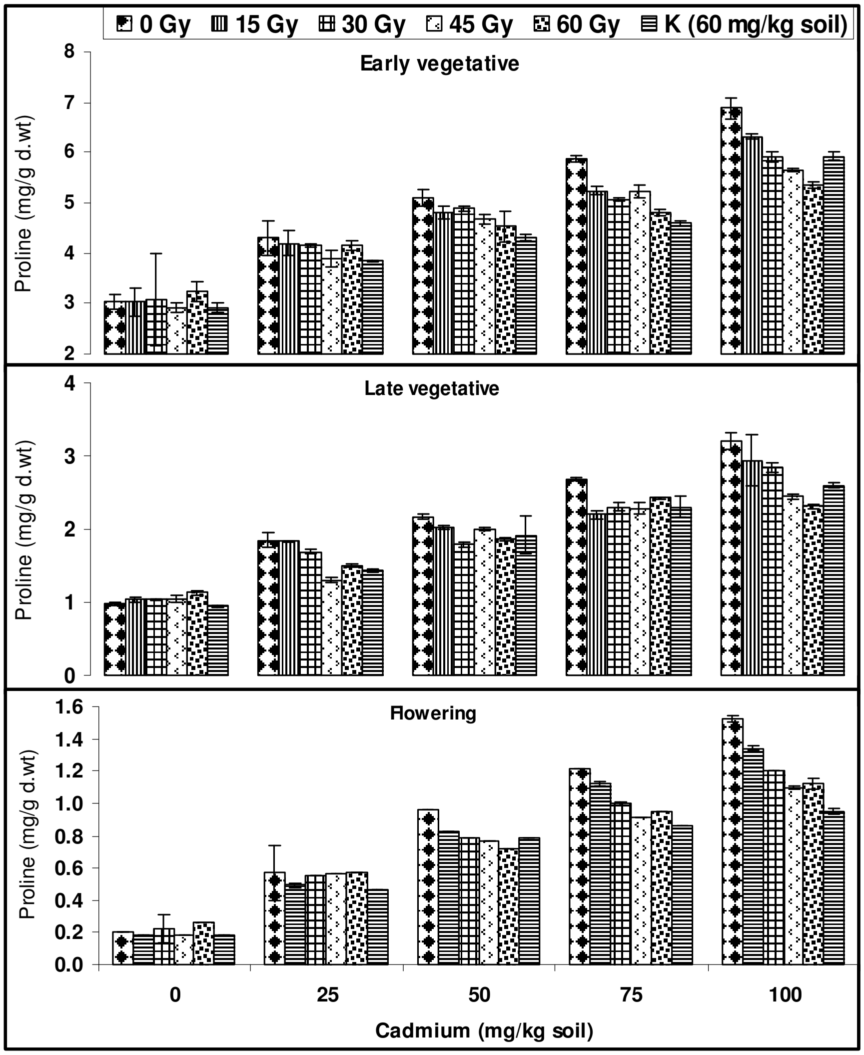

Figure 2. The interactive effect of gamma irradiation and potassium application on the root free proline content (mg/g d.wt) of cadmium stressed *Brassica rapa* (L.) plant at the different growth stages.

increase in soluble protein content (Pankovic et al., 2000) which causes sequestration of the mobile form of Cd to the immobile form by binding to some protein molecules. Increase in protein content as a result of cadmium toxicity is possible due to de novo synthesis of stress proteins provoked by metal exposure (Verma and Dubey, 2003). Gamma doses and KCl single treatments decreased the root soluble protein content at the early and late vegetative stages, but significantly increased it at flowering stage. Combination of $CdCl_2$ either with gamma doses or KCl treatment had increased the root soluble protein content at the various growth stages compared with the content of plants singly treated with $CdCl_2$. Owing to gene expression altered under gamma stress, increase in total soluble protein content was obvious (Corthals et al., 2000). On the other hand, the used gamma irradiation doses in most cases have decreased root soluble protein content. This decrease may be attributed to irreversible changes in protein conformation by radiation at the molecular level by breakage of covalent bonds of polypeptide chains (Kume and Matsuda, 1995). The interaction between cadmium treatments and potassium had increased root soluble protein content. Gupta et al. (2011) stated that potassium is required for maximum protein yield in *Brassica* species. Mengel (1980) demonstrated that the transport of amino acids is enhanced by higher potassium levels, especially the transport of amino acids to developing seeds.

