Nickel-Induced Changes in Nitrogen Uptake in Wheat Plants *(Triticum aestivum)* Grown on Sandy Loam Soil

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ABSTRACT

A greenhouse experiment was conducted to investigate the effect of Ni (0,5,10,15 and 20 mgkg^{-1}) on the dry matter (DM) yield of wheat plants grown on N-treated soils (0,30,60 and 120 mgkg^{-1}), to describe the relationships between Ni concentration and dry matter yield of wheat in the N-treated soils, and to study the utilization of N by wheat plants. The results indicated that high concentrations of Ni (15 and 20 mgkg^{-1}) added to the soil had negative effects on the above-ground dry matter yield, concentration and uptake of nitrogen in wheat plants, but the low concentrations (5 and 10 mgkg^{-1}) had positive effects on these parameters. The polynomial quadratic model accurately described the relationship between the above-ground dry matter yield of wheat plants grown on N-treated soils with different rates and application rates of Ni. The highly significant ($P \leq 0.001$) interaction between N and Ni concentrations points out that the tissues N concentrations and uptake depended on kind of element added and rate of its application. A significant N x Ni interaction at $P \leq 0.001$ on tissue Ni concentration indicated that the application of Ni to soil resulted in the accumulation of Ni in wheat tissue in treatments where no N was applied. The increase in Ni uptake with the concurrent decrease in tissue Ni concentration with increasing N supply in soil is attributed to the high DM yield.

Key Words: nickel, nitrogen, uptake, wheat.

INTRODUCTION

Nitrogen is considered to be a vital nutrient determining the growth and the productivity of plants. Nickel is an essential element for some plants, in small quantities, and has been reported to improve crop yield and quality (Brown *et al.,* 1990; Atta-Aly, 1999; Gerenda's *et al.,* 1999) and low concentrations of Ni are necessary in the N metabolism and the germination of plants, such as cereals and cowpeas (Brown *et al.,* 1987 and 1990; Dalton *et al.,* 1985; Walker *et al.,* 1985; Krogmeier *et al.,* 1991). However, similar to other microelements, at excess concentrations, this metal becomes toxic for most of the plant species. Phytotoxicity of Ni has been attributed to its deleterious effect on photosynthesis, respiration (Yang *et al.,* 1996), mineral nutrition (Barcelo and Poschenreider, 1990), enzymatic activity (Krogmeier *et al.,* 1991; Pandolfini *et al.,* 1992), decreasing plant growth (Bingham *et al.,* 1986), water uptake (Pandolfini *et al.,* 1996), and chlorophyll concentration (Pandolfini *et al.,* 1992; Brune and Dietz., 1995), plant water status, and transport of assimilates (Samarakoon and Rauser, 1979; Tripathy *et al.,* 1981; Parida *et al.,* 2003; Llamas *et al.,* 2008; Llamas and Sanz, 2008) as well as its ability to induce oxidative stress (Gajewska and Skłodowska, 2007, 2008). Although mechanisms of Ni toxicity to plants have been investigated by many researchers, relatively little is known about the influence of this heavy metal on nitrogen metabolism. The aims of this study were : to investigate the effect of Ni on the dry matter yield of wheat plants grown on N-treated soil to describe the relationships between Ni concentration and dry matter yield of wheat in the N-treated soils, and to study the utilization of N by wheat plants.

MATERIALS AND METHODS

Characterization of Soil

A sandy loam soil (*Typic torripsamments*) was selected for the study and sampled (0-15 cm depth) from El-Bostan, El-Behaira Governorate, Egypt. Sub-samples of the air-dried soils were ground to pass a 2-mm sieve prior to the following chemical analysis: electrical conductivity (EC) was determined in soil-paste extract (Richard, 1954). The pH was measured in 1:2.5 soil-water suspensions (Richard, 1954). The organic matter content was determined by dichromate oxidation method (Nelson and Sommers, 1982). Cation exchange capacity (CEC) was determined by 1N NaOAc (Rhoades, 1982). Particle size analysis was determined by the hydrometer method (Day, 1965). Calcium carbonate content was determined using calcimeter (Nelson, 1982). Available nitrogen was determined by the Kjeldahl method (Bremner and Mulvaney, 1982). Available P was extracted by 0.5M $NaHCO_3$ test (Olsen and Sommers, 1982). Available K was exracted by ammonium acetate (1N) method (Knudsen *et al.,* 1982). Selected properties of the studied soil are summarized in Table (1).

