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Effect of different concentrations of nickel chloride in biochemical and enzymatic parameters of *Cucumis sativus* L. imbibed 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) were studied. These concentrations affected on content of total carbohydrates, protein, glutathione, allantoin the activity of α-amylase, protease, and urease of *Cucumis sativus* L. seeds at imbibition stage. At concentration 1×10^{-5} M the total carbohydrates content and urease activity increased significantly comparing with control then begin to decrease gradually with increasing in concentration of $NiCl_2$, while protein content, the activity of both α-amylase and protease decreased by lowest concentration then still decreased with increasing concentration until concentration 0.1M which was more inhibition for these parameters. Content of glutathione and allantoin increased with increasing concentrations of $NiCl_2$.

Key words: Imbibed seed, nickel chloride, biochemical and enzymes estimation.

Introduction

Nickel is one of the important heavy metals for plants, it's a micronutrient but is toxic at high concentration [1;2]. Increasing concentration of heavy metals is one of the environmental pollution which effect on seeds "a protective stage of plant life against stresses", but after a short period of imbibition and beginning of vegetative stage the seeds become sensitive for outer circumstances as light, heat, and nutrition status [3]. The germination stage is more sensitive for heavy metals because low mechanical defends [4], and especially it's more sensitive for nickel toxicity [5]. The high concentration of nickel caused inhibition in seed germination and seedling growth for many plant species [6]. [7] referred that the causing of this inhibition belong to the low product of protein and decreasing in the activity of some enzymes that responsible on transport and storage the nutrients through germination. The differs in plant acceptance for heavy metals depend on seed type, sensitivity, resistance and the soil which effect on germination at a high range [8]. Nickel is important for urease activity to decay the urea, and seeds need it for nitrogen storage as urieds or arginine to use it at the early stage of seedling [9]. There were, the nickel deficient lead to decrease the activity of urease and then low absorption of nitrogen [10]. Many studies referred to inhibit the germination, seedling growth, the main enzymes which important in assimilation as protease, α and β-amylase, synthesized of protein and carbohydrates in varied plants by high concentrations of nickel [11;12]. The aim of this study is illustration the nickel important at different concentrations in the imbibition stage of *Cucumis sativus* L. seeds by determination the total carbohydrates, protein, glutathione, allantoin, the activity of α-amylase, protease, and urease.

Materials and Methods

Source of seeds

In this study used the seeds of cucumber *(Cucumis sativus* 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 0.1, 1×10^{-5}, 5×10^{-5}, 1×10^{-4}, 2×10^{-4}, 4×10^{-4}, 8×10^{-4} and 1×10^{-3} 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 °C, humidity 60-70 % and continuous light with intensity 3500 lux.

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 seeds at 27 h. age were dried with absorbent paper before measuring.

Biochemical and Enzymatic Measurements

After splitting the peel of seed, take the core to determine the contents of each the following: total carbohydrates [13], protein by Biuret method, glutathione [14], and allantoin which determined using HPLC technique. Determination the activity of the following enzymes: α-amylase [15], protease [16], and urease [17].

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

Figure 1 shows the total carbohydrate in cucumber seeds treated by different concentration of $NiCl_2$ for 27hours in addition to the control in which of 529 mg/g.

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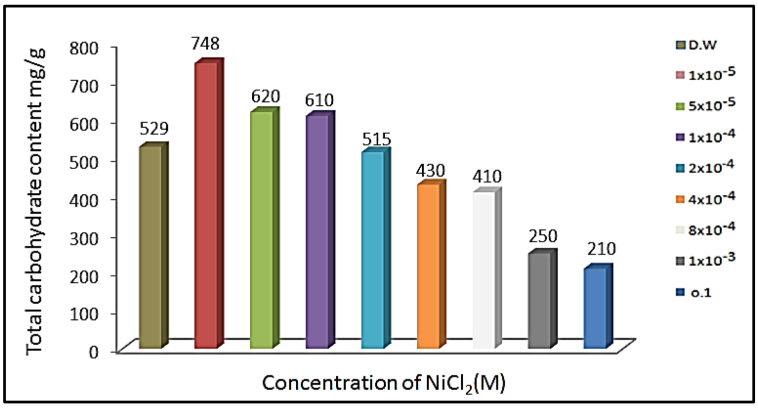

Fig. 1: **Total carbohydrate content (mg/g) in cucumber seeds treated with different concentrations of $NiCl_2$ (M). LSD (0.05) =17.15**

