

Effects of nickel on growth and the reproductive organs of *Vicia faba* plants

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Abstract. High concentration of nickel (Ni) could provoke numerous toxic effects in plant tissues. The present study was undertaken to determine the effects of nickel (Ni) treatment on agronomic and production parameters of bean plants (*Vicia faba*). For that, plants were treated with four increasing concentrations of Ni: control: 0 mg/kg, C1: 150 mg/kg, C2: 250 mg/kg, and C3: 500 mg/kg. The effects of these Ni concentrations on growth, dry matter, pollen germination and viability, flower number and yield per plant were determined in bean plants. Our data demonstrated that Ni caused threats to plant growth and development. Also, our results showed a substantial reduction of pollen germination and viability in different concentrations of Ni loads. Furthermore, a clear negative effect of nickel was observed in fruit weight and seed set. Our study must be carefully considered in view of soil contamination and its subsequence effect on crop production.

Keywords: Nickel; *Vicia faba*; Pollen germination; Pollen viability; Production parameters.

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Introduction

Heavy metal contamination is becoming a widespread environmental issue because they are persistent in nature (Järup, 2003) and their concentrations are being increased consistently by natural and anthropogenic sources including the use of fertilizers, pesticides, agricultural runoff, streams from mines smelters and industries (Shikazono et al., 2008; Jiang 2016; Hasanuzzaman et al., 2018). Nowadays, agricultural soils are increasingly contaminated by heavy metals (Hasanuzzaman and Fujita, 2013). These metals include essential elements such as copper (Cu), manganese (Mn), zinc (Zn), Ni (Nickel) and non-essential elements such as cadmium (Cd), lead (Pb), and mercury (Hg) (Wang and Shi, 2001; Bradl, 2002).

Nickel is a metal that plays an important role in the cellular physiology of some eukaryotes and prokaryotes (Poonkothai and Vijayavathi, 2012). In fact, low concentrations of nickel (0.01 to 10 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight), are necessary in nitrogen metabolism (Fabiano et al., 2015) and plants germination (Dalton et al., 1985; Brown et al., 1990). However, during the last decades, nickel (Ni) has become a serious concern as its concentration has reached up to 1.933 million tons in polluted soils (INSG, 2015). In addition, atmospheric emissions of nickel from natural sources are approximately 8.10^6 kg/year (Schmidt and Michel, 1980; Alloway, 1995). The main sources of nickel pollution are mining activities, disposal of Ni-Cd batteries, burning of diesel oil and metal industry (Nnorom and Osibanjo, 2009; Wuana and Okieimen, 2011; Wang et al., 2015). Toxic concentrations of Ni in soil could induce alterations of plant metabolism consequently leading to the inhibition of growth (Awasthi and Sinha, 2013), and other common symptoms such as chlorosis, necrosis and wilting (Madhava Rao and Sresty, 2000; Baligarx, 2012). Also, it causes disturbances on several physiological processes like photosynthesis (Tripathy et al., 1981; Hasanuzaman et al., 2018), mineral nutrition (Atta-Aly, 1999; Parida et al., 2003) and carbohydrate transport (Chen et al., 2009). Soil contamination with Ni has become a worldwide problem, leading to losses in agricultural yield and production according to various mechanisms (Guo and Marschner, 1995; Salt et al., 1995; Gajewska et al., 2006).

On the other hand, literature showed that pollen germination is increasingly used in the sensitive botanical indicators of metal pollution (Wolters and Martens, 1987). In fact, DalCorso (2012) showed that heavy metals could affect pollen germination and tube growth even at low concentrations. These metals could be released into the atmosphere through vaporization and affect pollen during pollination. Indeed, González et al. (2006) demonstrated that the performance of pollen could highly influence fruit size and quality.

The present study aimed to study the effect of Ni on agronomic and reproductive parameters in bean plants. Also, we investigate their effect *in vitro* pollen performance and on the subsequent reproductive output evaluated in fruit and seed set.

