Foliar application of seed water extract of *Nigella sativa* improved maize growth in cadmium-contaminated soil

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Abstract

Cadmium (Cd) is a widespread heavy metal, which commonly exert negative impacts on agricultural soils and living organisms. Foliar application of seed water extract of black cumin (*Nigella sativa* L.) can mitigate the adverse impacts of Cd-toxicity in plants through its rich antioxidants. This study examined the role of seed water extracts of *N. sativa* (NSE) in mitigating the adverse impacts of Cd-toxicity on maize growth. Two maize genotypes (synthetic ‘Neelum’ and hybrid ‘P1543’) were grown under 0, 4, 8 and 12 mg Cd kg⁻¹ soil. The NSE was applied at three different concentrations (i.e., 0, 10 and 20%) as foliar spray at 25 and 45 days after sowing. All Cd concentrations had no effect on germination percentage of both genotypes. Increasing Cd concentration linearly decreased root and allometric attributes, gas exchange traits and relative water contents of hybrid genotype. However, gas exchange traits of synthetic genotype remained unaffected by Cd-toxicity. Overall, hybrid genotype showed better tolerance to Cd-toxicity than synthetic genotype with better germination and allometric attributes and less Cd accumulation. Foliar application of NSE lowered negative effects of Cd-toxicity on all studied traits, except relative water contents. In conclusion, foliar application of NSE seemed a viable option to improve maize growth in Cd-contaminated soil.

Introduction

Soil pollution by toxic metals has become one of serious global environmental concern [1]. Heavy metals are accumulated in agricultural lands as a result of anthropogenic activities, including the use of polluted effluents, industrial wastes, phosphate fertilizers, sewage slurry, herbicides and pesticides [2–4]. Cadmium (Cd) is commonly distributed heavy metal in earth crust and ranked at 7th position among the most toxic 20 metals [5–7]. The use of phosphatic fertilizers is the major cause of Cd contamination in agricultural soils [8]. The Cd
concentration < 0.5 mg kg\(^{-1}\) dry weight is regarded as non-toxic, while this concentration may reach up to 3.0 mg kg\(^{-1}\) depending upon soil parent material [9]. The Cd concentrations >5–10 μg Cd g\(^{-1}\) leaf dry weight is toxic for numerous plant species [10]; however, hyper-accumulator species have the ability to tolerate 100 μg Cd g\(^{-1}\) leaf dry weight [11].

Plants can easily uptake Cd from soil, which poses adverse effects on their morphological, physiological, structural and biochemical attributes [4, 12, 13]. The adverse impacts of Cd-toxicity include destruction of thylakoids [14], hindered development [3, 12, 15, 16], leaf chlorosis [12, 17, 18], changed chloroplast ultrastructure and decreased transpiration and photosynthetic rates [19–21]. Cadmium causes negative impacts on all life forms in the soil and moves into harvested parts of plants; thus, enters the food chain [22, 23]. Vegetables consumptions contributes 70–80% of total Cd intake in humans [24–26], which cause serious health hazards [27]. Grazing or ingesting the Cd-polluted fodder by animals and subsequently consumption of milk and meat products cause Cd exposure in human beings [25]. Therefore, lowering Cd uptake in different crops is mandatory to avoid adverse impacts on humans and animals' health.

Maize (Zea mays L.) is an important crop after wheat and rice in Pakistan. The hiking population growth rate of the country is a significant threat to future food security. Therefore, production of the most important crops must be improved to meet the food demands of rapidly growing population in the country. Cell wall of maize plants has a significant potential for binding and retention of toxic metals due to negative charge [28]. Moreover, maize has the ability to accumulate and tolerate a certain Cd concentration without exhibiting toxicity symptoms [3, 29]. Although maize is regarded as a hyper-accumulator, Cd is harmful at high concentration and negatively affect its growth and development [12, 20, 21, 30].

*Nigella sativa* L., a member of the Ranunculaceae, is known as a miracle herb because of its huge antioxidant potential and use in pharmacology [31, 32]. It is used in natural medications and mentioned in several religious books [33]. *Nigella sativa* is commonly used to ameliorate Cd-toxicity in animals [34–37]. Seeds of *N. sativa* contain fixed (>30%) and volatile oil (0.40%-0.45%), comprising of 18.4 to 24% thymoquinone [38]. Thymoquinone (2-isopropyl-5-methyl-1, 4-benzo-quinone) is the main volatile component of the seeds [32, 34, 39], which exhibit antioxidant activities and help to overcome Cd-toxicity in animals.

Heavy metal tolerance of different crops is usually improved by chelation [40, 41], foliar application of various plant extracts [42, 43] and use of plants growth promoting rhizobacteria [44–46]. Several studies have reported the defensive role of *N. sativa* against harmful effects of heavy metals in animals; however, its role in alleviating Cd-toxicity in plants has been less explored. In a recent study, Ditta et al. [43] reported positive effects of foliar-applied seed water extract of *N. sativa* on maize growth grown in chromium (Cr) contaminated soil. Therefore, it was hypothesized that foliar application of *N. sativa* seed extract could also improve maize growth under Cd-toxicity.

The present study was conducted to infer the role of different concentrations of *N. sativa* seed water extract in improving the growth of maize genotypes under different Cd concentrations. It was hypothesized that i) maize genotypes will differ in their Cd-tolerance level, ii) increasing Cd concentration will suppress the growth of maize genotypes and iii) application of *N. sativa* seed extract will improve the growth of maize genotypes and lower Cd uptake. The results will help to improve maize growth on Cd-contaminated soils and help to lower Cd entry in food chain.

**Materials and methods**

**Experimental site**

This pot study was conducted in the wire house of Agronomy Department, Bahauddin Zakariya University, Multan, Pakistan during 2019 under natural environmental conditions. Round,
free-draining, plastic pots with 8 kg filling capacity were used in the experiment. Soil was collected from nearby agricultural lands with no known Cd-toxicity. For soil analysis, three random samples (0–20 cm depth) were collected. The soil analysis indicated that soil was clay-loam with 8.2 pH, 0.87% soil organic matter content, 1.51 dS m\(^{-1}\) EC, 0.031%, available nitrogen (N), 140 mg kg\(^{-1}\) available potassium (K), 8.00 mg kg\(^{-1}\) available phosphorous (P) with none-detectable Cd.

