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EFFECT OF DIFFERENT CONCENTRATIONS OF NICKEL CHLORIDE IN AN IMBIBITION, PHYSIOLOGICAL AND MOLECULAR PARAMETERS OF *CUCUMISSATIVUS* L. SEEDS

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Abstract

The effect of different concentrations of Ni (0, $1*10^{-5}$, $5*10^{-5}$, $1*10^{-4}$, $2*10^{-4}$, $4*10^{-4}$, $8*10^{-4}$, $1*10^{-3}$, 0.1 M) was studied. These concentrations affected on imbibition process by masseur the fresh weight of seed through different periods. The germination, growth and molecular parameter of *Cucumber sativus* L. seeds measured. All concentrations caused increasing in fresh weight of seeds with increasing in concentration and with increasing the periods too. The highest reading was at $1*10^{-5}$ M with fourth period and the lowest reading was at 0.1M with fourth period which is no significant change with control. Ni had significant decreasing ingermination at high concentration; there was a decrease in growth parameters, such as length of shoot and root, fresh and dry weights for shoot and root and for leaf area. Also, there was increased in the parameters of DNA damage by comet assay. The decreasing in all these parameters was proportional reversely with increasing of Ni concentrations.

***Key words*:** Imbibition, nickel chloride, physiological parameters, comet assay.

Introduction

Nickel (Ni) is discovered as essential elementrecently (1). Its metabolic function was determined well before it was determined as plant nutrients, its deficiency could disrupt the growth which observed in field situations in several perennial species (2). Although Ni is necessary for normal growth and development of plants as essential micronutrients, the high concentrations of it exhibit toxicity (3). The interest the role of nickel by plant scientists began after discovery of it in 1975 (4). It was a critical constituent of the urease enzyme in plant, which contains two nickel ions in the active site (5).

The anatomical and chemical characteristics of the seeds coat are important to control the diffusion of water and gases into seeds (6 & 7). Seed coat can be either permeable "soft" or impermeable "hard", depending on water, it is absorbed or not. A permeable seed absorbs water rapidly (within minutes to hours), whereas an impermeable seed does notimbibe water even after several days or weeks of being soaked and remains dormant (8 & 9). The nickel at lower concentration has been reported to play variety roles in plant growth and metabolism. However, it shows harmful effects at high concentrations (10). The toxic effects of excess nickel are evident through crop development, but the germination stage is regarded as the most sensitive to nickel toxicity (11). As example, the increasing in Ni^{2+} concentration caused inhibition in seed germination and seedling growth of different plant species (12). At single-cell level, the comet assay is a versatile and sensitive method for the evaluation of DNA damages and DNA repair capacity. The simplicity and sensitivity, also, the small number of cells required to obtain strong resultsb comet assay, which it has been used to study the genotoxic impact of radiation, chemicals including pesticides, phytocompounds, heavy metals, nanoparticles or contaminated complex matrices in plants (13). The aim of this study is to improve the important of Ni in an imbibition, physiological parameters and DNA degradation of *C. sativus* seeds.

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Materials and Methods:

Source of seeds: in this study used the seeds of cucumber *(Cucumissativus* L.) class BETH ALPHA F1 (Jordan).

Preparation of nickel chloride solution: Different concentrations of $NiCl_2$ prepared from the stock solution (0.1M), which were $1*10^{-5}$, $5*10^{-5}$, $1*10^{-4}$, $2*10^{-4}$, $4*10^{-4}$, 8*10-4, 1*10-3, and 0.1 M. In addition to the control treatment which was distilled water. Condition of experiments: all experiments was done in lab conditions which incubated in growth chamber with temperature 25 ± 2^0C and humidity 60-70 %.

Imbibition experiment: Five gram of cucumber seeds put in polyethylene plastic vial (100 ml volume); 25 ml of each concentration was added. The seeds incubated in growth chamber for 27 hour. The fresh weight of seeds measured every 3 h. for 3 times (periods). The last measured was the 4th at 27 h. The seeds were dried with absorbent paper before every measuring.

