

Effect of Cd and Pb on germination and early seedling growth of black seed (*Nigella sativa* L.)

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Abstract

The effects of cadmium and lead and their interaction on germination behavior and early seedling growth stage of the medicinal plant (*Nigella sativa* L.) were investigated. Seeds were grown in Petri dishes (9 cm diameter) in a complete randomized design with three replicates for each treatment. The heavy metals were Cd (100 and 300 mg/L), Pb (600 and 1500 mg/L) and all possible combinations. Results showed that final germination percentage and root length were greatly reduced at all heavy metals treatment. Reduction was markedly higher at higher concentrations. Root growth (length and fresh weight) was relatively higher than shoot growth at control. Dry weight showed another reduction with the increase in heavy metals concentrations. Cadmium was more toxic than lead where no growth measurements were recorded for seedlings treated with the high concentration of Cd whether applied alone or combined with Pb. The synergistic effect of Cd and Pb was stronger than their effects as single metals. The synergistic and antagonistic interactions of both heavy metals were discussed

Key words: *Nigella sativa* L., heavy metals, germination, early seedling growth

Introduction:

Toxicity with heavy metals is a problem which has been seriously increased with modern civilization through different anthropogenic activities (23; 16). Most of heavy metals are essential for plants growth but when present in excess amounts, they become harmful not only for plants but for animals and human health as well (14; 26). They reach to plants from soil through roots or from the air through leaves and consequently to animals and human through the food chain (15; 8).

Cadmium (Cd) and lead (Pb) are two common highly toxic pollutants which are added to the environment through many sources such as automobile exhausts; industry; mining; using pesticides and fertilizers which contain heavy metals etc (7; 36; 5; 20). For many plant species, it has been demonstrated that Cd and Pb negatively affect a wide range of physiological processes such as photosynthesis, respiration and induce a wide range of phytotoxic effects such as seeds germination inhibition; chlorophyll aberration at very high concentrations; alternation of respiration metabolic pathway; reduction of the mitotic index in root cells; stimulation of some abnormalities in synthesis of DNA; RNA; in function of membranes by changing lipid composition and in nucleus structure (37; 10; 27; 43; 33) which finally inhibit plant growth, production and may cause death at very high concentrations.

Although, the effects of the individual heavy metals on plants have been evaluated by many studies (ex. 38; 24; 40), limited information is available on the effects of heavy metal combination on plant species. In fact, as most of heavy metals present together in an environment at the same time or on the same environment at different times, focusing on their combined effects on plants may become important (27; 16).

Nigella sativa L. (Ranunculaceae) seeds, commonly known as black seed or black cumin, have been traditionally employed for thousands of years as a spice, food preservative, and curative remedy for numerous disorders. Medically, black seed was described by Prophet Mohammad (peace and pleasant be up on him) as a remedy for every disease except death, and scientific works has proved that black seeds contain components of a broad spectrum medical advantages as anti-

Effect of Cd and P.....E. J. AL-Sanabani, F. A. Alhadi, K.M. Al-Amary, M. A. Hussain histaminic, anti-diabetic, anti-hypertensive, anti-inflammatory, antimicrobial activities, and anti-tumor and (17; 2; 3; 44; 1; 13).

It is commonly known that germination and early growth stages are important to determine plant survival and successful establishment (29; 8; 34), so the present study aims at to investigating the synergistic and antagonistic effects of lead and cadmium on that two important growth stages of *N. sativa* under laboratory conditions.

Materials and methods:

Seeds of *N. sativa* were purchased from a local market in the old Sana'a city and species was then identified by the taxonomist Dr. Hassan Ibrahim, the Herbarium of Faculty of Science, Sana'a University, Yemen. For surface sterilization, seeds were soaked in 5 % (v/v) sodium hypochlorite for 20 minutes then they were rinsed three times with distilled water (41). To induce seeds germination (primary experiment), seeds were hydro-primed by soaking in distilled water at laboratory temperature for 24hrs. in the darkness, rinsed once with distilled water and left between two layers of filter paper to dry. Different concentrations of Cd(NO₃)₂: (100 and 300 mg/L); Pb(NO₃)₂: (600 and 1500 mg/L) and all possible combinations: (100+600; 100+1500; 300+600 and 300+1500 mg/L) were prepared and used as a source of heavy metal stress. Distilled water was used as control.