**Experimental details and treatments**

The experiment consisted of three different factors, i.e., two maize genotypes, four Cd concentrations and three concentrations of *N. sativa* seed water extract (NSE hereafter). Maize genotypes were synthetic 'Neelum' and hybrid 'P1543'. Seeds of synthetic genotype were procured from the market and hybrid genotype were obtained from Pioneer Seeds Sahiwal, Pakistan. Four different Cd concentrations, i.e., 0, 4, 8 and 12 mg Cd kg\(^{-1}\) of soil were included in the experiment. Three concentrations of NSE, i.e., 0, 10 and 20% were tested. Cadmium chloride was used as Cd source and mixed well in the soil before the initiation of the experiment. *Nigella sativa* seeds were boiled for 45 minutes to get NSE. The 10 and 20 g seed powder was boiled in 100 ml of water to prepare 10 and 20% NSE, respectively. The NSE was applied as foliar spray at 25 and 45 days after sowing (DAS) in two equal splits. The plants in 0% NSE were sprayed with distilled water only. Four seeds of both genotypes were sown in each pot. The concentrations of NSE were chosen based on the study of Ditta et al. [43]. All treatments had four replications and two pots were considered as a single replicate. This experiment was laid according to completely randomized block design with factorial arrangement. Maize genotypes were main factor, Cd concentration sub factor and NSE was considered as sub-sub factor.

**Crop husbandry**

Nitrogenous and phosphatic fertilizers were applied at the rate of 100 and 75 mg/kg soil. The whole P amount was applied at sowing, whereas N was applied in two splits, first at sowing and second split 25 days after sowing. The plants were irrigated with 2 to 3 days interval keeping in view moisture needs. Four grains of furadan (Carbofuron, FMC product) were applied to control maize borers and shoot fly. The plants were harvested 75 DAS.

**Observation germination and early stand formation**

The number of germinating seeds were counted daily basis starting from two DAS following Seedling Evaluation Handbook of Association of Official Seed Analysts (AOSA) [47]. Four plants were sown in each experimental pot. The first appearance date of plant was measured as days to start germination for each treatment level. The time take to complete 50% germination (E\(_{50}\)) was computed according Coolbear et al. [48].

\[
E_{50} = t_i + \frac{N/2 - n_i}{n_j - n_i} (t_j - t_i)
\]

Where, \(N = \) final number of germinated seeds, and \(n_i\) and \(n_j\) = number of germinated seeds at two adjacent days when \(n_i < N/2 < n_j\).

The formula described by Ellis and Roberts [49] was used to calculate mean emergence time (MET).

\[
MET = \frac{\Sigma Dn}{\Sigma n}
\]
Where, \( n \) = number of germinated seeds per day \( D \), and \( D \) = number of days totalled from the start of germination.

The number of germinated seeds at final count were converted to percentage and regarded as final germination percentage.

**Growth-related traits**

Chlorophyll index was measured at 75 DAS by SPAD meter (Minolta Chlorophyll Meter SPAD-502DL) from all plants, averaged and presented as SPAD values. Likewise, height of all plants from each pot was measured at 75 DAS. Randomly selected one plant from each experimental pot was pulled up carefully at 75 DAS. The leaves were separated and their area was measured with the help of leaf area meter. Shoot fresh weight of uprooted plant was noted from each pot. After sun drying, the samples were placed in oven to dry at 70˚C for 48 hr. The shoot dry weight of each pot was noted.

**Relative water contents and gas exchange traits**

Leaf relative water contents (RWC) were recorded at 75 DAS. The young fresh leaf was detached from randomly chosen one plant in each replication and weighed fresh. The leaves were then soaked in deionized water for 24 hours to get them completely turgid and weighed to record turgid weight. The leaves were then dried in an over at 75 ± 5˚C until constant weight and weighed to record dry weight. Afterwards RWC were calculated by using below formula:

\[
\text{Leaf RWC} \% = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100
\]

Where, \( \text{FW} \) = fresh weight of leaf, \( \text{DW} \) = dry weight of leaf, \( \text{TW} \) = turgid weight of leaf

Infrared gas analyser LCI-SD portable photosynthesis system (ADC Bio Scientific Ltd. United Kingdom) was used to measure gas exchange traits such as net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs) of a single leaf from each pot during 9:00–10:30 am.

**Acid digestion and cadmium analysis**

Cadmium accumulation was determined by using the leaves used for the determination of RWC. By using grinding mill, the dried leaves were ground to fine powder. After two-step acid digestion method [50], the samples were analysed on Atomic Absorption Spectrometer (iCE9 3000 SERIES) to determine Cd accumulation and presented as mg kg\(^{-1}\) of plant biomass.

**Statistical analysis**

The recorded data of all attributes were tested for normality by Shapiro-Wilk normality test [51], which showed a normal distribution. Thus, the analysis was performed on original data. Fisher’s analysis of variance (ANOVA) technique was used to determine the significance in the data. Two-way ANOVA was used for determining the significance in seedling germination data (since no NSE was applied at germination stage). Data relating to allometric parameters, gas exchange traits, RWC and Cd accumulation were analysed by three-way ANOVA [52]. The LSD (least significant difference test) at 5% probability was used to separate the means where ANOVA showed significant differences. The ANOVA was computed on SPSS version...
21 [53]. The data were graphically expressed by Microsoft Excel program through incorporation of standard errors of means.

**Results**

**Seed germination and early stand formation**

Days to start germination, mean emergence time and final germination percentage of both maize genotypes were not altered by all Cd concentrations. However, the highest Cd concentration significantly increased time taken for 50% germination in both genotypes compared to control (Table 1).

