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# Role of Nitrogen and Nitrogen Oxide in Reducing the Toxicity of Nickel on Wheat Plant

**Mohmmad K. Okla[1]*, Walid Soufan[2], Mohamed E. El-Zaidy[1] and Mohamed Bakir[3]**

[1]Botany and Microbiology Department, College of Science, King Saud University,P.O. Box 2455, Riyadh 11451, Saudi Arabia.
[2]Agricultural Research and Experimental Station, College of Food and Agriculture Sciences, King Saud University, Saudi Arabia.
[3]College of Hydrology and Water Resources, Hohai University, No. 1 Xikang Road, Nanjing 210098, China.

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**The study was conducted on the wheat plant *Triticum aestivum* L in order to determine the effect of nitrogen (N) and nitric oxide (SNP) on nickel (Ni) and reducing its toxicity in the plant. The experiment was carried out in College of Science laboratories, King Saud University, and the following factors has been applied. The wheat was planted in a plastic pots measuring (20 × 20 cm) each pot contains about 15 pills. The randomized complete blocks design was followed with three replications, each replicate includes three pots, data were analyzed using statistical analysis software (SPSS) and averages was compared by testing less significant difference LSD at 5% significance level. The results showed that nickel reduced plant height and leaf area, but it led to increase the amount of peroxides and catalysis and cause little reduce in wet and dry weight of the plant. Also it was found that the nitrogen and nitric oxide increases the length of the plant, the vegetative area, the wet and dry weight and the quantity of each of peroxides and catalysis which mean they reduced the toxicity of nickel and its impact on the mentioned qualities.**

**Key words:** The enzyme extracts, Heavy metals, Nickel, Nitrogen, Nitric oxide.

The wheat plant belongs to the superfamily *Triticum aestivum L*, Gramineae, and is considered as a wild herb, Mesopotamia in the Arab Middle East is its native places.

Many studies have shown that the heavy metals have negative impact on most plants, including wheat plant where the treatment of wheat seeds with heavy metals causing inhibition of germination. The degree of inhibition depends upon both the type and concentration of the heavy metal and the type of plant, for example, the toxic effect of the high nickel concentration discourage the activities of Mitosis (Madhava Rao and Sresty, 2000) but the presence of nickel in the plant in low concentration about 0.05 - 10 mg is considered essential for the plant (Ragsdel, 1998). The increase of nickel concentration in most plants are common now a days (Alloway, 1995).

Nitrogen has a significant impact on the growth characteristics of the wheat plant as it increases the plant height, length of internodes, the thickness of the stems and leaf area, the number of green leaves and dry matter. The increasing of fertilization using nitrogen to the optimal degree increases plant growth and productivity (Joel *et al* 1997) and also increases the rate of photosynthesis on leaves (Makino *et al* 1992) by increasing the amount of proteins and the amount of chloroplasts in the leaves (Bungard, *et al* 1997).

The aim of this study is to identify the role of nitrogen and nitric oxide in reducing the toxicity of nickel and its direct impact on the wheat plant.

* To whom all correspondence should be addressed.
Mohmmad K. Okla

## MATERIALS AND METHODS

### Experimental material and cultivation

Fresh wheat *Triticum aestivum* from the department farm have been planted after soaked in water for 24 hours with ventilation in plastic pots measuring (20 × 20) cm filled with perlite by 15 pills for each pot, irrigated by distilled water and Hoagland nutrient solution then placed in the incubator (or the germination room) at 25 °C and humidity of 75% in the presence of light source and let to grown suitable conditions for germination.

### Treatments

The following factors have been applied on the wheat plant pills for one week:

| | | |
|---|---|---|
| 1 | Control | 100 ml distilled water |
| 2 | SNP | 100 ml SNP (100mM) |
| 3 | N | 100 ml of urea (60mg / L) |
| 4 | Ni | 100 ml Nickel (60mM) |
| 5 | SNP+Ni | 50 ml Ni (150mM) + 50 ml SNP (100mM) |
| 6 | N+Ni | 50 ml Ni (150mM) + 50 ml Urea |
| 7 | SNP+N+Ni | 50 ml Ni (150mM) + 25 ml urea + 25 ml SNP (100mM) |

### Sampling

After 15 days of Agriculture the following characteristics of plants have been measured and evaluated; plant height, surface area, dry and fresh weight chlorophyll and some enzymes such as peroxidase (POD) and Catalase (CAT).