The proline content of *Brassica rapa* under the various treatments at the different growth stages was shown in Figure 2. The free proline content of the plant root was gradually decreased by the age from early vegetative to the flowering stage. Cadmium treatments significantly stimulated the accumulation of free proline at

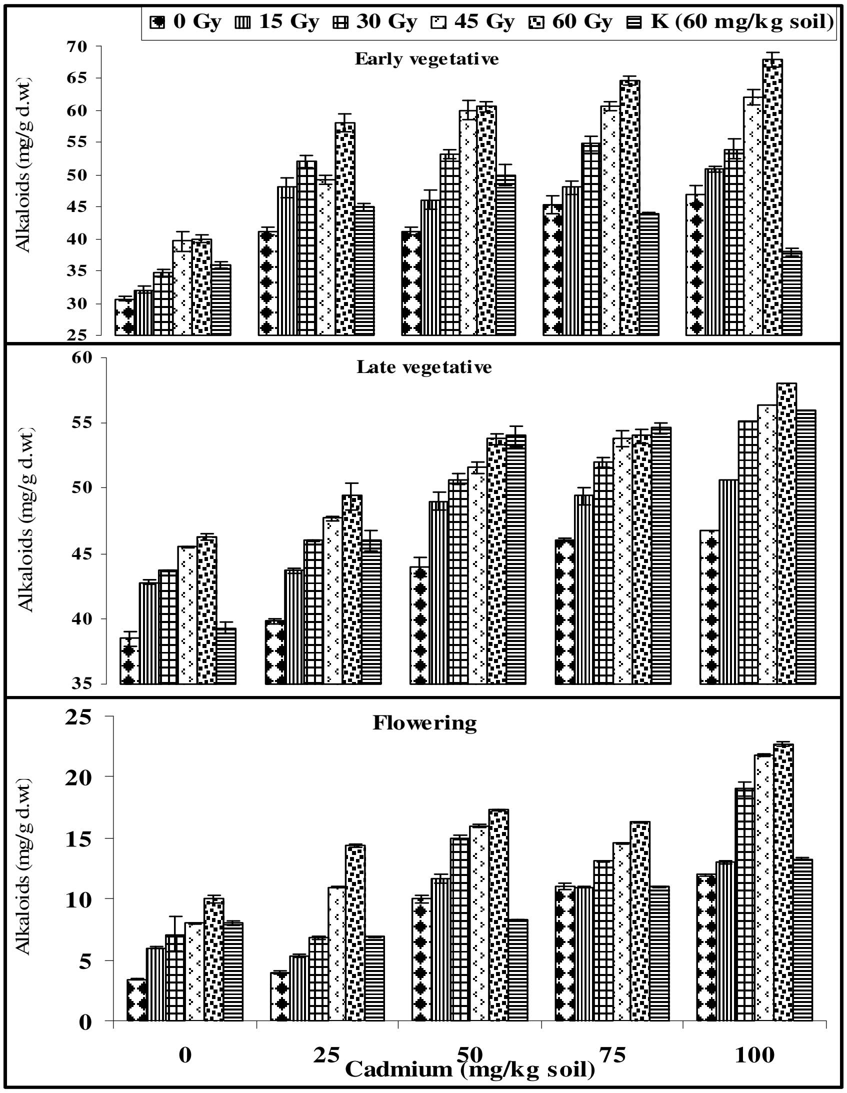

Figure 3. The interactive effect of gamma irradiation and potassium application on the root total alkaloid content (mg/g d.wt) of cadmium stressed *Brassica rapa* (L.) plant at the different growth stages.

the various stages of growth. These results are in agreement with the findings of Shaw and Rout (2002), Al-Hakimi (2007) and Chai et al. (2012) who suggested that proline protects plant tissues against heavy metal stress by acting as N-storage compound, osmotically active solute and protectant for enzymes, other macro-molecules and cellular structures.