**Growth-related traits**

Maize genotypes, Cd concentrations and foliar application of NSE had significant effect \((p<0.05)\) on allometric traits (chlorophyll content, leaf area, shoot dry weight and plant height) of maize (Table 2). Two-way interaction between maize genotypes and Cd concentrations had significant effect, while all other two-way and three-way interactions had non-significant effect on entire growth traits (Table 2).

Growth-related traits were suppressed by increasing Cd concentrations; however, NSE mitigated the adverse effects to significant extent. Hybrid genotype had higher chlorophyll contents, leaf area and shoot dry weight as compared to synthetic genotype, while the trend was reverse in terms of plant height, relative water content and Cd accumulation (Table 3). The application of 20% NSE resulted in the highest SPAD value, shoot dry weight, plant height, relative water contents and Cd accumulation as compared to control (Table 3). Chlorophyll content, leaf area, shoot dry weight, plant height and relative water content were higher under lower Cd concentration (control) as compared to higher concentrations (Table 3). Mostly higher Cd concentration negatively affected the growth traits (Table 3).

**Relative water contents and gas exchange traits**

Individual effect of genotypes, Cd concentrations and NSE had significant effect \((p<0.05)\) on relative water contents, while photosynthetic rate, transpiration rate and stomatal conductance was non-significantly altered by genotypes, Cd concentrations and NSE application (Table 2).

| Treatments | Time to start emergence (days) | Mean emergence time (days) | \(E_{50}\) (days) | Final emergence percentage (%) |
|------------|--------------------------------|-----------------------------|------------------|-------------------------------|
| **Maize Genotypes (M)** | | | | |
| Synthetic | 5.2±0.2 A | 6.2±0.5 A | 5.1±0.2 A | 99.3±0.7 |
| Hybrid | 4.9±0.1 B | 5.7±0.3 B | 4.8±0.2 B | 100±0.0 |
| LSD value at 5% | **0.17** | **0.38** | **0.18** | **NS** |
| **Cadmium concentrations (mg Cd kg\(^{-1}\) soil)** | | | | |
| 0 | 4.9±0.2 B | 6.3±0.6 | 4.7±0.2 B | 100±0.0 |
| 4 | 4.9±0.1 B | 5.7±0.4 | 4.8±0.2 B | 98.0±1.4 |
| 8 | 5.2±0.2 A | 5.9±0.3 | 5.1±0.2 A | 100±0.0 |
| 12 | 5.1±0.0 AB | 5.8±0.1 | 5.1±0.2 A | 100±0.0 |
| LSD value at 5% | **0.24** | **NS** | **0.26** | **NS** |
| \(M \times Cd\) | NS | NS | NS | NS |

Means sharing the same case letter for a parameter within a column did not differ significantly from each other at \(p>0.05\).

\(E_{50}\) = Time taken to complete 50% emergence; **NS** = non-significant.

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The interactive effect of genotypes, Cd concentrations and NSE had significant effect (p<0.05) on photosynthetic rate, transpiration rate and stomatal conductance (Table 2).

Synthetic genotype showed higher relative water contents as compared to hybrid genotypes. Foliar application of 20% NSE was the most effective in increasing relative water content as compared to control. However, with respect to Cd concentrations, no Cd-toxicity (control) showed higher relative water content as compared to higher Cd concentrations (Table 3).

### Table 2. Analysis of variance of different growth and biochemical traits of different maize genotypes (synthetic vs hybrid) treated with various black cumin seed extract concentrations (0, 10 and 20%) under different Cd concentrations (0, 4, 8 and 12 mg kg\(^{-1}\)).