Germination experiment was carried out in 9 cm (diameter) Petri dishes lined with filter paper which were witted with each heavy metal treatment. Thirty surface sterilized seeds were grown in each dish. Treatments were replicated three times each and dishes were then distributed using completely randomized design (CRD) and were kept in the darkness under laboratory conditions (23°C ± 2). Germination process was observed four times throughout the germination period (20 days) and germination criteria was the detected radicles (roots) grown to 2 mm at least. At the end of this experiment, the following measurements were taken: (1) final germination percentage (G%) with the formula adduced by (6):

$$G \% = \sum \frac{n_i}{N} \times 100$$

Where: n is the number of germinated seeds till ith day and N is the total number of seeds, (2) Inhibition percentage of germination (In. %) with this equation (18) :

$$\text{In. \%} = \left[1 - \frac{\text{Ger. \% in sample}}{\text{Ger. \% in control}} \right] \times 100$$

Where: Ger. % is germination percentage in sample and in control. (3) Length: Five seedlings (ten-days-old) were collected from each treatment and lengths of roots and shoots were recorded with millimetric ruler, (4) Fresh weight: Seedlings were dissected into shoots and roots and their fresh weights were recorded using sensitive balance, (5) Dry weight: The samples were then put in an oven at 75-80°C for 48hrs. to detect their dry weights, (6) The phytotoxicity percentage (Phytotoxicity %) for root and shoot lengths of seedlings was calculated using the following formula (9):

$$\text{Phytotoxicity \%} = \frac{\text{length in control} - \text{length in treatment}}{\text{length in control}} \times 100$$

(7) Seedling vigor index (SVI) was also calculated with this equation (32):

$$\text{SVI} = \frac{\text{Ger. \%} \times \text{mean (root length + shoot length)}}{100}$$

(8) Dry weight allocation was calculated using a formula after (4):

$$\text{Allocation} = \frac{\text{Dry weight of a plant part}}{\text{Total dry weight}}$$

The collected data were statistically analyzed with one-way ANOVA and other suitable statistical tests, using MINITAB for Windows, and all graphs were plotted using Microsoft Office Excel. Data in percentage was arcsine transformed before analyzing with ANOVA.

Results:

Germination traits:

The effect of different concentrations of Cd, Pb, and their interaction on final germination percentage of *Nigella sativa* was depicted in Figure 1. Results showed that germination percentage was significantly ($P < 0.05$) inhibited by the applied heavy metals treatments and the effect increased with the increase in heavy metal concentration. The highest percentage of germination was recorded in control (95.5%), while low values were recorded in the combination treatments of 300+600 and 300+1500 mg/L (Figure 1), which showed higher values of germination inhibition percentage (73.23% and 68.51%, respectively). Low inhibition percentages of germination were recorded at Pb 600 mg/L (6.89%) and the combination 600+100 mg/L (8.04%), where high percentages of final germination, were already obtained.

Velocity of germination of *N. sativa*, which was represented by days to 50% germination, also showed similar tendency where it progressively decreased with the increase in heavy metals concentrations. Seeds which were treated at control, Cd 100mg/L, Pb 600 mg/L, and 100+600 mg/L achieved more than 50% germination during the first five days of this experiment, while those seeds in the rest of treatments failed to reach 50% of germination till the end of the period of this experiment (Figure 2).

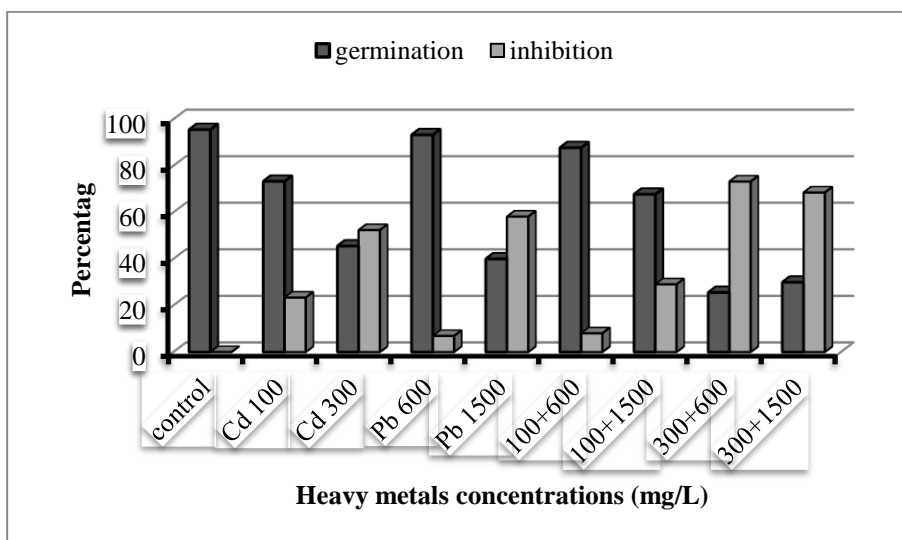


Figure 1: The effect of Cd, Pb and their combination on the percentages of final germination and inhibition of germination of *N. sativa* seeds.