Experimental Set-up:

A pot experiment was conducted with wheat (*Triticum aestivum* c.v.Gemmiza 7) as a test plant in polyethylene pots, each containing 3 kg of sandy loam soil. The soil was treated with five concentrations of Ni (0, 5, 10, 15, and 20 mg Ni kg^{-1} soil as $NiCl_2.6H_2O$) and four concentrations of N (0, 30, 60, and 120 mg N kg^{-1} soil as urea) in all possible combinations. A basal dose of 50, 63, 10, 10, 5, and 5 mg kg^{-1} soil of P, K, Zn, Fe, Cu, and Mn, respectively, was added to each treatment in solution form and mixed thoroughly with soil before sowing. The half dose of N was applied at sowing and the remaining half after 30 days of growth. Five seeds of wheat were sown per pot, which were thinned to three after germination. Distilled water was added to bring the soil moisture to 70% of field capacity. Each treatment was replicated fourth in a completely randomized design. The above-ground plants material was harvested after 10 weeks of sowing.

Plant Analysis

The above-ground plants material was harvested, and immediately were triple rinsed in distilled water to remove any adhering particles. Plants were oven dried at 65°C for 48 h and dry matter yield was recorded. Plant tissues were ground in a stainless steel mill. Subsamples of ground plant material were dry-ashed in a muffle furnace at 450°C for 6h. Ash was dissolved in 5 ml of HNO_3 (1: 1), diluted to a constant volume with distilled water and analyzed for Ni (Jones, 2001). Ni in the digested material was determined by atomic absorption spectrophotometry (Baker and Amacher, 1982). Another sub-sample of plant material was ashed and dissolved in hydrochloric acid solution (1:1, v/v), diluted to a final volume with double-distilled water, and analyzed for N (Jones, 2001) by Kjeldahl method (Bremner and Mulvaney, 1982).

Dry matter production is expressed on dry weight basis. Plant nutrient uptake was calculated from the concentrations in the above-ground plant material and its dry weight.

Statistical and Mathematical analyses

The two-way analysis of variance (ANOVA) was carried out to determine the statistical significance of the treatment effects on dry matter yield, N concentration, Ni concentration, and their uptake, with the Fisher's least significant difference procedure at a significant level of 0.05 (SAS Institute, 1994). The polynomial quadratic model was used to describe the relationship between Ni concentration in soil and dry matter yield of wheat plants grown on soils treated with different rates of nitrogen. The polynomial quadratic model used in the form:

$$Y_i = B_0 + B_1X_i \pm B_2X^2_i$$

Where Y_i is the expected dry matter yield corresponding to Ni application rate in each N-applied soil Xi, B_0 is the intercept and B_1, B_2 are the linear and quadratic coefficients, respectively. The efficiency of Ni (Ex_i) at different increment (X_i) was calculated (Thabet and Balba, 1994) using the following relationship:

$$Ex_i = B_1 - 2B_2X_i$$

RESULTS AND DISCUSSIONS

Dry Matter Yield and Polynomial Quadratic Model Application

Dry matter (DM) yield of wheat increased significantly ($P \leq 0.05$) with the increasing concentrations of N in soil at each concentrations of Ni applied to soil (Fig.1). The application of Ni, however, increased the DM yield noticeably ($P \leq 0.05$), up to 10 mg Ni kg^{-1} soil, and thereafter the depressive effect was evident at the 15 and 20 mg Ni kg^{-1} soil at all concentration of applied N to soil .The maximum DM yield (9.70 g per pot) was observed with a 120 mg N kg^{-1} + 10 mg Ni kg^{-1} soil treatment combination. The magnitude of increase in yield was greater for the first addition of Ni or N. The increase in DM yield with applied Ni to soil was higher in the presence than in the absence of added N to soil (N x Ni interaction significant at $P \leq 0.001$). For example, with the application of 10 mg Ni kg^{-1} soil , the increase in DM yield over their control values were 42 , 61 , and 72 % at 30, 60 , and 120 mg N kg $^{-1}$ soil concentrations , respectively, and the percentage of increase were 315, 491, 569, and 569 % at 0, 30, 60, and 120 mg N kg $^{-1}$ soil concentrations compared with control treatment (0 N, 0 Ni) .