| SOV                          | df | SS     | MS     | p VALUE | SS     | MS     | p VALUE |
|------------------------------|----|--------|--------|---------|--------|--------|---------|
| **Chlorophyll content**      |    |        |        |         |        |        |         |
| Maize genotype (M)           | 1  | 161.40 | 161.40 | 0.0010* | 1356016| 1356016| 0.0000* |
| Cadmium concentrations (Cd)  | 3  | 907.26 | 302.42 | 0.0000* | 2181782| 727261 | 0.0000* |
| Nigella sativa extract (N)   | 2  | 234.86 | 117.43 | 0.0005* | 36013  | 18007  | 0.0015* |
| M × Cd                      | 3  | 274.39 | 91.46  | 0.0006* | 45219  | 15073  | 0.0012* |
| M × N                       | 2  | 16.94  | 8.47   | 0.5251 NS| 35816  | 17908  | 0.0016* |
| Cd × N                      | 6  | 3.81   | 0.634  | 0.9999 NS| 40264  | 6711   | 0.021*  |
| M × Cd × N                  | 6  | 8.73   | 1.46   | 0.9947 NS| 110443 | 2401   | 0.3290 NS |
| **Leaf area**                |    |        |        |         |        |        |         |
| Maize genotype (M)           | 1  | 14.61  | 14.61  | 0.0000* | 100.35 | 100.35 | 0.0013* |
| Cadmium concentrations (Cd)  | 3  | 233.55 | 77.85  | 0.0000* | 2654.04| 884.68 | 0.0000* |
| Nigella sativa extract (N)   | 2  | 3.92   | 1.96   | 0.0138* | 477.03 | 238.51 | 0.0000* |
| M × Cd                      | 3  | 19.15  | 6.38   | 0.0000* | 146.49 | 48.83  | 0.0021* |
| M × N                       | 2  | 0.025  | 0.012  | 0.9706 NS| 12.03  | 6.01   | 0.4994 NS |
| Cd × N                      | 6  | 0.268  | 0.045  | 0.9952 NS| 96.75  | 16.13  | 0.1029 NS |
| M × Cd × N                  | 6  | 0.062  | 0.010  | 0.9999 NS| 18.64  | 3.11   | 0.8978 NS |
| **Stomatal conductance**     |    |        |        |         |        |        |         |
| Maize genotype (M)           | 1  | 0.00180| 0.00180| 0.0000 NS| 0.3850 | 0.3850 | 0.8101 NS |
| Cadmium concentrations (Cd)  | 3  | 0.00278| 0.00093| 0.0402 NS| 58.501 | 19.500 | 0.0420 NS |
| Nigella sativa extract (N)   | 2  | 0.00058| 0.00020| 0.3972 NS| 61.530 | 30.763 | 0.0143 NS |
| M × Cd                      | 3  | 0.00058| 0.00019| 0.6048 NS| 33.310 | 11.104 | 0.1834 NS |
| M × N                       | 2  | 0.00321| 0.00160| 0.9706 NS| 1.957  | 0.9784 | 0.8625 NS |
| Cd × N                      | 6  | 0.00624| 0.00104| 0.0077*  | 68.449 | 11.408 | 0.1352 NS |
| M × Cd × N                  | 6  | 0.00807| 0.00134| 0.0015*  | 169.87 | 28.312 | 0.0016*  |
| **Photosynthetic rate**      |    |        |        |         |        |        |         |
| Maize genotype (M)           | 1  | 0.4232 | 0.42320| 0.0000 NS| 1458.2 | 1458.2 | 0.0000 NS |
| Cadmium concentrations (Cd)  | 3  | 0.8764 | 0.29212| 0.0755 NS| 18844.3| 6281.45| 0.0000 NS |
| Nigella sativa extract (N)   | 2  | 0.0862 | 0.04310| 0.6987 NS| 593.1  | 296.57 | 0.0127 NS |
| M × Cd                      | 3  | 0.0711 | 0.02371| 0.8967 NS| 12928.3| 4309.45| 0.0000 NS |
| M × N                       | 2  | 1.1718 | 0.59590| 0.0117 NS| 1.0    | 0.49   | 0.9920 NS |
| Cd × N                      | 6  | 2.0633 | 0.34388| 0.0181 NS| 85.7   | 14.28  | 0.9642 NS |
| M × Cd × N                  | 6  | 3.1964 | 0.53274| 0.0012*  | 49.1   | 8.18   | 0.9915 NS |
| **Relative water contents**  |    |        |        |         |        |        |         |
| Maize genotype (M)           | 1  | 0.0338 | 0.0338  | 0.0000*  |        |        |         |
| Cadmium concentrations (Cd)  | 3  | 1.1358 | 0.3786  | 0.0000*  |        |        |         |
| Nigella sativa extract (N)   | 2  | 0.0271 | 0.0136  | 0.0000*  |        |        |         |
| M × Cd                      | 3  | 0.0074 | 0.0035  | 0.0065*  |        |        |         |
| M × N                       | 2  | 0.0006 | 0.0003  | 0.5677 NS|        |        |         |
| Cd × N                      | 6  | 0.0009 | 0.0002  | 0.9384 NS|        |        |         |
| M × Cd × N                  | 6  | 0.0244 | 0.0005  | 0.9762 NS|        |        |         |

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The highest values of gas exchange parameters, transpiration rate and stomatal conductance were observed under lower Cd concentrations (Figs 1 and 2). Application of 10% NSE improved photosynthetic rate of synthetic genotype, while applications of 10 and 20% NSE had no effect on transpiration rate and stomatal conductance. Gas exchange parameters of hybrid genotype had varied responses. The highest stomatal conductance of hybrid genotype was recorded under no Cd-toxicity, while higher transpiration rate was recorded under lower Cd concentration. However, the highest photosynthetic rate was recorded under higher Cd concentration (Figs 1 and 2). Furthermore, application of 10% NSE resulted in significant improvement in gas exchange traits of hybrid genotype. Overall, hybrid genotype performed better for all parameters than synthetic genotype.

Cadmium accumulation in shoot

Shoot Cd concentration was significantly affected (p<0.05) by genotype, Cd concentrations and NSE concentrations; however, all two and three-way interactions had non-significant effect on shoot Cd concentration (Table 2). Synthetic genotypes observed higher shoot Cd concentration compared with hybrid. Moreover, applications of NSE did not lower shoot Cd concentration in synthetic maize; however, application of 20% NSE significantly lowered the shoot Cd concentration in hybrid maize (Table 3). The higher Cd concentration resulted in higher shoot Cd concentration in both maize types as compared to control (Table 3).

Discussion

The results indicated that different Cd concentrations did not affect seed germination and early stand establishment traits of both genotypes. The possible reasons for non-significant results are that the seeds did not uptake Cd during the germination phase. Stefani et al. [54] suggested that seeds use their own reserves during germination; therefore, it is not affected by

Table 3. Effect of different cadmium concentrations on allometric traits, relative water contents and cadmium accumulation of maize genotypes.

| Treatments          | Chlorophyll content (SPAD value) | Leaf area (cm²) | Shoot dry weight (g) | Plant height (cm) | Relative water content (%) | Cadmium accumulation (mg kg⁻¹) | Shoot Cd concentration (mg Cd kg⁻¹ soil) |
|---------------------|----------------------------------|-----------------|----------------------|-------------------|---------------------------|-------------------------------|--------------------------------------|
| Maize genotypes     |                                  |                 |                      |                   |                           |                               |                                       |
| Synthetic           | 44.7±2.0 B                       | 1498.8±7.7 B    | 4.30±0.3 B           | 43.44±1.4 A       | 70.71±5.0 A               | 0.27±0.0 A                    |                                       |
| Hybrid              | 47.7±1.6 A                       | 1773.3±31.9 A   | 5.20±0.3 A           | 41.08±1.6 B       | 61.71±3.2 B               | 0.23±0.0 B                    |                                       |
| LSD at 5%           | 1.72                             | 23.24           | 0.31                 | 1.39              | 3.73                      | 0.01                          |                                       |

N. sativa seed water extract (%; w/v)

| Treatments          | Leaf area (cm²) | Shoot dry weight (g) | Plant height (cm) | Relative water content (%) | Cadmium accumulation (mg kg⁻¹) | Shoot Cd concentration (mg Cd kg⁻¹ soil) |
|---------------------|-----------------|----------------------|-------------------|---------------------------|-------------------------------|--------------------------------------|
| Synthetic           | 1629.2±24.1 A   | 4.47±0.3 B           | 39.00±1.5 C       | 62.48±4.8 B               | 0.22±0.0 C                    |                                       |
| Hybrid              | 1666.2±22.3 B   | 4.75±0.2 AB          | 42.50±1.6 B       | 66.69±3.8 AB              | 0.25±0.0 B                    |                                       |
| LSD at 5%           | 2.10            | 28.47                | 0.37              | 1.70                      | 4.56                          | 0.01                                 |