Material and methods

Plant material

Plants were grown in plastic pots (1 plant/pot) filled with 750 g soil and 100 mg gravel for 90 days (February, March and April). The soil was recovered from a biological culture in the Higher Institute of Agronomy of Chott Meriem, Tunisia. A first set of 10 control plants were maintained under optimum growing conditions not supplemented with Ni. A second set of plants was supplemented with C1 (150 mg/kg), C2 (250 mg/kg) and C3 (500 mg/kg) of Ni (Kamran et al., 2016) (10 plants for each treatment). Flowers from control plants, and Ni-treated plants were collected after 45 days of exposure for testing *in vitro* pollen germination and viability (5 flowers/plant). Samples of shoots and roots were taken after 90 days and used for analysis.

Estimation of seedling growth (cm) and dry matter

After 90 days of culture, each plant was harvested individually. The length of the shoots and the roots of the bean plants were measured immediately and the dry weight (DW) were measured after oven-drying of the plant material for 48 h at 105 °C.

The number of flower

The number of flowers was counted throughout the culture and the averages were indicated.

***In vitro* pollen germination assay**

This manipulation aimed to characterize the percentage of pollen germination from each treatment. The most suitable floral stages for evaluating the effect of nickel are the closed flower stages from plants aged 45 days. Anthers of each flower were removed and left to dry for 2 days at 25 °C. After that, germination was performed in polystyrene Petri dishes (35 mm × 10 mm) by scattering pollen on a solidified germination medium consisting of distilled water (1000 mL), agar (10 g), sucrose (100 g) and boric acid (0.01 g) (Xiong and Peng, 2001). In fact, 5 Petri dishes are prepared for each concentration. Then, they were incubated at 25 °C for 24 h. According to Visser (1955), pollen was scored as germinated when the length of the pollen tube exceeded the diameter of its pollen grain. Observations are made under the microscope 'Olympus Vanox' equipped with a photographic device.

Study of the pollen viability

In this microscopic preparation, a Stanley and Linskens reagent was used. It was possible to obtain a staining which overestimated fertility by considering fertile pollen grains those well colored red and those not colored, sterile (Colas and Mercier, 2000). In fact the acetic carmine reveals the presence of the genetic material and the potassium iodide which colors the starch, substance used in the first reactions of germination.

The yield of plants (g)

After 90 days, we evaluate the effect of nickel treatments on fruit number per plant, fresh fruit weight and seed number/fruit. The yield of each treatment was calculated as fruit number per plant × weight (g).

Statistical analysis

Statistical analysis was performed during SPSS for Windows Soft ware (Version 19.0). Analysis of variance test (ANOVA) was employed to test the significance of treatment effects. Values were considered statistically significant when $p < 0.005$.

Results

Growth of plants

The effect of Ni treatments on root and shoot growth of *Vicia faba* after 90 days are presented in Figure 1. Results presented a significant decrease in shoot length for C1, C2 and C3 with values respectively 62.14 ± 2.6 cm, 50 ± 4.54 cm and 40 ± 3.28 against a control value of 80.66 ± 3.58 cm (Fig 1a). Moreover, Fig 1b showed a significant decrease in root length with the increasing of Ni concentration. Indeed, means reached 47.16 ± 1.5 cm; 44.75 ± 2.28 cm and 39 ± 3.28 cm respectively for C1, C2 and C3 against a value of 54 ± 3.1 cm for the control plant.