In addition, the metal chelation property of proline is involved in plant tolerance to heavy metal stress (Siripornadulsil et al., 2002). Gamma irradiation doses slightly affected the free proline content, but in most cases these doses showed some increase in the free proline content. There was convincing evidence that osmolyte synthesis such as proline involved in protective mechanisms was altered with several environmental stresses including gamma irradiation (Al-Rumaih and Al-Rumaih, 2008). Also, the increase in proline content was reported to cope with the problem of oxidative stresses (Moussa, 2011). Treatment with KCl effectively lowered the free proline content in the plant root. Interactive combination of $CdCl_2$ either with gamma doses or with KCl relatively decreased the accumulation of free proline compared with cadmium single treatments, although its content remained higher than that of the control. Potassium treatment relatively lowered the proline content of the plant root. On contrast, proline content increased by potassium sulphate fertilizer in green faba been (Mona et al., 2011).

The data in Figure 3 indicate that different $CdCl_2$ concentrations, gamma irradiation doses, KCl application and their interactive combinations have variable effects on root total alkaloids content at the various growth

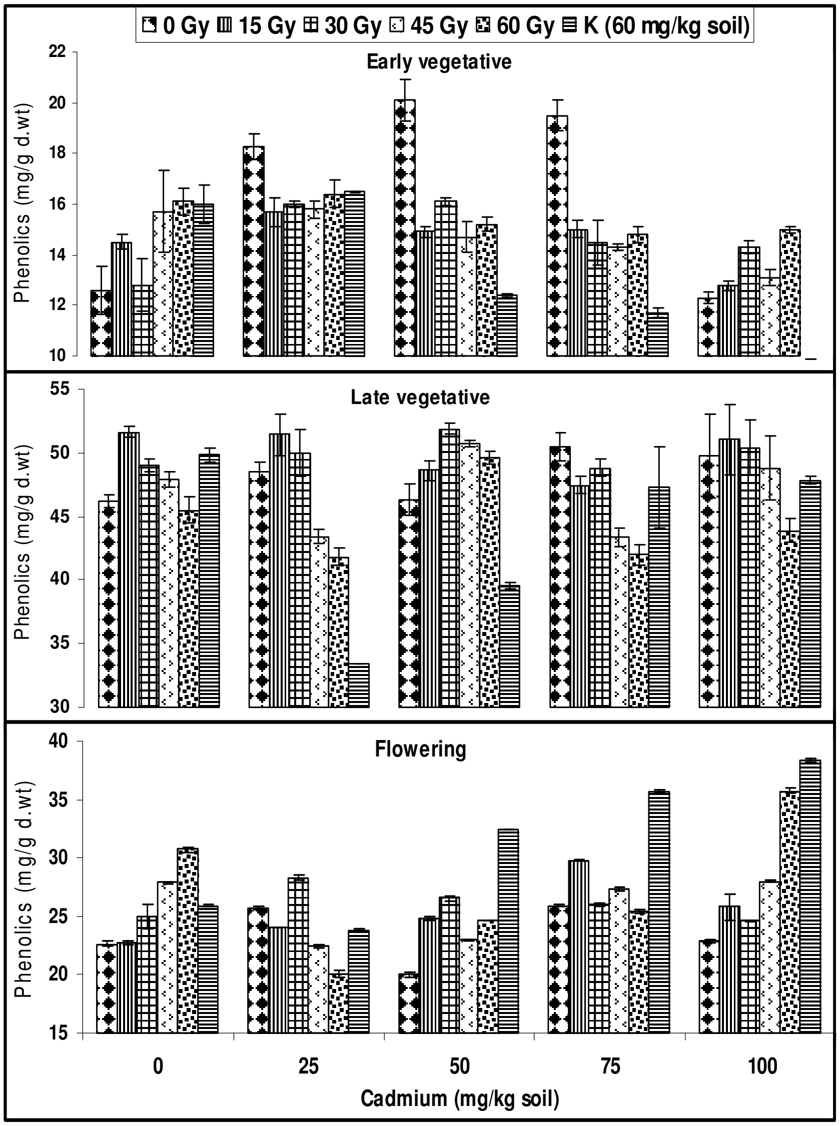

Figure 4. The interactive effect of gamma irradiation and potassium application on the root phenolic content (mg/g d.wt) of cadmium stressed *Brassica rapa* (L.) plant at the different growth stages.