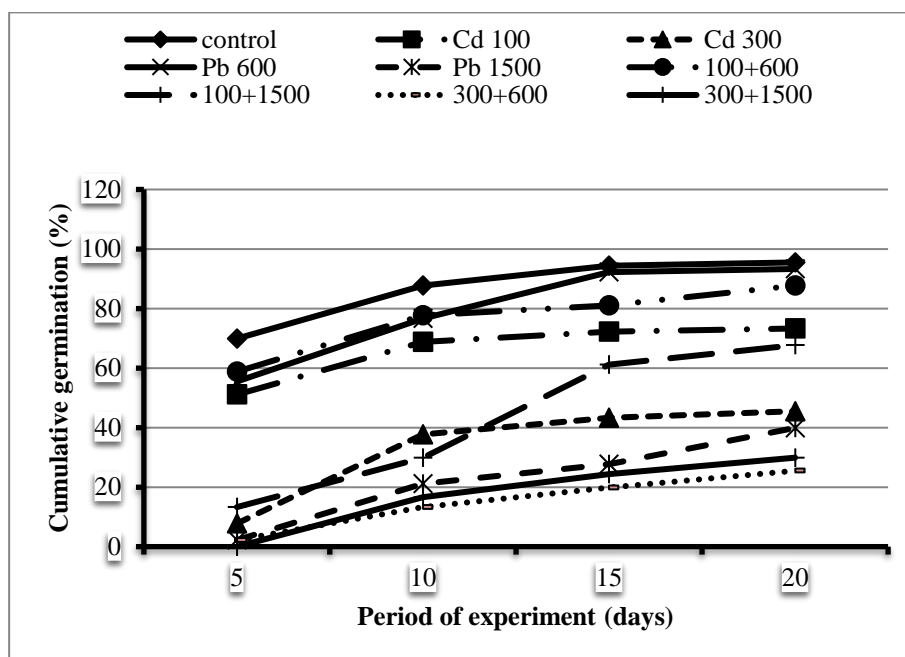


Figure 2: The cumulative germination percentage and days to 50% germination of *N. sativa* seeds which grown in different concentrations of Cd, Pb and their combination. The rectangle shape denotes treatments where seeds failed to achieve 50% germination till the end of germination period.

Early seedling growth measurements:

Root and shoot length:

A Number of early seedling growth measurements was recorded to evaluate heavy metal effect on early seedling growth stage. Results showed that, at the highest concentration of cadmium (300 mg/L), whether applied alone or combined with different concentrations of Pb, seeds could not grow well after root emergence which took a very long time to appear. In addition, roots were poorly developed (\approx 1mm in length) and the apexes of roots were brown. Most of shoots of seedling at those treatments were arrested in seeds coats till the end of the experiment (**Photo 1**) and, as a consequence, those treatments were excluded from the following results. In contrast, seedlings at Pb 1500 mg/L grew but most of them had abnormal cotyledons where they were unequal in size, and bigger than usual.

Roots and shoot lengths of *N. sativa* seedlings revealed high significant effect ($P < 0.05$) of the applied heavy metals treatments. Seedlings at control, Pb 600 mg/L and 100+600 mg/L , showed high values of root length, then it radically reduced as the concentration of heavy metals increased (Table 1). In contrast, low root length was obtained at Pb (1500 mg/L) and 100+1500 mg/L. The maximum reduction in root length, which was represented by high percentage of length phytotoxicity, was observed at those two concentrations, i.e., 1500 and 100+1500 mg/L, where it reached to 96.57% (Table 1). In contrast, the minimum length phytotoxicity (70.41%) was recorded at the combination of low concentrations of the two heavy metals (100+600 mg/L).