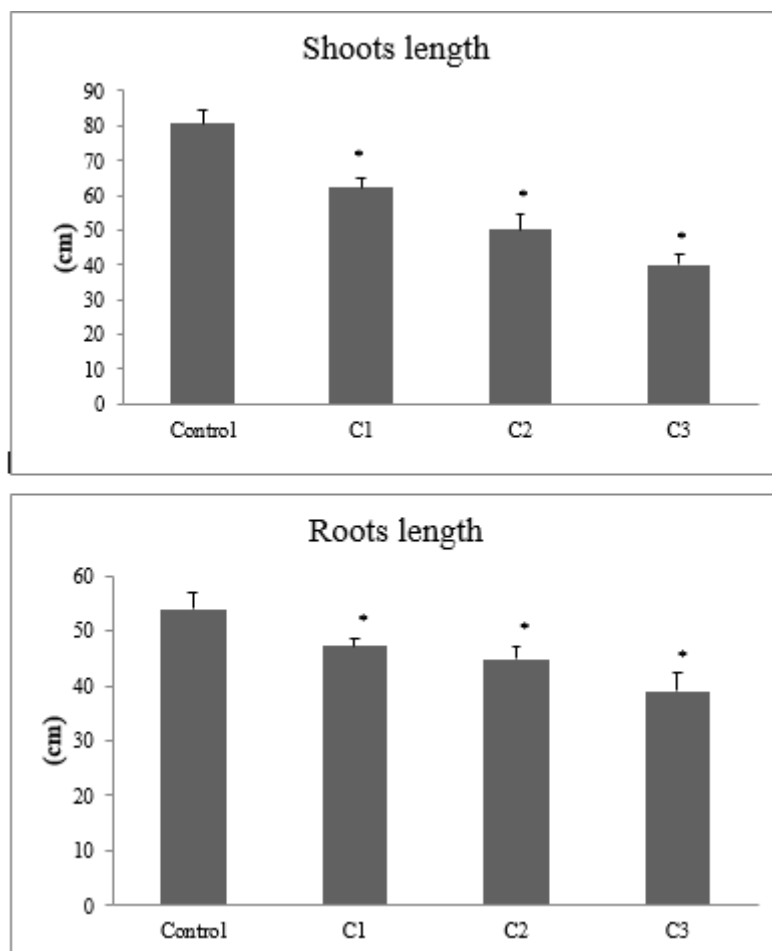


Figure 1. Effect of Ni on bean's roots and shootslengths (cm) after 90 days of culture exposure to: control (0 mg/kg), C1 (150 mg/kg), C2 (250 mg/kg) and C3 (500 mg/kg). *: significant difference in comparison to control.

Dry matter

The results of Figure 2a showed a significant decrease in shoot dry matter for C1, C2 and C3 concentrations with values $35.2 \pm 1.2\%$, $28.1 \pm 1.35\%$ and $24.1 \pm 2.63\%$, respectively, against a control value $48.23 \pm 1.2\%$. Also, Figure 2b showed a significant decrease in root dry after Ni contamination with C1, C2 and C3 where means reached respectively, $30.2 \pm 2.21\%$; $25.1 \pm 3.92\%$ and $23.7 \pm 2.45\%$ against a control value $39 \pm 1.18\%$.

