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The measurement of sprouting amount of dry and fresh weight in plant organs in environment due to polluting affect of cadmium in 8 varieties of *Brassica napus L.*

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ABSTRACT

Cadmium is one of the most polluting herbaceous, poison, heavy metal. Although it is not essential for plant's growth, it is simply absorbed by root and transferred to aerial organs. Gathered cadmium in plants is the main source of food poisoning for human and animals. Cadmium is an element that is found in crust naturally. Pure cadmium is a soft like- silver metal. Usually cadmium is not found as a free metal in environment but in combination with other elements, like cadmium oxide, cadmium chloride or cadmium sulfide and cadmium sulfate etc. Cadmium sulfide is used as pigments in plastic. The main factors that cause cadmium pollution in environment include:

- the burning of coal and house- waste rubbishes and extraction of metals from mines and that leads to increase cadmium in environment.
- house- waste and industrial waste waters introduce cadmium in potable waters, and under ground waters.
- chemical fertilizers specially phosphor have sufficient amount of cadmium. Fertilizers made from phosphate minerals is also one of the source of cadmium pollution. Agricultural lands in many parts of the world are polluted by cadmium as a result of the use of phosphate fertilizers for long time.

In this study the effects of cadmium chloride on a number of agricultural *Brassica napus* kinds is under taken, with following points.

1. Cadmium effect on sprouting in petri dishes.
2. The study of cadmium effect on the growth of *Brassica napus* embryo in Hogland food solution and the selection of *B. napus* types which are sensitive and resistant to cadmium chloride.
3. The effect of cadmium chloride is considered in respect of these aspects:
   Variation in amount of chlorophyll from leaves.
4. The study of role of cadmium in roots and aerial parts of the plant.
5. The study of amount of cadmium in the seeds due to green house cultivation of two sensitive and resistant varities *Brassica napus* kinds.

Key words: *Brassica napus*, cadmium tension, chlorophyll, dry and fresh weight.

INTRODUCTION

The effect of cadmium on absorbtion and translocation of solutes

In most of the plant species, cadmium is absorbed by roots, although the rate of transmission stem is different in various species. For example in sugar beet 10-20 percent of absorbed cadmium is transferred to aerial parts (H8) while in soybean only 2 percent of absorbed cadmium is transferred to leaves[26] absorbtion of poisonous metals and their transmission to aerial parts of plant and the resistance of plant to them, depend on their variation of metabolic processes. Various kinds of chelates and their reactions have a role in ion intercellular regulating. Cadmium absorption thought root system depends on many factors of soil like pH, revive potential, ion interchange capacity, organic matter and the presence of other metals.

Some of these factors like pH, can increase the ability of access of cadmium in soil for

plants. In soils, cadmium exist as free ion, or as complex or complex in inorganic soils. The movement of cadmium from soil to cell like other metals is accomplished by spreading phenomeno and mass flow, one part of cadmium joint to carbon oxide groups (mucilage uronic acid) of cell wall this part doesn't transfer to stem (8H). Due to the load of metal ions, it can not pass plasma material freely and their transmission is done by proteins of membrane called transmitters. Metabolic absorption of cadmium is low while non metabolic absorption of high. Some internal factors like light intensity, heat etc. may effect on metabolic procedure. Cadmium as an opportunist ion pass through the membrane by transmitters of bi cations such as Zn, Cu, Fe, Ni etc. There are other evidence that show that cadmium can arrive in cytosol via calcium channels. Because of similar characteristics of cadmium and zinc, it is suggested that absorption and transmission of these metals can accomplished via similar routes.

In 1983 Cataldo and his colleagues showed that zinc prevent the absorption of cadmium. So they suggested that cadmium enters to cell is through zinc transmission system. They also suggested that 20-25 percent of absorbed cadmium is banded to root surface and is non-exchangeable. In a research that accomplished on absorption of Cd and Pb in rice roots, it is determined that how the absorption and transmission of Pb and Cd is effected by Ca and Mg, which prevent transmission of Cd to rice root and decrease the poisonous effect of cadmium on plant's growth. These elements directly prevent entry of heavy metals Pb and Cd may compete with di cations for transferring to rice root. It is determined in this study that the amount of cadmium in root is much more than stem. So there is a prevention for transferring of cadmium from root to stem.

MATERIAL AND METHODS

Preparation of seed

The seed of eight varieties of *Brassica napus* named Slm, Opr, Okp, Li, Tl, Hol 60, Hol 401 and Hol 308 were prepared from Bily Seeds company. The name of these kinds and their abbreviation marks are shown inTable 1.