Cadmium levels (mg Cd kg⁻¹ soil)

| Treatments          | Leaf area (cm²) | Shoot dry weight (g) | Plant height (cm) | Relative water content (%) | Cadmium accumulation (mg kg⁻¹) | Shoot Cd concentration (mg Cd kg⁻¹ soil) |
|---------------------|-----------------|----------------------|-------------------|---------------------------|-------------------------------|--------------------------------------|
| Synthetic           | 1857.6±23.6 A   | 7.60±0.2 A           | 49.94±1.8 A       | 80.73±4.1 A               | 0.42±0.0 D                    |                                       |
| Hybrid              | 1731.6±30.9 B   | 4.79±0.2 B           | 45.44±1.3 B       | 75.89±3.3 A               | 0.31±0.0 C                    |                                       |
| LSD at 5%           | 24.2            | 3.82                 | 0.43              | 1.96                      | 5.27                          | 0.02                                 |

Means sharing the same case letter for a parameter within a column did not differ significantly from each other at p>0.05.

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the presence of heavy metals in the soil. Due to the reason, germination was not altered by various Cd concentrations included in the current study. Similar results for maize germination under heavy metal stress have been reported in an earlier study [55]. Inhibition of seedling development under Cd-toxicity is dependent on metal concentration in soil [56, 57]. Seed germination of plant species is altered by various environmental factors [58, 59] and large differences exist in different ecotypes of the same species for seed germination [59, 60].

Growth-related parameters of both genotypes were adversely affected by increasing Cd concentration. The highest negative effects on number of roots and root elongation rate were noted in both genotypes under higher Cd concentration. The findings of the current study are supported by other researchers who reported that Cd caused negative effects on maize roots
Alteration in micronutrient uptake in plants under Cd-toxicity is responsible for decrease in protein activity and inhibition of root development \[63\]. Plant growth rate and plant height of both genotypes were significantly altered by higher Cd concentrations. Similar results have been reported in numerous earlier studies \[3, 16, 64\]. Cadmium alters protein activity by disturbing the hydrogen-sulphur bond \[65\]; thus, plants uptake less nutrients and water from roots which hinder development \[66\]. The growth and productivity of plants are decreased under heavy metals’ stress due to insufficient antioxidant enzymes scavenging ability \[67–69\].

Higher Cd concentrations caused significant reduction in relative water contents in both genotypes. Decreased relative water contents under Cd stress has also been reported by Farouk et al. \[70\]. It has been reported that Cd-toxicity causes physiological drought in most plants by disturbing the water relationship between plants, which is responsible for decreased relative water contents \[4\]. Chlorophyll contents and gas exchange parameters of synthetic and hybrid genotype showed different responses. In synthetic genotype, only photosynthesis was slightly affected by higher Cd concentration. However, chlorophyll contents, transpiration rate and stomatal conductance were not affected under increased Cd concentration. Photosynthetic rate of hybrid genotype remained unaffected by higher Cd concentration; however, chlorophyll contents, transpiration rate and stomatal conductance were negatively affected. Similar results related to Cd-induced decrease in gas exchange traits have been reported for maize and many other plant species \[64, 71, 72\]. Wu et al. \[73\] reported that photosynthetic activity of plants is decreased under Cd-toxicity as it disturbs the opening and closing of stomata and damage metabolic processes. Under Cd-toxicity, alteration in gas exchange parameters of maize genotypes may be because of difference in the hereditary material of the maize plants \[74\].

Foliar application of NSE ameliorated toxic effects of Cd-toxicity and improved growth-related traits and gas exchange parameters of both maize genotypes. Several previous studies have reported that maize and different plant species improved growth and gas exchange parameters by applying foliar application of several substances under metals stress \[42, 43, 72\].

![Fig 2. Effect of different concentrations of Nigella sativa seed extracts on transpiration rate ± standard errors on different maize genotypes grown under various cadmium concentrations. Means sharing the same case letter for a parameter did not differ significantly from each other at p > 0.05. Here, NSE = N. sativa seed extract; DAS = days after sowing.](https://doi.org/10.1371/journal.pone.0254602.g002)
However, the capacity of NSE to overcome toxic effects of Cd in crop plants has been less explored. A recent study have reported that NSE improved maize growth and related traits under Cr stress [43].

Foliar application of NSE significantly enhanced growth and gas exchange parameters of maize genotypes under Cd-toxicity. The uptake of Cd by cereal crops caused serious health hazards in humans such as different skin issues, kidney failure, high blood pressure and mental variations etc. [4]. *Nigella sativa* has been extensively used in pharmacology [31, 75] and natural remedies [33, 76]. The seeds of *N. sativa* possess strong antioxidant activities to scavenge free radicles due to its bioactive components thymoquinone TQ [77, 78]. *Nigella sativa* has been reported to overcome heavy metals toxicity in several animals [34]. Foliar application of NSE mitigated Cd toxicity, which might be due to TQ. However, effective mechanism of NSE involved to improve Cd tolerance needs to be further studied.

The Cd uptake was increased in both genotypes with increasing Cd concentration. Cadmium accumulation in synthetic genotype was higher than hybrid genotype. Similar results are also reported for maize crop [79, 80]. Difference in Cd uptake of maize genotypes may be because of variation in hereditary material [12, 74, 80]. The accumulated Cd even at low concentration is consider as harmful for human ingestion.