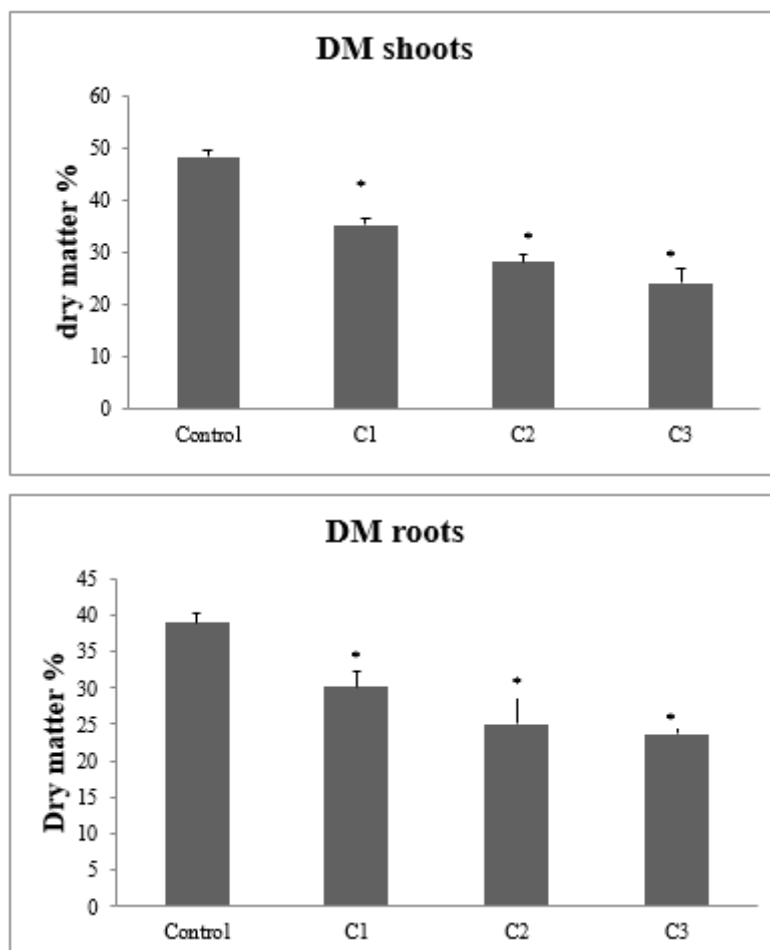


Figure 2. Effect of Ni on bean's shoots and roots dry matter (DM) after 90 days of exposure to control (0 mg/kg), C1 (150 mg/kg), C2 (250 mg/kg) and C3 (500 mg/kg). *: significant difference in comparison to control.

The number of flowers

The number of flower decreased with the different Ni concentrations. The lowest number was noted in plants contaminated with the highest concentration C3 (50 mg/kg) with value of 21 ± 1 against a control value 50 ± 1.1 (Figure 3).

***In vitro* pollen germination assay**

The *in vitro* pollen performance expressed as the percentage of germinated pollen in presence of the various nickel concentrations are represented in Figure 4. A significant decrease of the percentage of germinated pollen was observed at the three nickel concentrations C1, C2 and C3 with average values of $27.5\% \pm 0.807$, $20.5\% \pm 1.707$ and $17.5\% \pm 0.707$ respectively, compared to the control ($78 \pm 2,12\%$).

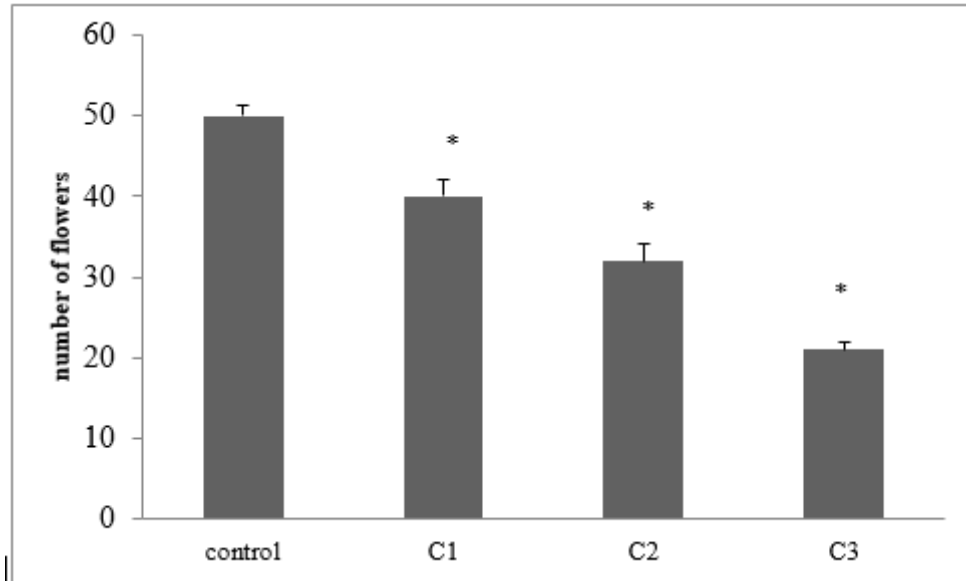


Figure 3. Effects of different nickel treatments on the number of flowers per plant compared to: control (0 mg/kg), C1 (150 mg/kg), C2 (250 mg/kg) and C3 (500 mg/kg). *: significant difference in comparison to control.