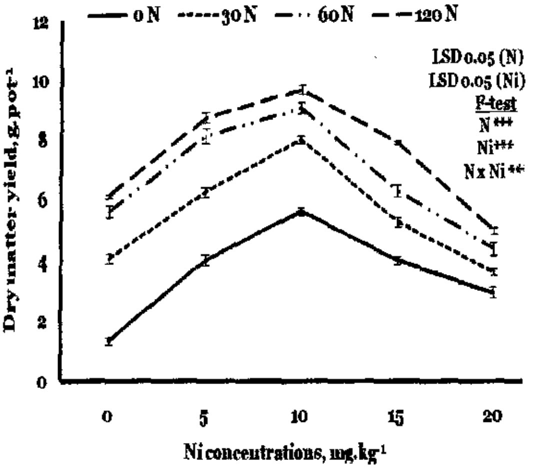

Fig.1: Dry matter yield as influenced by an application of N and Ni to soil.

Table 1: Some physical and chemical properties of the studied soil (means ± SD) #

EC	pH	$CaCO_3$	CEC,	Clay	Silt	Sand	Texture ¶	O.M†	Available-K (1N NH_4OAc-K)	Available-P (0.5 N $NaHCO_3$)	Available –N (2M KCl)
dSm^{-1}		gkg^{-1}	$Cmol(+).kg^{-1}$	———	gkg^{-1}	———		gkg^{-1}	———	$mgkg^{-1}$	———
0.82±0.06	7.82±0.08	18.25±0.14	13.95±0.28	100.00±8.00	84.35±0.65	815.65±6.16	S.L	8.00±1.90	55.00±2.10	3.19±0.11	39.00±1.06

Means of three samples ± SD.
† O.M: organic matter
¶ S.L: santy loam

Application of 15 and 20 mg Ni kg^{-1} soil decreased the DM yield of wheat both in the absence and presence of added N to soil as compared to 10 mg Ni kg^{-1} soil. The increase in yield may be attributed to the stimulatory effect of a low concentration of Ni on soil urease activity, releasing more ammonium from urea hydrolysis for absorption by plant roots. Polacco (1977) reported growth promotion in soybean tissue cultures on urea as the sole nitrogen source by low levels of nickel sulfate and concluded that Ni was essential for converting urea directly utilizable inorganic ammonia. The magnitude of reduction in DM yield with a 15 mg Ni kg $^{-1}$as compared to a 10 mg Ni kg^{-1}soil treatment increased from 1.66 to 2.76 g.pot^{-1} with increasing concentrations of applied N to soil from 0 to 30 mg N kg^{-1} soil, then it was decreased to 1.76 at 120 mg N kg^{-1} soil. The DM yield reduction amounted to 29.00% at 0, 34.00 % at 30, 34.00% at 60, and 18.00 % at 120 mg N added kg $^{-1}$soil. These results showed that the increasing supply of N at 120 mg N added kg $^{-1}$soil minimized the Ni toxicity. The depressive effect of high Ni supply on DM yield could be attributed to other factors such as suppression of root growth (Robertson, 1985; Gajwska and Sktodowska, 2009), reduction of cholorophyll and increased amounts of protocholoriphyll (Anderson *et al* .,1979) , imbalance in plant nutrition (Catalado *et al* ., 1978) , reduction in photosynthesis and enzymatic activity (Sheoran *et al* 1990), or a combination of these effects .

The polynomial quadratic model was used to describe the relationship between dry matter yield and Ni concentration in soil treated with different rates of nitrogen. The method of the least squares using the experimental results of each pot was used to calculate the values of B_0, B_1 and B_2 in the polynomial model. Thus 4 polynomial quadratic models were established to express the relationship between the dry matter yield of wheat plants and Ni concentration in all N-treated soils. The four models were:

$$Y_{N0} = -2.702 + 4.903\ X - 0.765\ X^2,\ R^2 = 0.92,\ SE = 0.113$$

$$Y_{N30} = -0.122 + 5.091\ X - 0.880\ X^2,\ R^2 = 0.88,\ SE = 0.187$$

$$Y_{N60} = 1.684 + 4.997\ X - 0.905\ X^2,\ R^2 = 0.92,\ SE = 0.095$$

$$Y_{N120} = 1.558 + 5.604\ X - 0.987\ X^2,\ R^2 = 0.99,\ SE = 0.092$$

The calculated DM values were close to the experimental DM values as shown from the values of standard error of estimates (SE) and determination coefficient (R^2).