**Conclusion**

Germination of both maize genotypes remained unaffected under all Cd concentrations, while increasing Cd concentration significantly decreased growth-related traits, gas exchange attributes and relative water contents in both maize genotypes. Gas exchange traits of both genotypes showed different response under Cd-toxicity. Foliar application of NSE ameliorated the toxic effects of Cd-toxicity to significant extent in both genotypes and lowered Cd accumulation. Hybrid genotype had better performance for all traits than synthetic genotype, indicating that hybrid genotype better tolerated Cd-toxicity. Nonetheless, NSE and other plants extracts must also be investigated for their prospective effect in mitigating Cd-toxicity in maize and other crops. However, differential response of gas exchange traits of maize and effective mechanism of NSE involved to improve Cd-tolerance needs to be further studied.

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References

1. Gratão PL, Monteiro CC, Tezotto T, Carvalho RF, Alves LR, Peters LP, et al. Cadmium stress antioxidant responses and root-to-shoot communication in grafted tomato plants. Biometals. 2015; 28: 803–816. https://doi.org/10.1007/s10534-015-9867-3 PMID: 26077192

2. Adrees M, Ali S, Rizwan M, Zia-ur-Rehman M, Ibrahim M, Abbas F, et al. Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. Ecotoxicol Environ Saf. 2015; 119: 186–197. https://doi.org/10.1016/j.ecoenv.2015.05.011 PMID: 26004359

3. Rizwan M, Ali S, Rizvi H, Rinklebe J, Tsang DCW, Meers E, et al. Phytomanagement of heavy metals in contaminated soils using sunflower: A review. Crit Rev Environ Sci Technol. 2016; 46: 1498–1528. https://doi.org/10.1080/10643389.2016.1248199

4. Haider FU, Liqun C, Coulter JA, Cheema SA, Wu J, Zhang R, et al. Cadmium toxicity in plants: Impacts and remediation strategies. Ecotoxicol Environ Saf. 2021; 211: 111887. https://doi.org/10.1016/j.ecoenv.2020.111887 PMID: 33450535

5. Choppala G, Saifullah, Bolan N, Bibi S, Iqbal M, Rengel Z, et al. Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. CRC Crit Rev Plant Sci. 2014; 33: 374–391.

6. Du Y-L, He M-M, Xu M, Yan Z-G, Zhou Y-Y, Guo G-L, et al. Interactive effects between earthworms and maize plants on the accumulation and toxicity of soil cadmium. Soil Biol Biochem. 2014; 72: 193–202.

7. Pan L, Ma J, Wang X, Hou H. Heavy metals in soils from a typical county in Shanxi Province, China: levels, sources and spatial distribution. Chemosphere. 2016; 148: 248–254. https://doi.org/10.1016/j.chemosphere.2015.12.049 PMID: 26807946

8. Kubier A, Wilkin RT, Pichert T. Cadmium in soils and groundwater: a review. Appl Geochemistry. 2019; 108: 104388. https://doi.org/10.1016/j.apgeochem.2019.104388 PMID: 32280158

9. Vahter M, Berglund M, Slorach S, Friberg L, Sarić M, Zheng X, et al. Methods for integrated exposure monitoring of lead and cadmium. Environ Res. 1991; 56: 78–89. https://doi.org/10.1016/s0013-9351(05)80111-2 PMID: 1915192

10. Hooda PS. Trace Elements in Soils. Hooda PS, editor. Trace Elements in Soils. Wiley; 2010. https://doi.org/10.1002/9781444319477

11. Verbruggen N, Hermans C, Schat H. Mechanisms to cope with arsenic or cadmium excess in plants. Curr Opin Plant Biol. 2009; 12: 364–372. https://doi.org/10.1016/j.pbi.2009.09.001 PMID: 19501016

12. Xu X, Liu C, Zhao X, Li R, Deng W. Involvement of an antioxidant defense system in the adaptive response to cadmium in maize seedlings (Zea mays L.), Bull Environ Contam Toxicol. 2014; 93: 618–624. https://doi.org/10.1007/s00128-014-1361-z PMID: 25154813

13. Fahad S, Hussain S, Khan F, Wu C, Saud S, Hassan S, et al. Effects of tire rubber ash and zinc sulfate on crop productivity and cadmium accumulation in five rice cultivars under field conditions. Environ Sci Pollut Res. 2015; 22: 12424–12434. https://doi.org/10.1007/s11356-015-4518-3 PMID: 25903182

14. Qureshi MI, D’Amici GM, Fagioni M, Rinalducci S, Zolla L. Iron stabilizes thylakoid protein–pigment complexes in Indian mustard during Cd-phytoremediation as revealed by BN-SDS-PAGE and ESI-MS/MS. J Plant Physiol. 2010; 167: 761–770. https://doi.org/10.1016/j.jplph.2010.01.017 PMID: 20199821

15. Bagheri R, Bashir H, Ahmad J, Baig A, Qureshi MI. Effects of cadmium on leaf proteome of Spinacia oleracea (spinach). Int J Agri Food Sci Technol. 2013; 4: 33–36

16. Rizwan M, Meunier J-D, Davidian J-C, Pokrovsky OS, Bovet N, Keller C. Silicon alleviates Cd stress of wheat seedlings (Triticum turgidum L. cv. Claudio) grown in hydroponics. Environ Sci Pollut Res. 2016; 23: 1414–1427. https://doi.org/10.1007/s11356-015-5351-4 PMID: 26370813

17. Gill SS, Tuteja N. Cadmium stress tolerance in crop plants: probing the role of sulfur. Plant Signal Behav. 2011; 6: 215–222. https://doi.org/10.4161/pstb.6.2.14680 PMID: 21330784

18. Márquez-García B, Horernans N, Cuypers A, Guizez Y, Córdoba F. Antioxidants in Erica andevalensis: A comparative study between wild plants and cadmium-exposed plants under controlled conditions. Plant Physiol Biochem. 2011; 49: 110–115. https://doi.org/10.1016/j.plaphy.2010.00.007 PMID: 21074447

19. Li Y, Chen Z, Xu S, Zhang L, Hou W, Yu N. Effect of Combined Pollution of Cd and B [a] P on Photosynthesis and Chlorophyll Fluorescence Characteristics of Wheat. Polish J Environ Stud. 2015; 24.
20. Lysenko EA, Klaus AA, Psyhbytko NL, Kusnetsov V V. Cadmium accumulation in chloroplasts and its impact on chloroplastic processes in barley and maize. Photosynth Res. 2015; 125: 291–303. https://doi.org/10.1007/s11120-014-0047-z PMID: 25315190

21. Tauqeer HM, Ali S, Rizwan M, Ali Q, Saeed R, Iftikhar U, et al. Phytoremediation of heavy metals by Alternanthera bettzickiana: growth and physiological response. Ecotoxicol Environ Saf. 2016; 126: 138–146. https://doi.org/10.1016/j.ecoenv.2015.12.031 PMID: 26748375

22. Liu H, Hussain S, Peng S, Huang J, Cui K, Nie L. Potentially toxic elements concentration in milled rice differ among various planting patterns. F Crop Res. 2014; 168: 19–26.