Physiological parameters: Twenty seed from each concentration of imbibed seeds after 27 h. put in Petri dish (9cm in diameter) with filter paper, and then 20 ml of distilled water added for all treated seeds. The experiment continues for 10 days. The percentage of germination measured after 6 day of incubation. Also, the coefficient velocity percentage of germination measured along 6 days of experiment age. The length of shoot and root was measured for 3 replications for each treatment. Then the fresh and dry (dried at 60-70 ^{0}C for 24 h.) weight for shoot and root was measured by sensitive balance (Sartorius BP 3013). The leaf area was determined by using digitalplanimeter (Placom).

Molecular parameters: Comet assay (single cell gel electrophoresis) introduced by Singh (14), comet shaped structure were measured by epifluorescence microscopy, the comet assay parameters considered in this study were comet length, comet high and comet moment.

Statistical analysis: ANOVA table used to analyze the data depending LSD with probability = 0.05, which used to compare the average of 3 replications for each treatment. The experiments was completely randomize designed.

Results and discussion

In general, there was graduated increasing in fresh weight of imbibed seeds by concentrations $5*10^{-5}$-$8*10^{-4}$ M. Depending on the period of soaking, there were four periods. The fourth period was the highest (7.388g) and caused significantly increasing in fresh weight of cucumber seeds compared with control, but it not changed significantly with the third period. The best fresh weight of seeds was with fourth period at concentration $1*10^{-3}$ M (7.62g). Some researchers have noted that imbibed seeds of *Anadenanthera colubrine* showed after one hour increase in biomass by 12.6%, but when macerating for 8 hours, there was increasing by 76% indicating an increase of seeds for solutions absorption (9). The fourth period at concentration 0.1 M gave lowest value (6.63 g) which is not changed significantly with control at 1st, 2nd, and 3rd periods. Depending on the last result that the high concentration caused dehydration in seeds after imbibed especially at fourth period with concentration 0.1 M.

The inhibition in fresh weight of seeds at fourth period with concentration 0.1 M reflected on other physiological parameters as showing in table 2. The germination percentage of the control sample was 90%, but has increased insignificantly by the low concentration of Nickel ($1*10^{-5}$ M) which was 95, and began to decline significantly with increasing Niconcentrations even reached to the lowest at concentration 0.1 M (50%). The coefficient velocity percentage of germination was increased significantly in the low concentration ($1*10^{-5}$ M) of Ni (43%) compared with the control sample which was 38% and began to decline significantly with increasing concentrations until it reached to 25% by concentration 0.1 Mof Ni.

All other parameters such as root and shoot length, fresh and dry weight of them, and leaf area) increased by low concentration of Ni ($1*10^{-5}$ M), and began to decrease gradually with increasing the concentrations. The high concentration of Ni (0.1M) causedno growth of plumule.

These results were compatible with some researchers. When exposed the seeds of a plant *Macrotylomauniflorum* to five concentrations of nickel (0-100mg/l), the low concentrations were good for germination and speed germination coefficient, shoot and root length, fresh and dry weight of shoot and root, then it began to decline with the increasing of nickel chloride concentration (15). Nickel at higher levels may decrease the root growth directly by cell division inhibition, or cell elongation inhibition or both, resulting in uptake limitation and translocation of nutrients and water, then mineral deficiencyinduced. The dry phytomass yield decreased at higher levels of nickel might be due to poor growth of seedling. At higher concentration, Ni act as a toxic metal (16 &17). Similar results were detected on the effect of cobalt. The high concentrations of cobalt may have caused a decrease in germination and growth of rice (*Oryza sativa* L.) seedling plant (17). Also some non-nutrients, such as high concentrations of cadmium may effect on the germination and growth of Triticumaestivum seedling (18). As well as, with high concentrations of

Table 1: Fresh weight (g) of *C. sativusimbibed* seeds in various periods with different nickel chloride concentrations (M).