The efficiency of Ni treatments (Ex_i) at different increment (X_i) was calculated (Thabet and Balba, 1994). The effect of Ni rates addition on efficiencies (Ex_i) of dry matter yield of wheat plants grown on N-treated soils is shown in Table (2). In general the efficiencies decrease with increasing the increments addition of Ni in all N-treated soils (Table 2), and the efficiencies of Ni were positive at the first two increments and negative at the third and fourth increments.

Table 2: Effect of Nickel treatments on efficiencies (Ex_i) of dry matter yield of wheat plants grown on soils treated with different rates of nitrogen fertilizer

No of Ni Units	**Ex_i**			
	N treatment, mg.kg^{-1}			
	0	30	60	120
1	3.37	3.33	3.19	3.63
2	1.84	1.57	1.38	1.66
3	- 0.31	- 0.19	- .043	- 0.32
4	- 1.22	- 1.95	- 2.24	-2.29

Ex_i is calculated from polynomial quadratic model (Thabet and Balba, 1994) and its unit is (g dry matter)/ (mg Ni.kg^{-1} soil).One unit of Ni= 5 mg.kg^{-1} soil.

The proceeding discussion indicated that the polynomial quadratic model accurately described the relationship between the above-ground dry matter yield of wheat plants grown on N-treated soils with different rates and application rates of Ni.

Nitrogen Concentrations and Uptake

The concentration and uptake of N in wheat tissues increased considerably ($P \leq 0.05$) with increasing concentrations of N in soil, irrespective of added Ni to soil (Figure 2 and Table 3). This increase was primarily due to an increased concentration of N in soil. At all N rates applied to soil, the concentration of N in plant tissues significantly increased ($P \leq 0.05$) with an increasing supply of Ni in soil up to 10 mg Ni kg^{-1} soil, but it decreased at 15 and 20 mg Ni kg^{-1} soil treatments. This suggested that low amounts of Ni may be beneficial in N absorption by plants as reported by Brown *et al.*,(1990) ;Krogmeier *et al.*, (1991); Gajewska and Sklodowska, (2007). The magnitude of increase in tissue N content was kept on decreasing with the increasing levels of Ni supply in soil, whereas the magnitude of decrease at higher Ni levels (>10 mg Ni kg^{-1} soil) showed a reverse trend. At 10 mg Ni kg^{-1} soil concentration, the increase in tissue N concentration over control was 64 %. At low amount of Ni in the nutrient solution increased the intake of N by increasing urease activity (Eskew *et al.*, 1983; Palacious *et al.*, 1998; Gajewska and Sklodowska, 2007) and thus prevented the accumulation of urea to toxic levels. On the contrary, a high amount of Ni in soil may disturb the

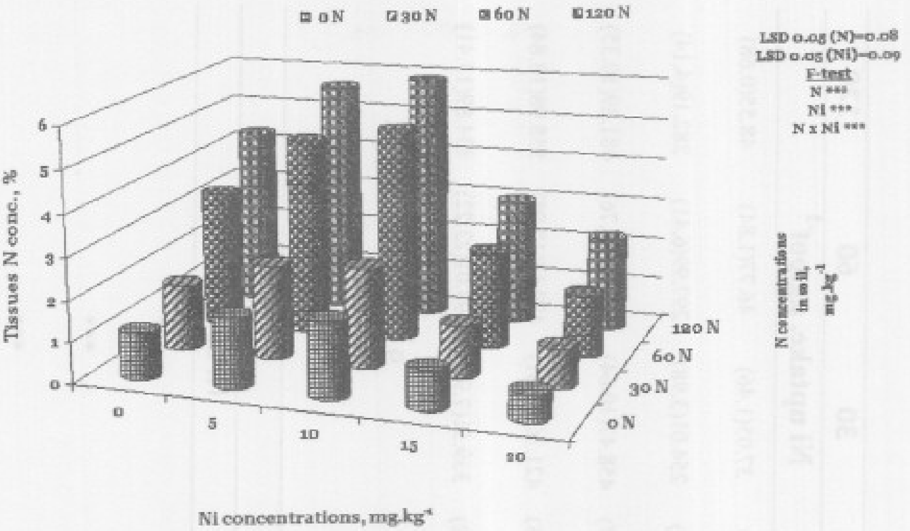

Fig.2: Effect of soil-applied N and Ni on the tissues concentrations of N in wheat plants