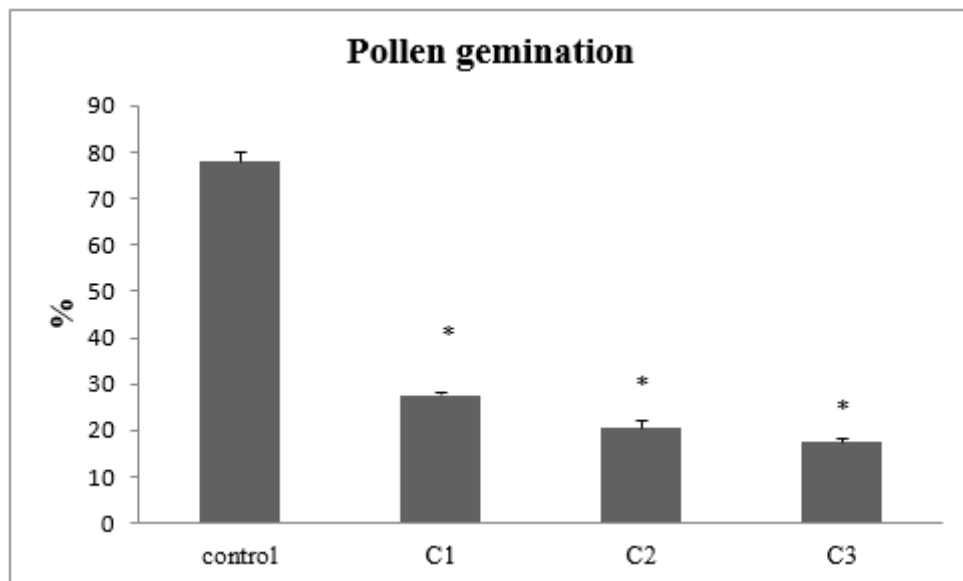


Figure 4. Effects of different nickel treatments on pollen germination *in vitro* compared to: Control (0 mg/kg), C1 (150 mg/kg), C2 (250 mg/kg) and C3 (500 mg/kg). *: significant difference in comparison to control.

Pollen viability assay

The percentage of pollen Viability showed a significant decrease with the concentrations C1, C2 and C3 with values, respectively, $92.5 \pm 0.7\%$; $89.5 \pm 0.93\%$ and $86.5 \pm 2.12\%$ respectively against the control ($97.5 \pm 1.06\%$) (Figure 5).

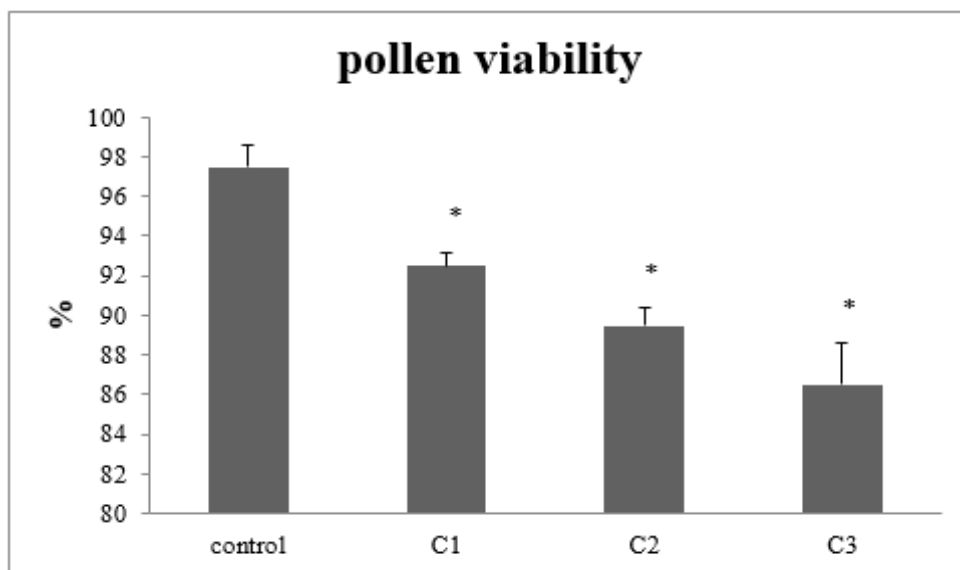


Figure 5. Effects of different nickel treatments on pollen Viability *in vitro* compared to: Control (0mg/kg), C1 (150mg/kg), C2 (250mg/kg) and C3 (500mg/kg)*: Significant difference in comparison to control.