Table 1: The type and trade names of seeds

SLM	Opra	Okapi	Licord	Talaye	Haula 60	Haula 401	Haula 308	Variety nams
Slm	Opr	Okp	Li	Tl	Hol 60	Hol 401	Hol 308	name

Experiments on the effect of various concentration of cadmium chloride on sprouting of embryo of eight varieties of *Brassica napus* in petri dishes were carried out.

Preparing the seeds

Firstly, the seeds were disinfected by 10% sodium hypo chloride solution for 10 minutes. Then they were washed several times by clean water.

Sprouting and growth of seeds

Petri-dishes were used for cultivating the seeds. 15 intact and monotonous(uniform) seeds of each type was selected and after disinfection, they were put between two layer of purity paper in petri-dishes. 15 ml cadmium chloride solution with 0, 25, 50 and 100 micromolar concentration was added to petri-dishes contained seeds. In control group, instead of cadmium chloride solution, distilled water is used. The dishes contained seeds were kept in incubator with 22 +2 ^{o}C temperature and budded seeds counted after 3 days. The criterion for sprouting in this experiment is coming out of small root with 2 millimeter length.

The experiments of the effect of various concentration of cadmium chloride on dry and fresh weight of stem and root length of *Brassica napus* plants in Hogland food solution:

The preparation of Hogland food solution

At first food solution of ½ Hogland power

as basal nutrient medium is prepared and used for determination and selection of sensitive and resistant figures. Because in this research we selected sensitive and resistant figures, which better reflect the effect in presence of various cadmium concentrations in ¼ food solution and control leaves remain green so food solution of ¼ Hogland power is used.

In order to provide iron in food solution, iron secestron 6%) is used. Because for one liter full power Hogland food solution contain 10 mg iron and for one liter of ½ power solution 5 mg iron and for one liter of power solution 2.5 mg iron are needed.

So in lieu of every ½ liter of power food solution, 4.83 mg iron secestron is used and for preparing ¼ power food solution 7.41 mg iron secestron is used.

MATERIAL AND METHODS

In this experiment, first the seed of *Brassica napus* were disinfected. Then the seeds were spreaded on culture baskets. After that, these baskets were kept in plastic dishes that contained pure water in an manner that seeds were in contact with water dishes, which were kept in darkness and laboratory temperature for 3 days. Then dishes were transferred and kept for 10 hours in light and 8 hours in darkness. After 5 days small plants were transferred to 1 liter plastic culture dishes that contained ½ Hogland solution.

After 2 days, cadmium chloride solution was added to culture dishes in 0, 25, 50 and 100 micromolar concentration. pH of culture medium solutions were regulated to pH=5 by using 1 normal HCl and 1 normal KOH. Food solutions were aerated 10 minutes in each hour in order to prevent oxygen shortage in root medium. After one week the length of root and stem and wet weight of root and stem of small plants were measured, then the root and the stem of small plants were dried separately for 48 hours in oven at 70 °C. After that small plants become dry completely dry, weight of roots and stems were measured in this experiment for each attendance 3 replicates were used.

The experiments of the effect of various concentrations of cadmium chloride on chlorophyll amount of sensitive and resistant kinds of *Brassica napus*.

Required materials

In this experiment, 15-days young plants and 80% acetone was used.

Chlorophyll measurement

First the seed of sensitive and resistant kinds were disinfected and transferred to culture medium and then to Hogland solution. Then small plants were attended by cadmium chloride with 0, 25, 50 and 100 micromolar concentrations, and pH of daily culture medium solution were regulated after one week of pieces of plants leaves were separated by chance and 200 mg of leaf tissues were weighted and settled in a china mortar. After adding an amount of 80% acetone leaf pieces were pulverized completely and its volume with acetone was made to 25 ml. resulting solution was centrifuged for 20 minutes with H800 round per minutes speed. Upper layer was used for measuring chlorophyll. For this reason, the light absorption was measured in 645 and 603 nanometer by a spectrophotometer system that was regulated before by 80% acetone.

Data Statistical analysis

All of data are analyzed with 5 SPSS 11 statistical program by DunCans multiple range test and on the level of α=0.05, Excell 2000 program is used for drawing graph.