Periods / Concentration (M)	1st period after 3h	2nd period after 6h	3nd period after 9h	4th period after 27h	average of concentration
Control (distill water)	6.38	6.51	6.89	7.51	6.825
$1*10^{-5}$	6.39	6.5	6.62	7.33	6.713
$5*10^{-5}$	6.45	6.70	6.94	7.49	6.902
$1*10^{-4}$	6.46	6.64	6.91	7.48	6.878
$2*10^{-4}$	6.44	6.56	6.72	7.51	6.810
$4*10^{-4}$	6.39	6.52	6.90	7.45	6.819
$8*10^{-4}$	6.41	6.65	6.96	7.43	6.866
$1*10^{-3}$	6.45	6.56	6.88	7.62	6.883
0.1	6.50	6.54	6.86	6.63	6.637
Average of periods	6.434	6.579	6.858	7.388	

LSD= 0.05, Concentrations average = 0.1416, Periods average= 0.0944, Interaction between concentration & period= 0.2832

Table 2: Effect of different concentrations of nickel chloride (M) in physiological parameters of *C. sativusimbibed* seeds.

Physiological parameters / Concentrations (M)	Germination %	Coefficient velocity of germination %	Length of root cm	Length of shoot cm	Fresh weight of root mg	Fresh weight of shoot mg	Dry weight of root mg	Dry weight of shoot mg	Leaf area cm^2
Control (distill water)	90	38	7.9	5.2	133	176	4.1	67.9	2.2
$1*10^{-5}$	95	45	14.9	5.8	111	265	10.9	71.3	2.5
$5*10^{-5}$	90	43	12.4	5.3	111	241	11.1	70.1	2.3
$1*10^{-4}$	80	41	11.5	4.5	111	240	12.3	63.1	1.6
$2*10^{-4}$	70	33	11.1	4.0	80	158	5.3	45.7	1.6
$4*10^{-4}$	70	31	5.9	2.4	68	138	4.6	14.4	1.2
$8*10^{-4}$	65	30	5.3	2.3	67	126	3.3	12.8	0.9
$1*10^{-3}$	60	29	4.3	2.1	66	116	1.2	12.4	0.7
0.1	0	50	25	1.3	0	61	0	1.1	0
L.S.D. (0.05)	8.58	2.21	0.82	1.18	5.9	5.9	0.2	0.2	0.60

Table 3: Effect of different concentrations of nickel chloride (M) in DNA damage of *C. sativus* imbibed seeds.

DNA parameters (μm) / Concentration (M)	Comet High	Comet Length	Comet moment
Control (distil water)	4.2	4	0.075
$1*10^{-5}$	14.6	22	0.174
$5*10^{-5}$	10.1	26	0.390
$1*10^{-4}$	11.6	30	1.047
$2*10^{-4}$	14.0	54	1.996
$4*10^{-4}$	12.4	72	3.004
$8*10^{-4}$	22.3	98	1.255
$1*10^{-3}$	68.6	119	6.978
0.1	77.8	169	3.822
L.S.D (0.05)	2.687	3.132	0.571

chromium which caused inhibition in the growth and germination in seedling plant of *Salvia sclarea* (19). Also, there was increasing in seed germination of corn plant exposure to the low concentrations of nickel while by high concentrations has aninhibition in germination (16). The decline in the germination of corn seed can be attributed to the collapse in the food stored in the seed due to the absorption of large quantities of nickel (20 & 21). The seeds of *Arabidopsis thaliana*that exposing for many heavy metals, which are showed effect on it germination and seedling growth. It was observed that copper does not effect on germination but it effects on seedling growth while Hg was toxic and inhibiting for germinating and seedling growth. This variation in response of *Arabidopsis* seeds for various metals and their effects inhibitory for germination and growth is similar to a large extent the seeds of *C. sativus*was exposed to many heavy metals, where was Hg more inhibition for germination of them (22 & 23). Seed of soybean that exposed to the cobalt and lead led to decrease in leaves area (24). The leaf area decrease in plants at higher concentration of heavy metals may be caused decreasing in activities of many