stages. All the applied $CdCl_2$ concentrations have resulted in an accumulation of total alkaloids in turnip root. Wu et al., (2004) reported that cadmium could induce alkaloids biosynthesis and their accumulation. It has been known that under stress condition plants generally shift a major portion of their metabolic activities towards secondary metabolite synthesis, as alkaloids (Pandey et al., 2007). A biotic stresses as cadmium stress may result in increasing the level of endogenous methyl jasmonate, which can stimulate the activity of enzymes involved in the biosynthesis of alkaloids leading to enhanced alkaloids accumulation (Moons et al., 1997). All the used gamma irradiation doses have resulted in an accumulation of the total alkaloids in turnip root. The interactive combination of all gamma doses with all $CdCl_2$ concentrations has been resulted in raising the level of root alkaloids higher than that of $CdCl_2$ or the control treatments. The most effective dose of gamma irradiation was 60 Gy; which with all $CdCl_2$ concentrations supported the highest alkaloids content in the plant root at all growth stages. The response of plants against radiation induced reproductive and metabolic disorder may be due to the accumulation of several bioactive constituents like alkaloids (Padhya, 1986), which may act through different mechanisms such as inhibition of lipid peroxidation (Goel et al., 2004). Addition of KCl had increased the root alkaloids compared with the control treatment at the different growth stages. The combination of KCl with $CdCl_2$ treatments caused relative fluctuation in the root alkaloids content. The increase in total alkaloids content in response to potassium is recorded by Marchand (2010). On the other hand, many authors observed that potassium application lowered the total alkaloids content (Gremigni et al., 1999).

Phenolic compounds content of the root markedly increased from the early vegetative to the late vegetative stage then dropped again down at the flowering stage under the control and other treatments (Figure 4). Single treatment with $CdCl_2$ accumulated the phenolic compounds in the plant root at the various growth stages, except 100 and 50 mg $CdCl_2$ at the early vegetative and flowering stages. Phenolics are known as non-enzymatic antioxidants (Sharma and Dietz, 2009). The antioxidant activity of phenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Thirugnanasampandan and Jayakumar, 2011). Phenolics may contribute, together with ascorbate, to H_2O_2 destruction (Polle et al., 1997), and thus protect from oxidative stress. Furthermore, phenolic structures can function as metal chelators (Vasconcelos et al., 1999).

Gamma irradiation doses have stimulated the accumulation of phenolic compounds in the plant root at the different growth stages. On contrast, the combination of gamma doses with 100 mg $CdCl_2$ at the same stage increased the phenolic compounds content. At late vegetative stage, combination of all gamma doses with 50 mg $CdCl_2$ increased the phenolic compounds content compared with $CdCl_2$ alone, while the other combinations during this stage were suppressive. At flowering stage, combination of 15 45 and 60 Gy with 25 mg $CdCl_2$ and 60 Gy with 75 mg $CdCl_2$ had lowered the total phenolics content; meanwhile other combinations have increased it compared with $CdCl_2$ single treatments. Tan and Lam (1985) found that gamma irradiation stimulated the synthesis of PAL and hence increased the concentration of phenols. The used KCl concentration also increased phenolic compounds in the root at all growth stages. Phenolic compounds content was decreased by combination of KCl with $CdCl_2$ at the vegetative stages. On the other hand; at the flowering stage, all of combinations have increased the total phenolic compounds content higher than those only treated with $CdCl_2$. When plant growth increases and more photosynthates are produced at higher potassium rates, increased phenolic concentrations may correspondingly occur due to allocation of excess fixed carbon to the shikimic pathway (Shaw et al., 1998 and Crozier et al., 2006).

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