There are a significant increasing in seeds carbohydrates content at low concentrations. The largest value was 748 mg/g at concentration of 1×10^{-5} M, whereas the decrease was not significant at concentration 2×10^{-4}M with carbohydrate content of 515 mg/g. The concentration range between 4×10^{-4} to 0.1 M caused a significant decreasing in comparison with control. These results agree with [18] which soaked the seeds of *Phaseolus aconitifolius* with different concentrations of heavy metals, they found that low concentrations of Ni increases the carbohydrate content, while in higher concentrations, the content inhabited. [19] noticed the deficient of the carbohydrate in seedling of *Vigna radiata* with increasing in $CdCl_2$ concentration.The results shown in figure 2 which represent the protein content in cucumber seeds. There was a significant decreasing in protein content in seeds exposed to $NiCl_2$ in comparison with the control treatment (36 mg/g).

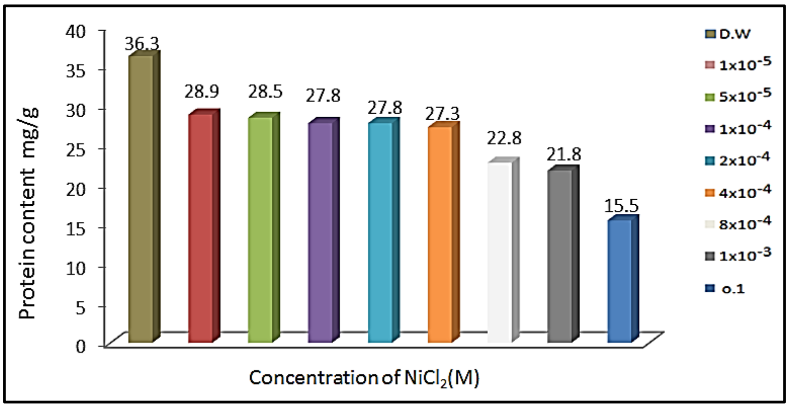

Fig. 2: **Protein content (mg/g) in cucumber seeds treated with different concentrations of $NiCl_2$ (M). LSD (0.05) =1.618**

The seeds exposed to different concentrations of $NiCl_2$ undergoes from gradual decreasing in the protein content from 28.9 mg/g at 1×10^{-5}M to 15.5 mg/g at 0.1 M. A fair correspondence of above results with [3], they exposed the seeds of *Macrotyloma uniflorum* to various concentrations of Ni that led to decrease in protein content with increased Ni concentration. This decrease in protein content due to decrease in protein metabolism through seeds germination, thus, the shortage of protein caused by its decay by Ni [20].

Figure 3 shows the glutathione content in cucumber seeds soaked in different concentrations of $NiCl_2$, it is obvious that there was a significant increase in glutathione content (23.3 μg/g) at 1×10^{-5} M in compared with control treatment (20.8 μg/g).

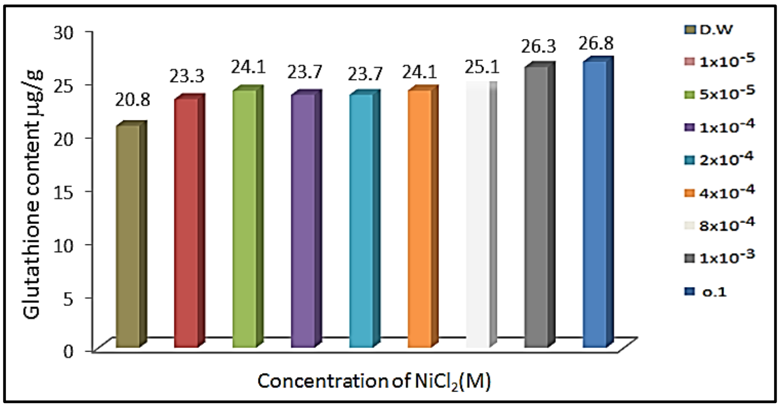

Fig. 3: **Glutathione content (µg/g) in cucumber seeds treated with different concentrations of $NiCl_2$ (M). LSD (0.05) =1.72**

This increase was continuous with increasing in concentration to reach 26.8 μg/g at 0.1 M. As well known that the glutathione is one of non-enzymatic anti-oxidant which provide chemical protection to plants and increase the thiol concentration in cytoplasm and plastids. [21] mentioned that exposure of *Groenlandi dense* to different concentration of Ni causes a significant increase of glutathione content.

Figure 4 represents the allantoin content in cucumber seeds. There was a gradual increase in allantoin content as treatment concentrations increases until the treatment 4×10^{-4} M (598μg/g), then the allantoin content rapidly decreased as treatment concentration progress forward reaching to largest concentration (0.1 M) where the value become 207μg/g, but the values are still higher than the control (99μg/g) significantly.