pattern of nutrient uptake (Knight and Crooke, 1956; Khalid and Tinsley, 1980; Pandey and Sharma 2002; Parida *et al.,* 2003). The mean N uptake by wheat at 120 mgkg^{-1}-treated soils decreased from 575.24 mg.pot^{-1} in the presence of 10 mg Ni kg^{-1} soil to 113.18 mg.pot^{-1} in the presence of 20 mg Ni kg^{-1} soil and this amounted 80% decrease. This decrease in N uptake could be due to toxic effects of the high amounts of soil applied Ni on root cells resulting in impaired N absorption process. The interaction effects of N and Ni rates on tissues N concentrations and N uptake were generally significant ($P \leq 0.001$) (Figure 2 and Table 3). The highly significant interaction between N and Ni concentrations points out that the tissues N concentrations and uptake depended on kind of element added and rate of application.

Nickel Concentrations and Uptake

The concentrations of Ni in wheat tissues increased considerably ($P \leq 0.05$) with the addition of Ni in soil but decreased noticeably ($P \leq 0.05$) with the increasing concentrations of N in soil (Figure 3). A significant N x Ni interaction at $P \leq 0.001$ on tissue Ni concentration indicated that the application of Ni to soil resulted in the accumulation of increased amounts of Ni in wheat tissue in treatments where no N was applied (Figure 3 and Table 3). The highest Ni concentration in wheat tissue was 102 mgkg^{-1} of DM with the application of 20 mg Ni kg^{-1} soil. This increase in tissue Ni concentration was primarily attributed to increased Ni supply in the soil. The depressive effect of increased N supply in soil on tissue Ni was significant ($P \leq 0.05$) at all Ni concentrations in soil. This reduction in tissue Ni concentration with an increasing N supply in soil may be due to Ni utilization during metabolism of N in plants (Dixon *et al.,* 1975; Parida *et al.,* 2003) and increased DM yield of wheat.

The uptake of Ni by wheat was significantly increased ($P \leq 0.05$) with applied Ni and N in soil (Table 3). The magnitude of the increase in Ni uptake by wheat due to additions of Ni to soil was more in the presence than in the absence of applied N (N x Ni interaction significant at $P \leq 0.001$; Table 3). The maximum uptake of Ni (434.88 µg.pot^{-1}) was at 120 mg N kg^{-1} along with a 20 mg Ni kg^{-1} soil treatment combination. The increase in Ni uptake with the concurrent decrease in tissue Ni concentration with increasing N supply in soil is attributed to the dilution effect of the high DM yield. These results were agreed with the results of Dahiya *et al.,* (1993); Palacious *et al.,* (1998); Gajewska and Sklodowska, (2007).

CONCLUSIONS

The results of this experiment clearly demonstrated that high concentrations of Ni added to the soil had negative effects on the above-ground dry matter yield, concentration and uptake of nitrogen by wheat plants. The polynomial quadratic model accurately described the relationship between the above-ground dry matter yield of wheat plants grown on N-treated soils with different rates and application rates of Ni. The highly significant interaction between N and Ni concentrations points out that the tissues N concentrations and uptake depended on kind of element added and rate of application. A significant N x Ni interaction at $P \leq 0.001$ on tissue Ni concentration indicated that the

Table 3: Effect of soil-applied nitrogen and nickel uptake by wheat plants

Ni concentrations, mg.kg^{-1}	N treatment, mg.kg^{-1}							
	0	30	60	120	0	30	60	120
	N uptake, mg.pot^{-1}				Ni uptake, µg.pot^{-1}			
0	15.23(1.64)	65.83(6.82)	185.78(12.62)	266.53(10.49)	13.87(1.73)	37.05(1.46)	46.77(1.84)	48.55(0.68)
5	69.04(3.12)	144.14(14.29)	386.74(17.62)	496.38(36.77)	178.52(1.53)	254.01(3.98)	297.99(6.41)	282.19(4.14)
10	105.04(2.48)	196.34(6.69)	467.45(12.23)	575.24(12.39)	361.05(16.37)	458.47(10.64)	483.27(16.76)	481.89(10.35)
15	37.83(3.01)	67.16(3.54)	149.50(1.03)	240.20(4.35)	328.05(21.85)	421.30(14.81)	479.15(17.98)	558.98(18.84)
20	19.36(2.88)	33.95(2.27)	67.03(4.16)	113.18(3.32)	299.67(11.12)	339.51(7.99)	393.01(23.22)	434.89(13.41)
$LSD_{0.05}$ (N)	8.45				9.23			
$LSD_{0.05}$ (Ni)	9.45				10.33			

ANOVA	F-test	
	N uptake	Ni uptake
Nitrogen(N)	***	***
Nickel(Ni)	***	***
N x Ni	***	***

*** Significant at the 0.001 probability level.
Data are the average (n=4).
Numbers in parenthesis indicate the standard deviation.

