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Effect of Cadmium Levels on Seed Germination and Seedling Growth of Spinach (*Spinacia oleracea*) Under Salinity Stress

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Abstract: Cadmium is highly toxic heavy metal. Soil factors such as pH, caution exchange capacity, $CaCO_3$ content and salinity have a significant influence on Cd uptake by plants. Moreover, soil salinity has a great impact on crop yield in Iran. Therefore, the effect of four salt levels (0, 45, 90, 180 mM) of NaCl solutions and four concentrations (0, 15, 30, 60 ppm) of Cadmium sulfate solutions on seed germination of spinach was studied in two separate experiments. Treatments were arranged in a factorial manner in base of completely randomized design with four replications. The most seed germination percentage decreased significantly with increasing after 180mM NaCl concentration under levels of Cd. Increased salt concentration caused a decrease in germination percentage and rate. In comparison with the control treatment, the lowest germination rate values determined at 180 mM NaCl salinity and 60 ppm Cd. Shoot and root length was reduced significantly with a rise in NaCl and Cd levels. Cd concentration had a more depressing effect on the root than on the shoot growth. Interactive effects between Cd and NaCl on dry weight and root length was significant. According to this study the interactive effect of both stresses were more than additive.

Key words: Cadmium · Germination · Salinity · Spinach *(Spinacia oleracea*)

INTRODUCTION

Heavy metal contaminations in soil are associated with biological and geochemical cycles and are influenced by anthropogenic activities such as agricultural practices, industrial activities and waste disposal methods [1-3]. Cadmium (Cd), being a highly toxic metals pollutant of soils, inhibits root and shoot growth and yield production, affects nutrient uptake and homeostasis and is frequently accumulated by agriculturally important crops and then enters the food chain with a significant potential to impair animal and human health [4].

Soil salinity is a major constraint limiting agricultural productivity on 20% of the cultivated area and half of the irrigated area worldwide [5]. Excess salt in soil and water has detrimental effect on crop yields and results in substantial losses of arable soil, especially in the arid and semi-arid areas [6].

Seed germination takes the most important part in life cycle of plants [7, 8]. In many plant types, germination and seedling-growing phase is very sensitive to salt stress [9].

There are studies that indicate that spinach *(Spinacia oleracea*) act as good metal and toxin accumulators [10, 8].

The aim of present study is to assess the interaction of different concentrations of Cadmium and NaCl on germination of spinach (*Spinacia olareceae L.).*

MATERIALS AND METHODS

Seeds of spinach (*Spinacia olareceae L.*) were sterilized by soaking in 0.6% sodium hypochlorite (v/v) for ten seconds to avoid fungal contamination. After that, the seeds were washed with deionized water. The treatment included four concentrations (0, 45, 90, 180 mM) of sodium chloride (NaCl) and four concentrations (0, 15, 30, 60 ppm) of cadmium sulfate solutions ($3CdSO_4.8H_2O$). The germination of seeds were tested at temperature of 18±1°C.

Fifty seeds of similar size were randomly selected and placed within a 9 cm-diameter petri dish containing filter paper and then 8 ml of each concentration treatment was added to each petri dish. For the control, only distilled

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water was added to petri dishes. Four replications of each test solution were prepared. The number of germinated grains count every 2 days once. The germinating seeds were counted when the plant had grown to over 2mm. At the end of experiment (after two week) 15 plants from each petri dish were selected and root and shoot length were recorded. In order to dry them and measure their dry weigh, put them in oven with 75°C temperature for 24 hours. This experiment was arranged in a factorial manner in base of completely randomized design. The germination percentage and rate, root and shoot length and dry weight were analyzed using MSTAT-C software. Duncan analysis was used for classification of data in 0.01% probable level.

RESULTS AND DISCUSSION

Variance analyses of interaction effects between cadmium and NaCl on germination percentage and rate of spinach has been showed in table 1.

Germination Percentage and Rate: Increased salt concentration caused a decrease in germination percentage. Significant reduction was observed mainly at the higher level of salt concentration compared to control. Germination percentage decreased significantly with increasing after 180 mM NaCl treatment. The most germination percentage was at 15 ppm Cd (87.50%). Increased Cd concentration caused a decrease in germination percentage (after 15 ppm Cd). According to results of variance analysis, effect of Cd levels on germination percentage was non-significantly ($p<0.01$). As you see (Table 1) the most germination percentage was high (87.50%) and germination percentage decreased significantly with increasing after 180 mM NaCl concentration under Cd treatments.

Germination rate was highest at control and 45 mM salt concentrations and decreased significantly with increasing after 180 mM NaCl concentration. Increased Cd concentration caused a decrease in germination rate. Germination rate decreased significantly with increasing after 60 ppm Cd concentration. In comparison with the control treatment, the lowest germination rate values determined at 180 mM NaCl salinity and 60 ppm Cd.