RESULTS

The effect of various concentrations of cadmium chloride on sprouting the seeds of *B. napus*

The result of the experiments of the sprouting of eight kinds of *Brassica napus* seeds in the persence of various concentrations of cadmium chloride are shown in Fig. 1. The sprouting amount of H kind in 25 and 100 micromolar concentrations of cadmium chloride showed full defference in α=0.05 level with regard to control group, the sprouting amount of the kinds in various concentrations of cadmium chloride don't show meaningful defference with control group, all of the kinds have the most sprouting amount in 50

micromolar concentration of cadmium chloride. H308 kind in 50 micro molar concentration of cadmium chloride has the most amount (143%) and H_{401} kind has the least amount (87%) in the light of sprouting. The most percentage of sprouting in 100 micromolar concentration of cadmium chloride relate to slm kind (127%).

The effect of various cancerntrations of cadmium chloride on dry weight of *Brassica napus* root in Hogland food produntion

The results obtained from the study of the effect of various concertrations of cadmium chloride on dry weight of 8 kinds of *Brassica napus* are shown in Fig. 3 in slm, H60, T1 and okp in the α=0.05 level meaningfull difference was not seen among dry weight of root in various concentrations of cadmium chloride, but in opr kind this defference is meaningful in Li, H_{Hol}, H308 in 100 micromolar concentration of cadmium chloride the meaningful difference have been seen with control group. In all of the kinds with increasing of cadmium chloride and percentage of root decreasing of cadmium chloride, the percentage of dry weight of root decrease gradually relative to control group. The most percentage of decreasing in 100 mm concentration of cadmium have seen in H_{Hol} (8.38%) and the least percentage of decreasing in 100 mm concentration seen in H60 kind (11%) and slm/lind (12%). In 50 mm concentration of cadmium chloride the most percentage of decreasing have seen in opr kind (4.33%).

The effect of various concentrations of cadmium chloride on fresh weight of stem in *Brassica napus* kinds in Hogland food solution

In various concentrations of cadmium chloride only slm kind doesn't show meaningful

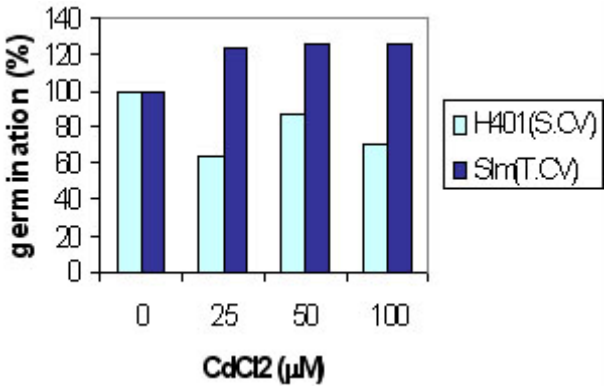

Fig. 1: The comparison of seeds sprouting percentage of two sensitive and resistant kinds of *Brassica napus* relative to control group in presence of various concentrations of cadmium chloride

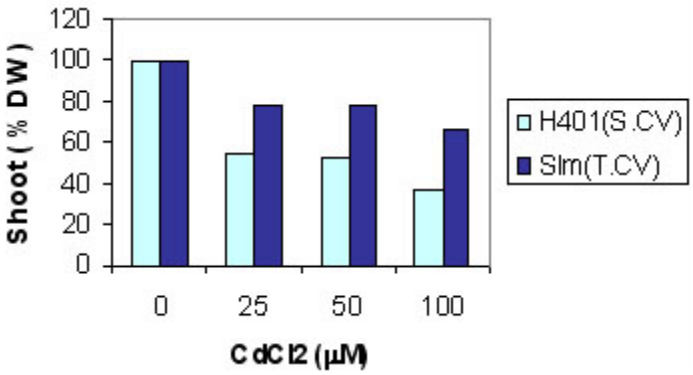

Fig. 2: The comparison of root length percentage of two sensitive and resistant kinds of *Brassica napus* relative to control group in presence of various concentrations of cadmium chloride

difference at α=0.05 level with control group in the height of wet weight of stem. Wet weight of stem in other kinds at the level of α=0.05 have meaningful difference relative to control group. In H60 and T1 wet weight of stem in 50, 25 and 100 mm concentrations at cadmium.

Chloride doesn't show meaningful difference. All the kinds with increasing of cadmium concentration show decreasing relative to control group in the light of average percentage of wet weight of stem. The most percentage of decreasing stem wet weight in 100 mm concentration of cadmium chloride is in P1 (65%) and H_{Hol} (62%) and the least percentage of decreasing relatested slm kind (27%).