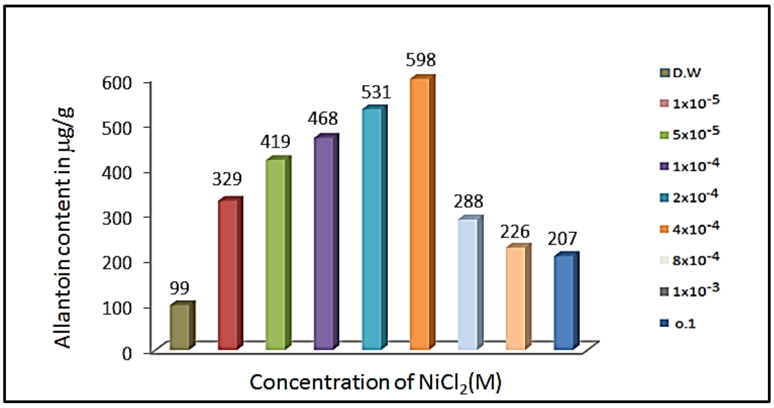

Fig. 4: **Allantoin content (µg/g) in cucumber seeds treated with different concentrations of $NiCl_2$ (M). LSD (0.05) =32.242**

According to [22] observations, the levels of allantoin were increased due to inhibition of the allantois enzyme which crashed the allantoin that provided protection mechanism or support against stresses exerted by heavy metals, this led to relief the side effects resulted from different types of stresses. Beyond the peak allantoin content its began in declined rapidly with high concentrations of $NiCl_2$ [22-25]. Some researchers suggested that allantoin works as anti-oxidant and reduced the damages of oxidant activities [26;27]. Figure 5 shows the α-amylase activity in cucumber seeds caused by various concentrations of $NiCl_2$ treatments. It is evident in figure 5, the significant decrease of activity of α-amylase enzyme with different concentrations of NiCl2, this inhibition increased as treatment concentrations increased compared to the control (30 mg/min.) reaching to its lower value of 6 mg/min. at 0.1 M.

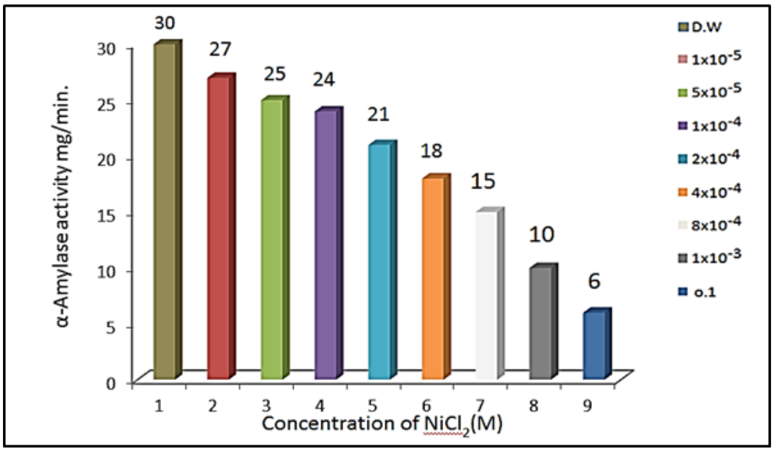

Fig. 5: **α-Amylase activity (mg/min.) in cucumber seeds treated with different concentrations of $NiCl_2$ (M). LSD (0.05) =1.72**

The exposure of *Zea maize* to various concentrations of Cd inhibited seeds germination and stop seedling growth, also inhibited the transport of starch from endosperm because of the α-amylase enzyme play a vital role in transport the stored nutrients in the endosperm of seedlings by decomposition it into carbohydrates, proteins and fats[28]. This inhibition in α-amylase enzyme led to reduce the carbohydrate decomposition and implies to increases its accumulation in the plant as shown in figure 5 as well as illustrated in figure 1 above, in addition to reduce decomposing of glycolysis which eventually leads to reduced germination and seedling growth, this conclusion supported by [29]. Figure 6 shows the reduction in protease activity in cucumber seeds when treated with different $NiCl_2$ concentrations, this reducing was increased as treatment concentration increases compared by the control (82.4 unit/ml). The significant enzyme activity was 46.4 unit/ml at 1×10^{-5}M. The activity of enzyme began to reduce significantly, as treatment concentration increases to reach its smallest value (7.7 unit/ml) at 0.1 M.