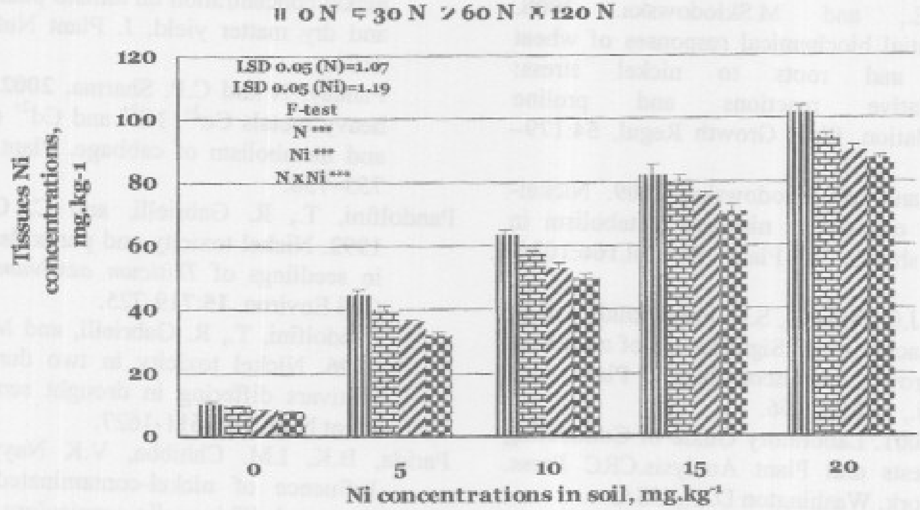

Fig. 3: Effect of soil-applied N and Ni on the tissues concentrations of Ni in wheat plants.

application of Ni to soil resulted in the accumulation of increased amounts of Ni in wheat tissue in treatments where no N was applied. The increase in Ni uptake with the concurrent decrease in tissue Ni concentration with increasing N supply in soil is attributed to the high DM yield.

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الملخص العربي

أثر النيكل على حدوث تغيرات فى إمتصاص النيتروجين بواسطة نباتات القمح النامية فى أرض لومية رملية

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أجريت هذة الدراسة فى الصوبة الزراعية بهدف إستكشاف تأثير عنصر النيكل المضاف للأرض بتركيزات مختلفة (صفر،٥ ،١٠، ١٥ ، ٢٠ مجم/كجم أرض) على محصول المادة الجافة لنبات القمح (صنف جميزة ٧) النامى فى أراضى معاملة بتركيزات مختلفة من عنصر النيتروجين (صفر، ٣٠ ،٦٠، ١٢٠ مجم/كجم أرض) وكذلك وصف العلاقة بين تركيزات النيكل و المادة الجافة و لدراسة إستخدام النيتروجين بواسطة نبات القمح . أوضحت نتائج الدراسة أن التركيزات العالية من عنصر النيكل (١٥ و ٢٠ مجم/كجم أرض) المضافة للأراضى المعاملة بالنيتروجين لها تأثير سلبى على محصول المادة الجافة وعلى تركيز النتروجين فى الأنسجة و كذلك إمتصاصة بواسطة النبات بينما إضافة التركيزات المنخفضة من النيكل (٥ و ١٠ مجم/كجم أرض) كانت لها تأثير إيجابى عليهم. و تم وصف العلاقة بنجاح بين تركيزات النيكل المضافة و محصول المادة الجافة للقمح عند التركيزات المختلفة من النيتروجين بواسطة معادلة الدرجة الثانية كما يتضح من قيم معامل التقدير(R^2) و الخطأ القياسى للتقدير(SE). و أشارت الدراسة أيضا الى أن تأثير التداخل بين عنصرى النيتروجين و النيكل على تركيز النيتروجين و إمتصاصة بواسطة نباتات القمح يعتمد على نوع العنصر المضاف و كذلك معدل إضافتة. و تبين من الدراسة أن الزيادة التى حدثت فى إمتصاص النيكل بالرغم من إنخفاض تركيزه فى أنسجة النبات و ذلك مع زيادة تركيز النيتروجين كان سببها وجود زيادة فى محصول المادة الجافة.