The effect of various concentration of cadmium chloride on dry weight of stem in *Brassica napus* kinds in Hogland food solution

Stem dry weight in Li, H_{Hol}, H308, okp, T1 and H60 in various concentrations on the level of α=0.05 show meaningful difference relative to control group (Fig. 4). Average percentage of stem dry weight in *Brassica napus* kinds decreases relative to control group with increase of cadmium chloride concentration in this point slm has the least decrease and H_{Hol} has the most decrease with respect to obtained results from experiments in the light to dry weight percentage of root and stem, slm kind is introduced as the most resistant kind and H_{Hol} is introduced as the most sensitive kind of *Brassica napus*. The second resistant kind is H60 that has the most percentage of root dry weight in 100 mm concentration of cadmium chloride.

Comparison of sensitive and resistant kinds of *Brassica napus* in the light of sprouting percentage, root length, dry weight of root, stem length and wet and dry weight of stem.

The stage of sprouting percentage of two sensitive and resistant kinds of Brassica napus

Sprouting percentage in resistant kind increase gradually till 50 mm concentration of cadmium chloride. But in sensitive kind decrease

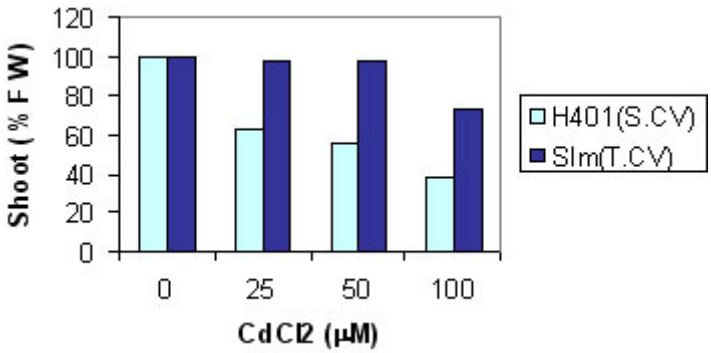

Fig. 3: The comparison of root dry weight percentage of two sensitive and resistant kinds of *Brassica napus* relative to control group in presence of various concentrations of cadmium chloride

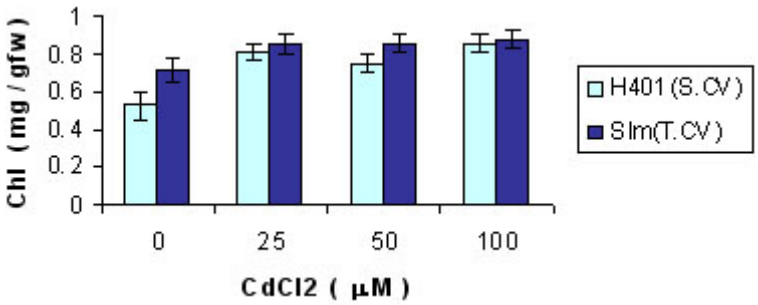

Fig. 4: The comparison of stem dry weight percentage of two sensitive and resistant kinds of *Brassica napus* relative to control group in presence concentrations of cadmium chloride

gradually till 100 mm concentration (Fig. 1-4) in 25, 50 and 100 mm concentrations of cadmium chloride, sprouting percentage of resistant kind is 3.58, 6.39 and 56% more than sensitive kinds sensitive and resistant kind show meaningful difference with each other in 25 and 100 mm concentration of cadmium chloride.

The study of root length percentage in various concentrations of cadmium chloride in relative to control group. Root length in 50 and 100 mm concentrations in resistant kind is 68.4 and 59.17 percent more than sensitive kind but in 25 mm concentration of cadmium chloride, in sensitive kind is 7.3% more than resistant kind.

The study of root dry weight percentage of two sensitive and resistant kinds of *Brassica napus*

Root dry weight percentage of resistant kind show increase in 25 and 50 mm concentrations of cadmium chloride relative to control group. But in sensitive kind meaningful decrease is seen (3-4 figure). Root dry weight in 25, 50 and 100 mm concentrations of cadmium chloride in resistant kind is 3.29, 4.28 and 1.27 percent more than sensitive kind. However in 100 mm concentration of cadmium chloride, resistant kind show 7.11 decrease relative to control group.

The study of stem weight percentage in two sensitive and resistant kinds of *Brassica napus*

Stem length decrease in both sensitive and resistant group gradually by increasing of cadmium concentration. Decrease percentage of stem length of resistant kind in 25 and 50 mm concentration of cadmium chloride is 10% and 8.7 % more than sensitive type. But sensitive type show more length decrease in 100 mm concentration of cadmium relative to resistant kind.