### Measuring the growth characteristics

plant height was measured using a meter scale, the plant weighted and recorded as weight of fresh plant, the samples then placed in the oven at degree of 70 $^{o}$C for 48 hours and again weighed to determine the dry weight of the plant.

### Estimating the antioxidant enzymes

### Preparation of extracting enzymes

Preparing of 500 mg of the plant green leaves with 100ml of phosphate buffer (pH 7) are grind together in a mortar and then placed in a centrifuge 10,000rpm for 30 minutes, are used in the enzymatic tests.

### Catalase

The activity of CA (EC 4.2.1.1) was determined by the method of Dwivedi and Randhawa (1974). Preparing the blank set by using 0.5ml of the extracted enzyme and 4.5ml of phosphate buffer, while the test set prepared by 0.5ml of the extracted enzyme and 3.25ml of phosphate buffer (pH 7.0) and 1.25ml $H_2O_2$ then mixed together and placed in a water bath at 37 °C for 15 minutes. 2ml of the mixture placed in test tubes and 2ml of potassium dichromate and glacial acetic acid are added, the test tubes placed in a water bath for 10 minutes and the measurements are done by using spectrophotometer at 570 nm.

### Peroxides

Peroxidase POD (E.C. 1.11.1.7) activity was determined by the method of Chance and Maehly (1955). The blank set prepared using 2ml of the extracted enzyme and 3.2 ml of phosphate buffer while test set prepared by 2ml of the extracted enzyme and 2ml of phosphate buffer (pH 6.0) and 1ml of pyrogallol and 0.2ml $H_2O_2$ then placed in a water bath for 10 minutes at 37 °C and measured using spectrophotometer at 430 nm.

### Design of the Experiment

The experiment was planted in a complete block randomized way with three replicates. 2 pots per each replicate were planted and thus the number of replicated pots = (3) number of replicates × (7) number of pots × (2) (number of pots per replicate) = 42 pots.

### Statistical analyses

Data were analyzed using statistical analysis software (SPSS) and averages was compared by testing less significant difference LSD at 5% significance level.

## RESULTS AND DISCUSSIONS

### The effect of nitrogen and nitric oxide on the length of the wheat plant under the influence of nickel toxicity

The addition of nitric oxide or nitrogen increases plant height compared to the control. The effect of nitric oxide alone was higher while the addition of nickel alone had a negative impact on plant height Figure (1). This is compatible with (Madhava Rao and Sresty, 2000) as they showed that the toxic effect of high concentrations of nickel reduces plant height.

From Fig (1) it showed that the addition of Nitrogen or Nitric Oxide or a mixture of both to grow plants under the toxicity of nickel led to a reducing the toxicity of nickel and increasing the plant height compared to the control.