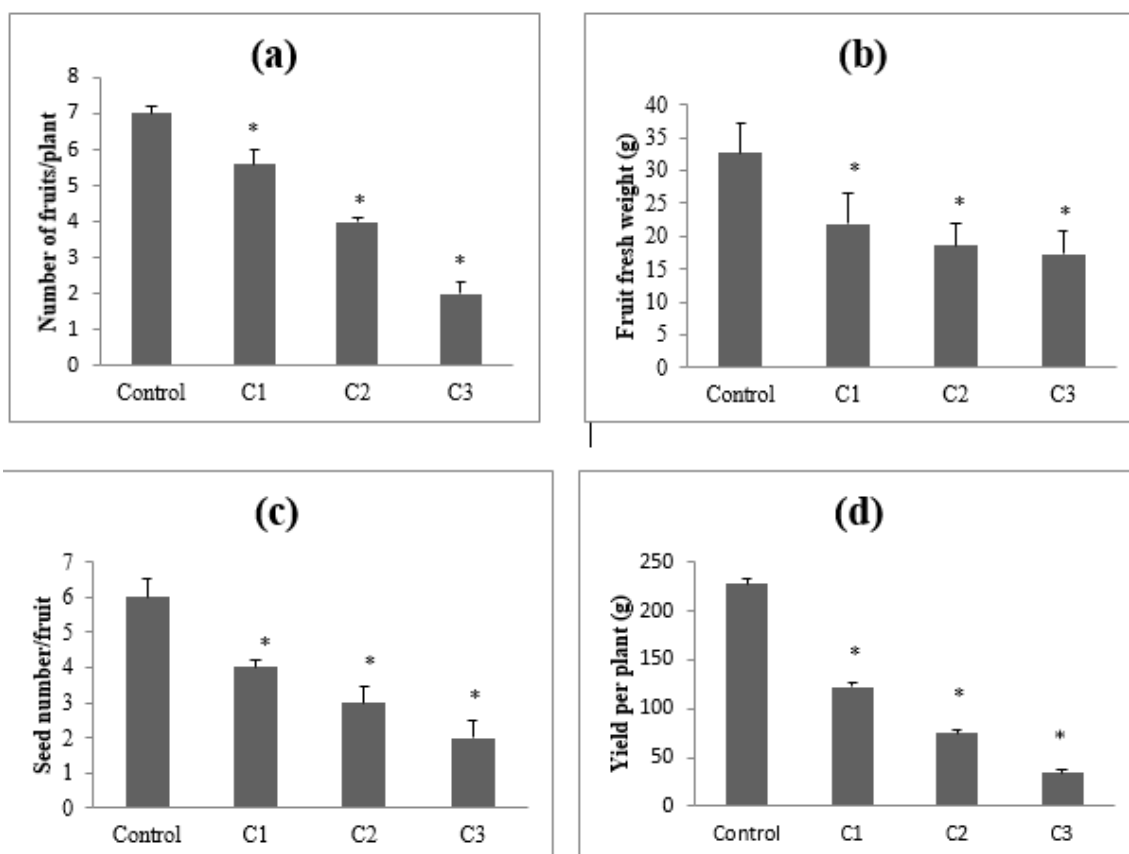


Figure 6. Effects of different nickel treatments (Control: 0mg/kg, C1: 150 mg/kg, C2: 250 mg/kg and C3: 500 mg/kg) on the number of fruits/plant (a), fruit fresh weight (g) (b), seed number/fruit (c) and Yield per plant (g) (d) (in comparison with the control. *: significant difference from in comparison to control.

The yield of plants

The effect of nickel exposure on the yield of plants was reported by their effects on the number of fruits/plant, fruit weight (g), seed number/fruit, and total yield/plant (Figure 6). Our results indicated a significant decrease in the number of fruits/plant, with average reduced to 5.6 ± 0.4 ; 4 ± 0.1 g and 2 ± 0.3 under the different Ni concentration C1, C2 and C3 respectively, compared to 7 ± 0.2 of fruits number from control plants (Figure 6a). Moreover, a significant decrease of the fruit weight (g) was noted with the different Ni concentrations C1, C2 and C3 with values of 21.9 ± 4.5 g; 18.6 ± 4.5 g and 17.2 ± 3.52 g respectively compared to the control value of $(32.6 \pm 4.42$ g) (Figure 6b). Then, the application of the different nickel concentrations (C1, C2 and C3) significantly decreased the number of seeds per fruit (4 ± 0.23 seeds per fruit), (3 ± 0.47 seeds per fruit) and (2 ± 0.51 seeds per fruit), respectively, when compared to the control (6 ± 0.51 seeds per fruit) (Figure 6c). Finally, the average yield per plant decreased significantly after 90 days of culture in soils contaminated with C1, C2 and C3 where values reached respectively 122.64 ± 4.5 g, 74.4 ± 3.43 g and 34.4 ± 3.52 g against a control value 228.2 ± 4.42 g (Figure 6d).