The study of stem weight percentage in sensitive and resistant kinds of *Brassica napus*

Stem wet weight in both sensitive and resistant kinds shows meaningful decrease in response of cadmium attendance relative to control group. That the decrease amount of two sensitive types accomplished gradually. Stem wet weight amount in resistant kind in 25 and 50 mm concentrations of cadmium chloride is approximately equal to wet weight of control group but shows 27% decrease in 100 mm concentration of cadmium chloride wet weight of resistant kinds shows 5.36 and 5.42 and 5.34 percent increase relative to sensitive kind in 25, 50, 100 mm concentrations of cadmium chloride.

The study of stem dry weight of sensitive and resistant types of *Brassica napus*

Stem dry weight in both sensitive and resistant kinds decrease with increase of cadmium chloride concentration. sensitive kind shows 6.23, 1.25 and 29 percent decrease relative to resistant kind in 25, 50 and 100 mm concentrations of cadmium chloride.

The effect of cadmium chloride on chlorophyll amount

The effect of various concentrations of cadmium chloride on chlorophyll amount in extremity leaves of stem in sensitive and resistant kinds of *Brassica napus*.

Chlorophyll amount in extremity leaves of stem that produced after creation of tension in absence of cadmium chloride, the chlorophyll amount in resistant kinds is more than sensitive kinds when cadmium chloride concentration increases, gradual increase in chlorophyll amount of resistant kinds was also observed.

It was also observed that, in sensitive kind cadmium chloride cause increasing of chlorophyll amount of leaves relative to control group. But chlorophyll amount is sensitive kinds more than resistant kind increase amount of chlorophyll in both kinds in various concentrations of cadmium chloride on α=0.05 level is meaningful relative control group. But difference in increase amount of chlorophyll among different attendance is not meaningful statistically in sensitive kind the percentage of increasing of chlorophyll relative to control group are 153%, 141% and 162% in 25, 30 and 100 mm concentrations of cadmium chloride while this amount is resistant kind are 48%, 49% and CCC.

The effect of various concentrations of cadmium chloride on length and dry weight of root and length and dry and wet weight of stem of 8 kinds of *Brassica napus* in Hogland foos solution

On the basis of present findings data, it

was determines that in all kinds, the amount of length and dry weight of root and also length and wet and dry weight of stem decreased by increasing of cadmium concentration in medina. From the results obtained, it was clear that cadmium has negative effect on the growth of *Brassica napus* kinds and in the light of root length. In most of the kinds there is a negative correlation between the increase of cadmium concentration in medium culture and root length. Root is the first plant part that is injured in contact with cadmium. Perhaps cadmium prevent plants's growth and esp root's growth due to production of active oxygen species. A conducted by Liu and Kottke in 2004, it is determined that in high concentrations at cadmium (1-10 mm) because of injury to cell members system and decrease of crystal surface of mitochondrium and the number of ribosumes, injured cells and these changes effect clearly on root growth. There is not any meaningful difference in slm, H60, T1, okp and opr in the light of the effect of various concentrations of cadmium chloride on longitudinal growth of root that it might be because of the existence of stronger antioxidants systems in these kinds. In the light of dry weight of root, the results show that cadmium has negative effect on dry weight of root. In slm kind in 25 and 50 mm concentrations of cadmium chloride 18% and 10% growth increasing is seen relative to control group. But in 100 mm concentration of cadmium chloride 11% growth decreasing is seen relative to control group. The results show that slm kind resistance matches for compairing against negative effect of cadmium on the growth.

The changes of the amount of leaves chlorophyll of two sensitive and resistant kinds of *Brassica napus* under the effect of cadmium chloride's various concentrations

On the basis of presented data in two sensitive and resistantkinds of *Brassica napus* with cadmium chloride concentration increasing. The gradual increase in chlorophyll amount of extremity leaves of small plants of *Brassica napus* that was produced after certain of tension is seen. In observations that are done after tension is seen clearly relative to control group. The results show that the negative effects of cadmium are more on plant growth. The reason of this growth decrease might be due to forming of small cells and small inter cellular space in leaves. It is recommended that the decrease of tell targor potential and the elasticity of wall cell leaf to forming small cells and decreasing of inter cellular space in the plants that are attended by cadmium. This object can be because of the decreasing of perpiration and water movement. The decrease of turgor potential is due to indisposition of water balance in plants and in most of researches the negative effects of cadmium on water absorption and transmission and transpiration are proved. The decrease of water absorption can be related to prevent of root growth.

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