23. Li Y, Tang H, Hu Y, Wang X, Ai X, Tang L, et al. Enrofloxacin at environmentally relevant concentrations enhances uptake and toxicity of cadmium in the earthworm Eisenia fetida in farm soils. J Hazard Mater. 2016; 308: 312–320. https://doi.org/10.1016/j.jhazmat.2016.01.057 PMID: 26852206

24. Olsson I-M, Bensryd I, Lundh T, Ottosson H, Skerfving S, Oskarsson A. Cadmium in blood and urine—impact of sex, age, dietary intake, iron status, and former smoking—association of renal effects. Environ Health Perspect. 2002; 110: 1185–1190. https://doi.org/10.1289/ehp.021101185 PMID: 12460796

25. Wang H-Y, Wen S-L, Chen P, Zhang L, Cen K, Sun G-X. Mitigation of cadmium and arsenic in rice grain by applying different silicon fertilizers in contaminated fields. Environ Sci Pollut Res. 2016; 23: 3781–3788. https://doi.org/10.1007/s11356-015-5638-5 PMID: 26498817

26. Yang Y, Chen W, Wang M, Li Y, Peng C. Evaluating the potential health risk of toxic trace elements in vegetables: Accounting for variations in soil factors. Sci Total Environ. 2017; 584: 942–949. https://doi.org/10.1016/j.scitotenv.2017.01.143 PMID: 28185733

27. Song K-H, Choi K-Y, Kim C-J, Kim Y-I, Chung C-S. Assessment of the governance system for the management of the East Sea-Jung dumping site, Korea through analysis of heavy metal concentrations in bottom sediments. Ocean Sci J. 2015; 50: 721–740.

28. Polle A, Schützendübel A. Heavy metal signalling in plants: linking cellular and organismic responses. Plant responses to abiotic stress. Springer; 2003. pp. 187–215.

29. Yang Y, Li Y, Zhang J. Chemical speciation of cadmium and lead and their bioavailability to cole (Brassica campestris L.) from multi-metals contaminated soil in northwestern China. Chem Speciat Bioavai-lab. 2016; 28: 33–41.

30. Metwali MR, Gowayed SMH, Al-Maghrabi OA, Mosleh YY. Evaluation of toxic effect of copper and cadmium on growth, physiological traits and protein profile of wheat (Triticum aestivum L.) and maize (Zea mays L.) and sorghum (Sorghum bicolor L.). World Appl Sci J. 2013; 21: 301–304.

31. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013; 3: 337–352. https://doi.org/10.1016/S2221-1691(13)60075-1 PMID: 23646296

32. Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F. Nigella sativa L. (Black Cumin): A promising natural remedy for wide range of illnesses. Evidence-Based Complement Altern Med. 2019; 2019: 1–16. https://doi.org/10.1155/2019/1528635 PMID: 31214267

33. Saha R, Bhupendar K. Pharmacognosy and pharmacology of Nigella sativa—a review. Int Res J Pharm. 2011; 2: 36–39.

34. El-Dakhakhny M, Madi N., Lember N., Lember T., Ammon HP. Nigella sativa oil, nigellone and derived thymoquino ne inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. J Ethnopharmacol. 2002; 81: 161–164. https://doi.org/10.1016/s0378-8741(02)00051-x PMID: 12065147

35. Kanter M, Coskun O, Gurel A. Effect of black cumin (Nigella sativa) on cadmium-induced oxidative stress in the blood of rats. Biol Trace Elem Res. 2005; 107: 277–287. https://doi.org/10.1385/BTER:107:3:277 PMID: 16286683

36. Kishwar F, Anwar H. Use of active ingredient of Nigella sativa to reduce toxicity of some trace elements (Fe (III), Cr (VI), Cu (II), V (IV) and Co (II)). FUUAST J Biol. 2012; 2: 95–101.

37. Kishwar F, Mahmood T, Mahmood I. Complexation of active ingredient thymoquinone of Nigella sativa (black seed) with chromium (VI). FUUAST J Biol. 2016; 6: 65–72.

38. Hosseini SM, Taghiabadi E, Abnous K, Hariri AT, Pourbakhsh H, Hosseinzadeh H. Protective effect of thymoquinone, the active constituent of Nigella sativa fixed oil, against ethanol toxicity in rats. Iran J Basic Med Sci. 2017; 20: 927. https://doi.org/10.22038/ijbms.2017.9116 PMID: 29085585

39. Badary OA, Taha RA, Gamal El-Din AM, Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol. 2003; 26: 87–98. https://doi.org/10.1081/dct-120020404 PMID: 12816394

40. Danish S, Kiran S, Fahad S, Ahmad N, Ali MA, Tahir FA, et al. Alleviation of chromium toxicity in maize by Fe fortification and chromium tolerant ACC deaminase producing plant growth promoting rhizobacteria. Ecotoxicol Environ Saf. 2019; 185: 109706. https://doi.org/10.1016/j.ecoenv.2019.109706 PMID: 31561073
41. Abbas A, Azeem M, Naveed M, Latif A, Bashir S, Ali A, et al. Synergistic use of biochar and acidified manure for improving growth of maize in chromium contaminated soil. Int J Phytoremediation. 2020; 22: 52–61. https://doi.org/10.1080/15226514.2019.1644208 PMID: 3133932

