The Quarterly Scientific Journal of Applied Biology
Vol.36, No.2, Sering.71, Summer 2022, p. 3-4
Journal homepage: https://jab.alzahra.ac.ir
10.22051/JAB.2021.34043.1392

Research Paper

Evaluation of nickel accumulation and tolerance by *Trifolium repens* L. in hydroponic culture

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Received: 2021.05. 24 **Accepted:** 2022.02.22

Introduction

Nickel at low concentrations (0.05-10 mg/kg dry weight) is an essential element for plant growth (Nieminen *et al*., 2007). However, excessive levels of nickel in plants are more common than deficiencies (Alloway, 1995). Excessive amounts of nickel have detrimental effects on plant germination characteristics (Rao & Sresty 2000), plant growth (Molas, 2002), nutrient uptake (Aggarwal *et al*, 1990), photosynthesis and respiration (Rahman *et al*., 2005) and leaf chlorosis and necrosis (Gajewska *et al*., 2006). White clover (*Trifolium repens*) is a widely cultivated forage crop with high biomass. On the other hand, nickel pollution on farms has increased. Accordingly, in this study, the effects of nickel were investigated on seed germination and the physiological characteristics of the plant.

Methods & Materials

In this study, the effects of nickel treatments (at 0, 10, 50, 100, 200, 500, and 1000 µM levels), in a completely randomized statistical design, were investigated on seedling length, germination percentage, germination rate, and seed vigor of white clover in a growth chamber and then on biochemical and physiological parameters in hydroponic culture. Lichtenthaler and Wagner methods were used, respectively, to measure photosynthetic pigments and anthocyanins. The amount of total soluble protein and carbohydrates were measured using the Bradford and phenol sulfuric acid methods, respectively. An enzyme mixture containing monobasic phosphate buffer, pyrogallol, enzyme extract, and hydrogen peroxide was prepared and its adsorption was measured by spectrophotometer, then peroxidase activity was calculated. Root and shoot concentrations of nickel, iron, zinc, and copper were determined using the atomic absorption spectrometry method, after acid digestion of plant samples. Data analysis was performed using SPSS and Excel software. Data mean comparison was performed using the Duncan test.

Results & discussion

The results showed that seed germination percentage and speed, seed vigor, and seedling length of White clover (*Trifolium repens*), increased and decreased with low and high levels of nickel, respectively. Seedlings' death was observed at 100 µM and higher concentrations of nickel. Heavy metals in the germination medium, impair seedling growth by affecting physiological processes and inhibiting cell division (Márquez-García *et al*., 2013). The reason for the decrease in the germination percentage of white clover seeds can be due to the increase of the osmotic potential of the solution and the decrease of water absorption power by the seeds. High levels of heavy metals lead to reduced seedling growth, which can be attributed to a decrease in the amount and activity of hydrolytic enzymes in the endosperm and cotyledons. When heavy metal affects the amount and activity of these enzymes, not enough organic matter reaches the growing seedling and thus affects the length of the seedling (Kabir *et al*., 2008)

Concentrations of 10 and 50 µM nickel in nutrient solution led to a significant gradual decrease in total soluble protein and carbohydrates of plant shoots compared to the control. Under heavy metal stress, oxygen radicals are produced and lead to oxidative damage to nucleic acids, fats, and proteins, disruption of cellular metabolism, photosynthesis, respiration, and growth retardation (Mishra *et al.*, 2006). Levels of 50 µM nickel in Hoagland's nutrient solution reduced the activity of white clover leaf peroxidase enzyme compared to the control treatment. Therefore, it seems that this plant is not able to use this antioxidant potential for nickel resistance. Nickel treatment led to a significant reduction in the amount of iron, zinc, and copper in white clover shoots and roots compared to the control. Nickel competes with other nutrients for absorption and transport. Therefore, when the plant is under nickel stress, concentrations of many nutrients such as iron, copper, zinc, magnesium, and manganese are usually reduced (Krupa & Basznski, 1995).

Nickel treatment had a decreasing effect on chlorophyll and anthocyanin and an increasing effect on carotenoids of white clover. Nickel toxicity in plants affects photosynthetic pigments (Seregin & Kozhevnikova, 2006). It seems that the white clover plant has tried to counteract the oxidative effects of nickel by increasing the production of carotenoids.

By increasing levels of nickel treatments, nickel concentrations increased in roots, and shoots. Plants readily absorb the available nickel and mainly accumulate it in the roots, although, in some species, the adsorbed nickel is also transported to the shoot (Alloway, 1995). In the present study, nickel metal accumulated more in the roots than in the shoot.

In comparison to the control, 10 and 50 µM nickel concentrations in the nutrient solution, led to an increase and decrease in stem dry weight, shoot length, leaf area, and leaf dry weight, respectively. Decreased growth indices of white clover under high concentrations of nickel can be due to reduced chlorophyll content and consequently reduced photosynthesis, reduced carbohydrate and protein content, accumulation of toxic amounts of nickel in the plant, and reduced concentration of plant zinc and iron.

Conclusion

In the current study, the effects of nickel treatments were investigated on some growth and biochemical and physiological parameters of White clover (*Trifolium repens*). It seems that 10 µM and 50 µM concentrations of nickel function as suitable and toxic levels for the growth of this plant, respectively. It also seems that white clover is a nickel-sensitive plant and not able to tolerate and accumulate nickel, and it is not a proper candidate for phytoremediation.

Keywords: *Heavy metal resistance, Nickel accumulation, Phytoremediation, Trifolium repens*

Acknowledgment: *The current research has been financially supported by Yasouj University.*

Declaration of conflict of interest: *The authors declare that they have no conflicts of interest.*