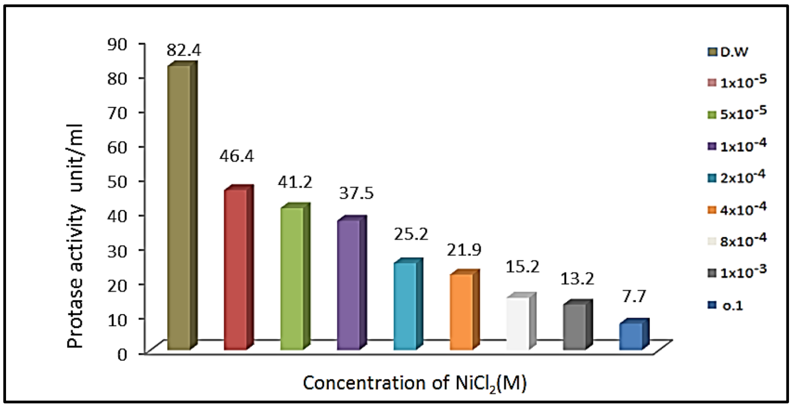

Fig. 6: **Protease activity (unit/ml) in cucumber seeds treated with different concentrations of $NiCl_2$ (M). LSD (0.05) =1.411**

The exposure of *Phaseolus vulgarise* L. seeds to various concentrations of $CuCl_2$ (which considered as a major elements in the plant growth processes) after that estimation of protease activity, they observed inhibition in enzyme activity reached to 60% in compare with distilled water[30]. The increase in $NiCl_2$ concentration causes the reduction protease activity in the rice seeding. It was commonly known that the activity of enzymes were inhibited in plant cells by deficiency of enzyme activity, Ni considered as separator element in the creation of complexes with carboxyl and Sulphur groups, thus the inhibition of protease activity which has been obviously noticed in our work probably was due to correlation between Ni and active groups of enzymes [31].

The results illustrated in figure 7 shows the effect of different concentrations of $NiCl_2$ on the urease activity in cucumber seeds.

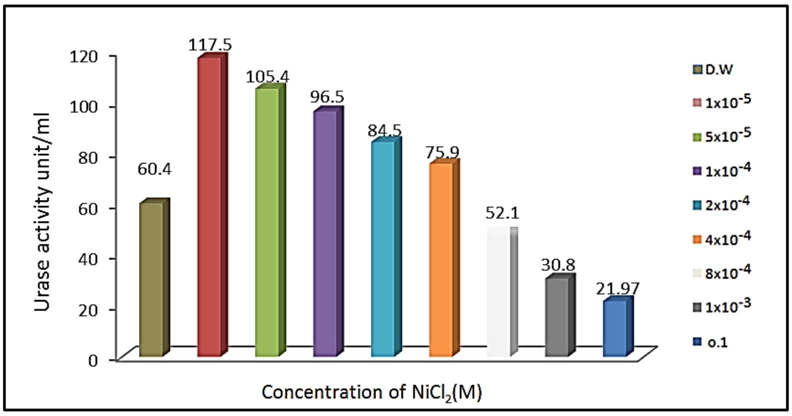

Fig. 7: **Urase activity (unit/ml) in cucumber seeds treated with different concentrations of $NiCl_2$ (M). LSD (0.05) = 0.88**

It was noticed that the significant activity of urease enzyme at low concentrations of $NiCl_2$(1×10^{-5}M) was 117.50 unit/ml in compare with control witch was 60.4unit/ml, then gradually decreased as $NiCl_2$ concentration increased in comparison by low concentration (1×10^{-5} M) but still significantly high at compare with control. The inhibition begin at concentration of 4×0^{-4}M and afterward significantly decreased compared with control to reach its smallest value (21.97 unit/ml) at 0.1M.

These results of present study agree with those obtainable by [32] who has treated *Lactuca sativa* L. seeds for different concentrations of Ni & Cd, in which the low concentrations increases the activity of urease while high concentrations inhibited it. These results were fairly approved with [33] for copper, [34] for zinc, and [35] for silver. The urease enzyme was closely related to the possibly released nitrogen by using mechanism of crash urea to form easily absorbed by plants.

Conclusions

(1) The cucumber seeds needs to Ni as a necessary element in life cycle of plant within range of 1×10^{-5}-4×10^{-4}M.

(2) Increasing concentration of Ni larger than 4×10^{-4}M caused inhibition in content of carbohydrates, protein, allantoin, the activity of α-amylase, protease, and urease.

(3) Increasing concentration of Ni larger than 4×10^{-4}M caused enhancement in glutathione content.

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