Discussion

Growth reduction is one of the most frequent and first responses when plants are under Ni contamination (Nakazawa et al., 2004). This is in accordance with the results obtained in this study with the different nickel concentration. Similar results were observed with other species such as *Oryza sativa* (Rizwan et al., 2018; Maheshwari and Dubey, 2007), *Triticum aestivum* (Gajewska et al., 2009; Siddiqui et al., 2011), *Glycine max* (Sirhindi et al., 2016) and *Zea mays L.* (Seregin et al. 2003). The growth inhibition induced by nickel could be related to the reduction of water uptake caused by Ni ions (Chen et al., 2009) and could be associated with the altered normal activity of different enzymes and availability of carbohydrates (Gajewska and Skłodowska, 2009).

Indeed, a significant reduction in dry matter was found with the different concentrations of nickel in shoots and roots. Similarly, a decrease was observed in the dry matter of *Solanum lycopersicum L.* treated with 5 mg/kg of nickel (Palacios et al., 1998). Also, Parida et al. (2003) indicated that the dry weight of *Trigonella foenum-graecum L.* was affected at the concentration of 40 mg/kg of nickel. This reduction in dry matter could be attributed to the interference of Ni with the metabolic and biochemical processes, such as protein and chlorophyll synthesis (Tripathy et al., 1981; Weis and Weis, 2004). Moreover, Bai et al. (2006) proved that high nickel concentrations had an effect disruption in amino acid distribution.

In addition, Gajewska et al. (2006) demonstrated that physiological changes could affect the reproduction of plants, according to various mechanisms. In fact, our results indicate a significant effect of nickel on the number of flower per plant. Ryser (2006) showed that sexual organs could be affected directly by heavy metals. Moreover, Ryser and Sauder, (2006) observed delays and inhibitions of flowering in *Hieracium piloselloids* growing on a soil contaminated with nickel. Furthermore, heavy metals could induce reduction in the size of flowers (Gill et al., 2015).

In the other hand, several authors have suggested the use of pollen as biological indicators (Feder, 1981). In general, pollen germination depends on two factors, the metal content of the soil and its translocation to the flowers. In this study, the analysis of nickel toxicity reveals the negative effects on pollen germination and viability. Similarly, Shivanna (2003) showed that heavy metals affect the extension of pollen tubes by disrupting the development of the cell membrane. Then, Mohanty et al. (2004) explain the high proportion of pollen infertility in *Oryza sativa* exposed to heavy metals by a high frequency of chromosomal aberrations. Also, Yousefi et al. (2011) showed that metal

contamination could reduce ovule and seed viability. In this work, we found that yield production is greatly reduced for the different Ni concentrations. Similar results were observed with other species like *Geranium carolinianum*, *Lepidium virginicum* (DuBay and Murdy, 1983) and *Solanum lycopersicum* (Balaguer et al., 1993). In fact, this reduction is mainly caused by a decrease in the viability of pollen grain. According to Ferraz et al. (2012) nickel could negatively affect plant metabolism and functioning. As well as, nickel contamination could influence the number of fruits through reducing cell division and cell development (Seregin and Kozhevnikova, 2008).

Conclusion

In summary, our results indicate that nickel negatively affects the growth of bean plants by disrupting numerous physiological mechanisms. Pollen germination and viability are relatively sensitive to nickel. These parameters were inhibited by different nickel concentrations, thus possibly resulting in failures in reproduction. The present study showed also that pollen germination in bean plants can provide a useful tool to assess the biological effect of heavy metal contamination.

Conflict of interest

Authors declare that there are no conflict of interest.

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