For shoot length, an increase over control (with no significant difference) was recorded at both Cd 100 mg/L and at its combination with the low Pb concentration (100+600 mg/L), while the lowest shoot length value, on the other hand, was recorded at Pb 1500 mg/L (Table 1). The maximum phytotoxicity of shoot length was 57.69% at Pb 1500 mg/L, while the minimum was - 31.54% at 100-600 mg/L. The present results also showed that, under control treatment, root length was much higher than that of shoot, but the situation was reversed under all other treatments where

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shoot length was the highest (Photo 1). In addition, the inhibitory effect was on root and shoot length but it was stronger on root length where it was reduced to 2.2 mm compared to 64.2 mm at control (Table 1).



Photo 1: Growth performance of *N. sativa* seedlings which were treated in concentrations of Cd, Pb and their combination

Fresh weight:

Statistical analysis of root fresh weight revealed high significant ($P < 0.05$) effect of heavy metals, treatments. The highest value of root fresh weight was found at control treatment, then it critically reduced as heavy metal concentrations increases, except at 100+600 mg/L which showed a humble and non significant improvement (Table 1). Shoot fresh weight showed a similar behavior where the effect of the applied heavy metals was significant ($P < 0.05$). In fact, the recorded values at all treatments, except at 1500 mg/L, were arranged between 15.4 ± 2.07 and 10.6 ± 3.85 mg/seedling and the difference between them was not significant ($P > 0.05$), so the source of significant difference is only the low value which was recorded at 1500 mg/L (Table 1).

Dry weight:

Results of this measurement indicated that neither root dry weight nor shoot dry weight showed significant effect ($P > 0.05$) of heavy metals treatments. The highest value of root dry weight was obtained only at control (1.3 mg/seedling), then it reduced as heavy metal concentration increase within a range from 0.8 to 0.6 mg/seedling (Table 1). Shoot dry weight showed 1.5 and 0.9 mg/seedling as maximum and minimum values with no significant difference (Table 1). Calculation of dry weight allocated to both root and shoot, showed similar trend where no significant effects of heavy metals on those measurements were found ($P > 0.05$ for both).

Table 1: Growth measurements of 21 day-old *N. sativa* seedlings exposed to different concentrations of Cd and Pb. Results were represented by the Mean of 5 samples ± SD

Root growth parameters					
Concentrations (mg/L)	Length (mm)	Phytotoxicity (%)	FW (mg/seedling)	DW (mg/seedling)	Allocation
Control	64.2±11	//	10.4±1.67	1.3±0.97	0.487±0.09
Cd 100	16±4.6	75.5±3.6	1.6±0.55	0.7±0.27	0.362±0.19
Pb 600	8.6±1.3	86.1±4.3	1.8±0.84	0.8±0.27	0.506±0.19
Pb 1500	2.2±0.4	96.5±0.86	1±0.00	0.6±0.22	0.373±0.13
100+600	19±8	69.8±12.7	3.4±2.61	0.7±0.27	0.433±0.09
100+1500	2.2±0.4	96.6±0.51	1±0.00	0.6±0.22	0.279±0.07
P value	0.000	0.000	0.000	0.188 ^{ns}	0.122 ^{ns}
Shoot growth parameters					
Control	26±6.1	///	12.6±2.79	1.3±0.67	0.513±0.09
Cd 100	32.2±3.3	-31.6±42.1	13.4±4.78	1.5±1.00	0.638±0.19
Pb 600	25.8±1.8	-4.5±28.8	15.4±2.07	1±0.61	0.527±0.17
Pb 1500	11±1.9	54.9±16.5	6.6±2.51	1.1±0.55	0.626±0.13
100+600	34.2±4.9	-41.2±53.4	14.2±2.78	0.9±0.22	0.566±0.09
100-1500	18.4±4.9	24.2±29.3	10.6±3.85	1.6±0.55	0.719±0.07
P value	0.000	0.005	0.004	0.465 ^{ns}	0.159 ^{ns}

ns= non significant

Seedling vigor index:

This measurement, in its turn, showed high significant difference between heavy metals treatments (P < 0.05). From Figure 3, it can be noticed that the highest value was recorded at control where the highest germination percentage and seedling length were obtained. In contrast, the lowest value was recorded for seedlings which were treated with the highest concentration of Pb, whether applied alone or combined with Cd, while the rest of treatments showed moderate effect.