In general, the highest germination percentage occurs in non-salty conditions and it decreases depending on the ascending salt concentrations [7]. The decrease in water intake of the seed in salty conditions and by the ion toxicity with accumulation of Na and Cl ions around the seed, prevents the seed germination [11]. Ascending salt concentrations not only prevent the germination of the seeds but also extend the germination time by delaying the starting of germination [12]. Increase in metal (Cd) concentration may increase suppression of seed germination [13].

Growth of Seedling Characteristics: Interaction effects between cadmium and NaCl on growth of seedling characteristics has been showed in table 2.

Root and Shoot Length: Root length decreased significantly with increasing at all NaCl treatments. There is a significant ($p>0.01$) effect on root length at all level of Cd treatments. Increased Cd concentration caused a decrease in root length. When seeds of spinach were treated with Cd and NaCl, the root length was significantly decreased. According to this study the interactive effect of both stresses were more than additives.

Salinity, declines shoot length and by increasing salinity levels these reduction increase. The most reduction in shoot length related to 180 mM NaCl.

Table 1: Effect and interaction of different concentration of Cd and salinity on germination percentage and rate

	NaCl concentration (mM)				
	0	45	90	180	Means
Cd concentration (ppm)	Germination percentage (%)				
0	86.50 a	84.50 a	62.00 abc	51.50 bc	71.13 A
15	87.50 a	85.00 a	67.50 abc	45.00 bc	71.25 A
30	86.00 a	82.00 a	66.00 abc	44.00 c	69.50 A
60	73.50 ab	65.50 abc	61.50 abc	40.00 c	60.13 A
Means	83.38 A	79.25 A	64.25 AB	47.13 B	
	Germination rate				
0	16.75 a	16.50 ab	13.50 bcde	10.75efg	14.38 A
15	15.75 abc	15.00 abc	13.25 cdef	9.750 g	13.44 A
30	14.25 abcd	13.75 abcde	11.75 defg	8.750 g	12.13 AB
60	11.25 defg	11.00efg	10.25 fg	9.00 g	10.38 B
Means	14.50A	14.06A	12.19 AB	9.563 B	

In each column and row values with the same letter are not different ($P<0.01$).

Table 2: Effect and interaction of different concentration of cadmium and salinity on growth of seedling characteristics

	NaCl concentration (mM)				
	0	45	90	180	Means
Cd concentration (ppm)	Shoot length (mm)				
0		36.90 a	32.06 ab	20.31 def	31.66 A
15	37.38 a	30.51 abc	28.01abcd	18.74 ef	27.26 A
30	31.78ab	29.01abcd	25.48bcde	12.68 f	24.38 AB
60	30.36 abc	21.66 cdef	17.83 ef	12.36 f	17.65 B
Means	18.75 ef	29.52 A	25.84 A	16.02 B	29.57 A
	Root length (mm)				
0	48.06 a	41.53 b	35.64 b	23.20 c	37.11 A
15	35.22 b	24.13 c	24.67 c	13.64 d	24.41 B
30	15.91 d	12.82 d	6.358 e	4.925 e	10.01 C
60	5.565 e	3.615 e	2.775 e	3.180 e	3.784 D
Means	26.19 A	20.52 AB	17.36 BC	11.23 C	
	Dry weight (mg)				
0	0.0670 a	0.0565 b	0.0374 e	0.0165 i	0.0443 A
15	0.0572 b	0.0467 d	0.0350 e	0.0152 i	0.0385 B
30	0.0507 c	0.0455 d	0.0372 e	0.0267 h	0.0400 B
60	0.0450 d	0.0377 e	0.0312 g	0.0097 j	0.0309 C
Means	0.0550 A	0.0466 B	0.0352 C	0.0170 D	

In each column and row values with the same letter are not different (P<0.01).

Shoot length of spinach generally decreased with increasinge in levels of Cd treatments. Significant reduction was observed mainly at the higher level of Cd (60 ppm Cd). Interaction effects between Cd and NaCl on shoot length of spinach has been showed in table 2. Strong reduction was observed mainly at the higher level of Cd concentration under salinity stress (Table 2).

Salt stress has been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species [14, 15]. The reduction in the root and shoot lengths of the seeds to varying metal treatments could have been brought about by the reduction of mitotic cells in the plants' meristematic zone [16]. Excessive amount of Cd may cause decreased uptake of nutrient elements, inhibition of various enzyme activities and induction of oxidative stress including alterations in enzymes of the antioxidant defense system [17].

Dry Weight: Impact of salinity stress treatment, on dry weight was significant (P < 0.01). Dry weight decreased significantly with increasing at all Cd treatments. Interactive effects between cadmium and NaCl on dry weight of spinach were significant (Table 2). According to this study, the interactive effect of both stresses was more than additives. Suppression of plant growth under saline condition may either be due to decreased availability of water or to the toxicity of sodium chloride. Also the reduction in dry weight under salinity stress may be attributed to inhibition of hydrolysis of reserved foods and their translocation to the growing shoots [18]. The reduction of biomass by cadmium toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis [19].

Most of the spinach seedling turned yellow at three days, before their death. The characteristic feature of toxicities in plants due to heavy metal is chlorosis and reduction in net photosynthetic rate which leads to decrease in plant growth and productivity [20].

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