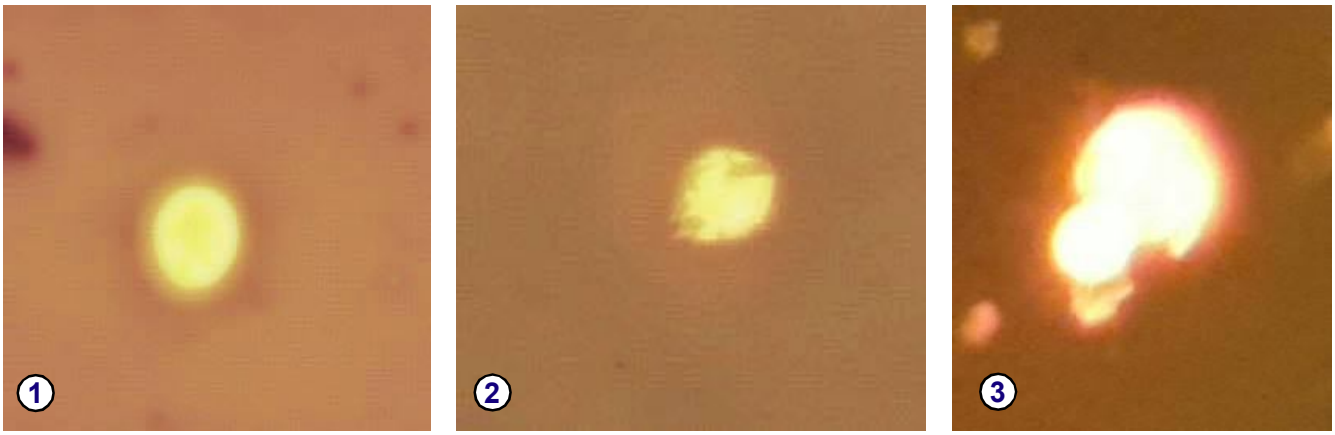

Fig. 1: Class of DNA damage in *C. sativus* L. seeds after treatment with different concentrations of Nickel chloride (M). 1: control (no damage), 2: medium DNA damage, 3: high DNA damage.

enzymes involved in the fixation of CO_2, inhibition of photosynthetic activities, changes in the thylakoid organization, reduction in chlorophyll contents, and cause disorder in the interaction of chlorophyll molecules into the stable complex (25).

Table 3 show the effect of nickel chloride on DNA damage. The high concentration of nickel chloride increased some parameters such as comet length, comet high and comet moment. The high concentrations of nickel chloride have led to a significant increase in the comet length from 4µm in control sample to 169 µm in the concentration 0.1 M, and also with other parameters such as comet high and comet moment.

These results coincided with some researchers. When the plants are exposed to many environmental stresses such as exposure to heavy elements, there searchers pointed out that the plant exposed to heavy metals lead to damage of DNA (26). It was the beginning of the damage during the formation of ROS, thus, the comet assay identified damage process breaking a single tape or a double tape of DNA (27). As well as several plants were a model to study the effect of radiation, toxic heavy metals, which it showed a change in some of the indicators as the damage of DNA with cellular responses to stressful factors (28).

As in table 3, the damage was increased gradually with Ni concentration increasing. Low concentrations were enhanced for imbibition (table 1) and for almost the physiological parameters (table 2) that reflect the low damage of DNA which is not effect on the physiological parameters. While the high concentration of Ni (0.1M) caused DNA damaged as in comet length (169µm) and comet high (77.8 µm) which is illustrated in figure 1 which showed the graduation in DNA damage as shown in pictures 2 and 3.

Conclusions

1- Imbibition process in seed is affected by Ni at different concentrations.

2- The low concentrations of Ni ($1*10^{-5}$ and $5*10^{-5}$M) were more enhanced for all the physiological parameters.

3- The Ni at higher concentration (0.1 M) caused no imbibed seeds in the fourth period (27h), no shoot emergence, and damage in DNA.

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