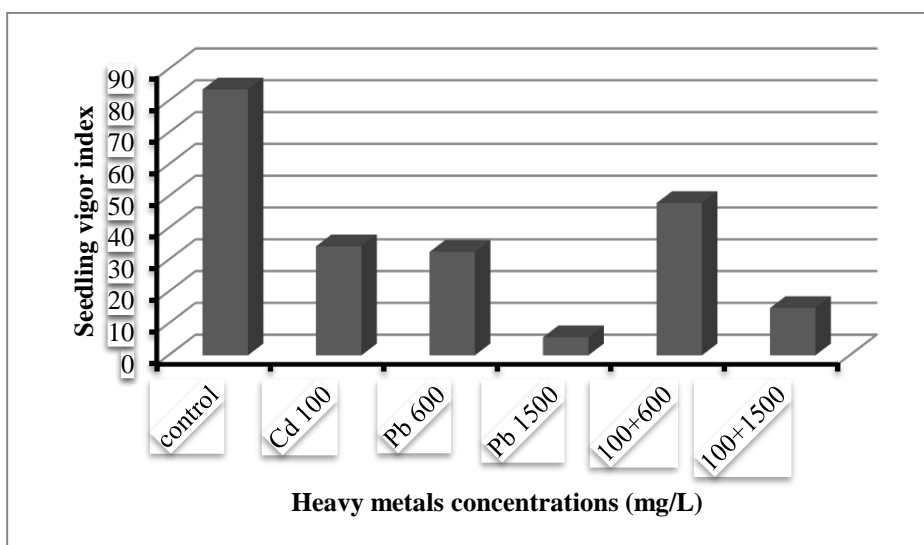


Figure 3: Effect of Cd, Pb and their combination on seedling vigor index of *N. sativa* seedlings.

Discussion:

The present study was confined to determine the effects of Cd(NO₃)₂ and Pb(NO₃)₂, applied as single or in combination, on germination behavior and early seedling growth traits of the medicinal plant *N. sativa*. Cadmium and lead clearly have inhibitory, and in some treatments toxic, effect on germination of seeds of black cumin (*N. sativa*). There was a continuous decrease in the final germination percentage with the increase of heavy metal concentrations whether applied alone or in combination. The present findings revealed low inhibition percentages of germination at low Pb concentration and at 100+600 mg/L which may indicate little or no harm effect (osmotically affected which cause low water uptake and transport) of those concentrations on seed viability.

It was evident that Cd at high concentration (300 mg/L) showed greater inhibitory, or may be toxic, effect on germination and early seedling growth measurements than Pb. Seedlings which were treated by that concentration of Cd, as single or combined treatments, could not grow well at all. Most of seeds at those treatments swelled, but no further growth features appeared till the end of the experiment. In fact, it has been reported that seedling growth and development is considered as being inhibited after imbibition if the seed coat was visibly broken, the root protruded from testa but the embryo growth was arrested beyond this point where no further growth of the embryo was noticed (31). Such observation could indicate that osmotic potential and water uptake were not negatively suppressed. Such drastic effects could be attributed to one or more than one reason such as (a) real harm effects on cell division and enlargements in the embryo, (b) an overall decrease in metabolic activity relevant to these steps Shaddad et.al.(38) and (c) the establishment of higher toxic effect syndrome (HTES) due to high accumulation of the metallic salts within the embryo tissues (24). The inhibition of germination caused by heavy metals has been reported by many workers on different plant species such as Shaddad et.al (38) on *Zea mays*, *Helianthus annuus*, and *Vicia faba*; Cavusoglu et.al. (8) on *Phaseolus vulgaris*; Patel et.al. (34) on *Phaseolus aureus* and Thakkar (42) on *Vigna sinensis*.

On the contrary to our findings, some studies revealed that some heavy metal treatments had no critical effect on germination percentage when compared with seedling growth stage as described by Li et.al.(31) on the model plant *Arabidopsis thaliana*; Jamal et.al. (25) on *Vigna radiata* and *Vigna sinensis* and Jamal et.al (26) on some cultivars of *Triticum aestivum*. In some other studies, such as Dukic et.al. (11) who worked on three tree species of *Ulmus pumila*, no significant effect of heavy metals was detected on seed germination and seedling growth stage. Such contrast results may emphasize the specialty of plant response to stress conditions which is affected by a number of factors such as plant itself (species, age and organs) and heavy metal salt (form, concentration and duration of exposure) (39; 28).