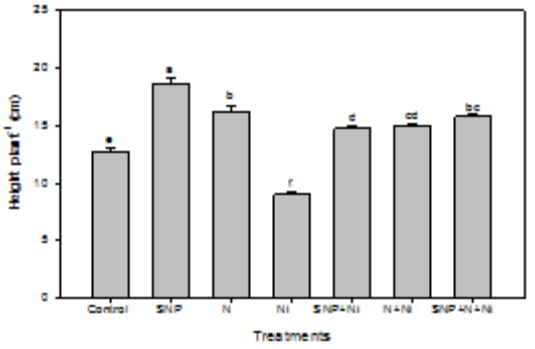


**Fig. 1.**

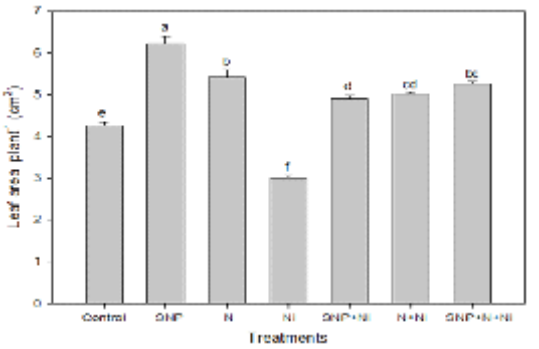


**Fig. 2.**

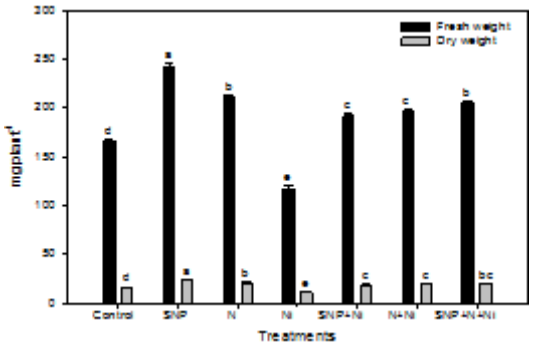


**Fig. 3.**

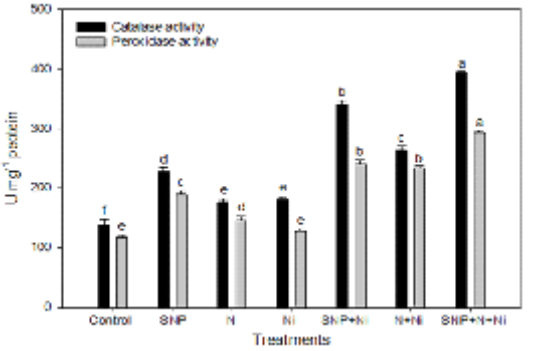


**Fig. 4.**

**The effect of nitrogen and nitric oxide on the leaf area of the wheat plant under the effect of nickel toxicity:**

Results showed that the addition of nitric oxide or nitrogen increases leaf area of the plant compared with control and the effect of nitric oxide alone was higher and by adding nickel alone, it had a negative impact on the leaf area of the plant. The addition of nitrogen or nitric oxide or a mixture of both to a grow plants under the toxicity of nickel, a reduction of the impact of the toxic and increasing of the leaf area of the plant occur figure (2) as nitrogen is a part of the proteins and nucleic acids composition.

**The effect of nitric oxide and nitrogen on the fresh and dry eights under the effect of the nickel toxicity:**

The addition of nitric oxide or nitrogen increases the fresh and dry weight of the plant compared with control. The effect of nitric oxide alone was higher while adding nickel alone showed a negative impact on the fresh and dry weight of the plant and this also was pointed out by several studies (J. Molas, 2002; Gajewska *et al*, 2006). Adding nitrogen or nitric oxide or a mixture of both to a plants growing under the influence of nickel toxicity, it was found that a reduction of the impact of nickel toxicity and an increasing of fresh and dry weight of the plant (Fig. 3).

**The effect of nitric oxide and nitrogen on the activity of the catalase and peroxidase enzyme under the influence of the nickel toxicity:**

By adding nitrogen or nickel increases the activity of the catalase and peroxidase enzymes and the effect on the peroxidase enzyme slightly higher. the higher effect resulted by adding nitrogen or nitric oxide or a mixture of both to plants that growing under the influence of the toxicity of nickel, where enzyme activity of catalase and peroxidase increased significantly Fig (4).

It is found that nickel adversely affect the plant growth and reduce its height and inhibits the growth of the vegetative, this is coincide with (Molas, 2002) also nitric oxide has positively impact

on the plant, where with nitrogen are causing the impact and working on increasing the plant height and the vegetative area which is coincide with (Laspina *et al*, 2005).

From this study it can be concluded that the nitrogen and nitric oxide have a clear role in the increase of plant height, the vegetation area and fresh and dry weight and dry weight. They also, positively impact on increasing the amount of peroxidase and catalase enzymes. Nitrogen and nitrogen oxide played a major role in mitigating the impact of the nickel toxicity and the heavy metals on the plant.

## ACKNOWLEDGMENTS

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