It was evident that root length of *N. sativa* seedlings was, significantly, and negatively affected by all the applied heavy metals treatments. This finding accorded to many authors who found strong inhibitory effect of Cd and / or Pb on root length, growth, and branching such as Jaja and Odoemena (24) on two tomato varieties; Jamal et.al (26) on *Prosopis juliflora* and Koleva et.al (30) on durum wheat. Inhibition of root growth could be referred to the toxic effect of both heavy metals and the inhibitory effect of Cd was more than that of Pb as was found in this experiment. In fact, it has been known that seedling growth and development is considered as being inhibited after imbibition if the seed coat was visibly broken, the root protruded from testa but the embryo growth was arrested beyond this point where no further growth of the embryo was noticed (31). The results of shoot length, on the other hand, revealed a non significant induction effect of heavy metals at low concentration of Cd and at its combination with low concentration of Pb. A similar result was found by Hatamzadeh et.al. (16) where shoot length of *Festuca rubra* seedlings were non significantly enhanced with low concentration (0.001% w/v) of heavy metals treatments. In fact, in preliminary experiment, we found a significant stimulatory effect of low heavy metal concentration on that growth measurement (data not shown). Similar results have been also reported by other investigators (9; 28). Cadmium and some other heavy metals have been regarded as non essential nutrient element, however, their low concentrations may increase some growth features or induce

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some desirable effects (26). By contrast, inhibitory effect of heavy metals on shoot length was recorded in some plant species, such as *Zea mays*, where shoot length was reduced at all concentrations of Pb (22).

Generally, root growth (length and weight) appeared to be more negatively affected by heavy metals than shoot length. The differential response of root and shoot to heavy metals has been widely demonstrated where it ascribed to the capability of roots to accumulate significant quantities of heavy metals more swiftly than shoots, or speed detoxification in the shoot than in roots (7; 8). Many mechanisms have been suggested to verify the suppression of general plant growth such as: (a) reducing rate of mitosis in the meristematic zones of roots especially through blocking the metaphase, (12; 35); (b) retarding cell division and elongation (9). (c) reducing meristematic cells present in this region and some enzymes contained in the cotyledon and endosperms (21); (d) inhibiting physiological processes, like chlorophyll synthesis and photosynthesis which directly affect biomass production (6; 21) and (e) blocking the entry of cations and anions into plant tissues which decline transpiration rate and plant water content (8).

Conclusion:

Our study revealed that Cd and Pb have a toxic effect on germination traits and early seedling growth stage of *N. sativa* and Pb was not as toxic as Cd. All concentrations of heavy metal tested, except low concentration, negatively and significantly affected germination percentage, length, and fresh weight of roots and shoots as compared with the control. Root growth, in general, was affected more by heavy metals than shoot. Germination percentage, length, fresh and dry weight of roots and shoots can be used as real and strong indicators of *N. sativa* response to Cd and Pb stress conditions. Reduction in plant growth and biomass production significantly depends on many reasons such as plant species and metal concentration.

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تأثير الكاديوم والرصاص على الأنبات والنمو المبكر لبادرات الحبة السوداء

(*Nigella sativa* L.)

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المخلص

تمت دراسة التأثير المستقل والتفاعلي للمعادن الثقيلة (الكاديوم والرصاص) على الإنبات والنمو المبكر لبادرات الحبة السوداء (*Nigella sativa*) في تجربة تحت ظروف المختبر في قسم علوم الحياة، كلية العلوم، جامعة صنعاء. المعاملات التي استخدمت هي نترات الكاديوم (100 و 300 ملجم/لتر)، نترات الرصاص (600 و 1500 ملجم/لتر) وكل التوافيق الممكنة. وضعت البذور في أطباق بتري (بقطر 9 سم) على أوراق ترشيح مبللة بالتراكيز المحضرة. وزعت الأطباق وفقاً للتصميم تام التعشية بواقع 3 مكررات لكل معاملة. اظهرت النتائج ان نسبة الإنبات النهائية وطول الجذر كانت أكثر القياسات متأثراً سلبياً في كل المعاملات المستخدمة حيث كان الانخفاض حاد وقوي في التراكيز المرتفعة. أفضل نمو للجذور (طول ووزن طري) لوحظ فقط في الكنترول. اظهر الوزن الجاف انخفاضاً متزايداً بارتفاع التراكيز. اظهرت التجربة ايضاً ان الكاديوم أكثر سمية من الرصاص حيث لم يحدث نمو للبادرات في التركيز المرتفع (300 ملجم/لتر) سواء استخدم مستقلاً او مع الرصاص. التأثير المساعد للمعادن المستخدمة كان اقوى من التأثير المستقل. تمت مناقشة التأثيرات المساعدة والمتضادة لكلا المعدنين في هذه التجربة.

الكلمات المفتاحية: *Nigella sativa*, معادن ثقيلة, انبات, نمو مبكر للبادرات.