42. Danish S, Tahir FA, Rasheed MK, Ahmad N, Ali MA, Kiran S, et al. Effect of foliar application of Fe and banana peel waste biochar on growth, chlorophyll content and accessory pigments synthesis in spinach under chromium (IV) toxicity. Open Agric. 2019; 4: 381–390. https://doi.org/10.1515/opag-2019-0034

43. Allah Ditta HM, Aziz A, Hussain MK, Mehboob N, Hussain M, Farooq S, et al. Exogenous application of black cumin (Nigella sativa) seed extract improves maize growth under chromium (Cr) stress. Int J Phytoremediation. 2021; 1–13. https://doi.org/10.1080/15226514.2021.1889965 PMID: 33631090

44. Islam F, Yasmeen T, Arif MS, Riaz M, Shahzad SM, Imran Q, et al. Combined ability of chromium (Cr) and water collected from Tanda Dam Kohat. J Pharm Sci Res. 2015; 7: 89.

45. Stambulska UY, Bayliak MM, Lushchak VI. Chromium (VI) Toxicity in Legume Plants: Modulation of ACC-deaminase activity improve nutrient uptake, chlorophyll contents and early seedling growth of wheat under PEG-induced osmotic stress. Intl J Agric Biol. 2019; 21: 1212–1220.

46. Danish S, Zafar-ul-Hye M, Hussain M, Shaaban M, Núñez-Delgado A, Hussain S, et al. Rhizobacteria with ACC-deaminase activity improve nutrient uptake, chlorophyll contents and early seedling growth of wheat under PEG-induced osmotic stress. Int J Agric Biol. 2019; 21: 1212–1220.

47. Association of Official Seed Analysts A. Rules for testing seeds. J Seed Technol. 1990; 12: 1–112.

48. Coolbear P, Francis A, Grierson D. The effect of low temperature pre-soaking treatment on the germination performance and membrane integrity of artificially aged tomato seeds. J Exp Bot. 1984; 35: 1609–1617. https://doi.org/10.1093/jxb/35.11.1609

49. Steel R., Torreij J, Dickey D. Principles and Procedures of Statistics A Biometrical Approach. 1997.

50. Nazir R, Khan M, Masab M, Rehman HU, Rauf NU, Shahab S, et al. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. J Pharm Sci Res. 2015; 7: 89.

51. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965; 52: 591–611.

52. Steel R., Torreij J, Dickey D. Principles and Procedures of Statistics A Biometrical Approach. A Biometrical Approach. 1997.

53. IBM C, IBM SPSS Inc. SPSS Statistics for Windows. IBM Corp Released 2012. 2012;Version 20: 1–8.

54. Stefani A, Arduini I, Onnis A. Juncus acutus: germination and initial growth in presence of heavy metals. Annales Botanici Fennici. JSTOR; 1991. pp. 37–43.

55. Mahmood S, Hussain A, Saeed Z, Athar M. Germination and seedling growth of corn (Zea mays L.) seeds: Implications for range expansion and management. Weed Sci. 2018, 66: 494–501. https://doi.org/10.1017/wsc.2018.20

56. Feroz I, Hammad M, Tangi S, Qaim M, Musharraf H, Maqbool M, et al. Effect of different seed treatments on the growth and seed yield of maize (Zea mays L.) under saline environment. J Agric Sci. 2011; 4: 60–65.

57. Diwan H. Characterization of chromium toxicity in food crops and their role in phytoremediation. J Bioremediation Biodegrad. 2012; 03. https://doi.org/10.4172/2155-6199.1000159

58. Önen H, Farooq S, Tad S, Özaslan C, Gunal H, Chauhan BS. The influence of environmental factors on germination of Burcucumber (Sicyos angulatus) seeds: Implications for range expansion and management. Weed Sci. 2018; 66: 494–501. https://doi.org/10.1017/wsc.2018.20

59. Farooq S, Önen H, Özaslan C, Baskin CC, Gunal H. Seed germination niche for common ragweed (Ambrosia artemisifolia L.) populations naturalized in Turkey. South African J Bot. 2019; 123: 361–371.

60. Özaslan C, Farooq S, Önen H, Özcan S, Bukun B, Gunal H. Germination biology of two invasive Physalis species and implications for their management in arid and semi-arid regions. Sci Rep. 2017; 7: 16960. https://doi.org/10.1038/s41598-017-17169-5 PMID: 29208989

61. Mihaescu LA, Mare-Rosca OE, Marian M, Bildar CF. Research on the growth intensity of the Zea mays L. plantlets aerial parts under cadmium treatment. Analele Univ din Oradea, Fasc Biologie (Brațlil). 2004; 59: 513–517.

62. Diwan H. Characterization of chromium toxicity in food crops and their role in phytoremediation. J Bioremediation Biodegrad. 2012; 03. https://doi.org/10.4172/2155-6199.1000159

63. Chen YX, He YF, Luo YM, Yu YL, Lin Q, Wong MH. Physiological mechanism of plant roots exposed to cadmium. Chemosphere. 2003; 50: 789–793. https://doi.org/10.1016/s0045-6535(02)00220-5 PMID: 12688492
64. Arshad M, Ali S, Noman A, Ali Q, Rizwan M, Farid M, et al. Phosphorus amendment decreased cadmium (Cd) uptake and ameliorates chlorophyll contents, gas exchange attributes, antioxidants, and mineral nutrients in wheat (Triticum aestivum L.) under Cd stress. Arch Agron Soil Sci. 2016; 62: 533–546.

65. Lin A, Zhang X-H, Chen M-M, Qing CAO. Oxidative stress and DNA damages induced by cadmium accumulation. J Environ Sci. 2007; 19: 596–602. https://doi.org/10.1016/S1001-0742(07)60099-0 PMID: 17911213

66. Anjum SA, Ashraf U, Khan I, Tanveer M, Shahid M, Shakoor A, et al. Phyto-toxicity of chromium in maize: Oxidative damage, osmolyte accumulation, anti-oxidative defense, and chromium uptake. Pedsphere. 2017; 27: 262–273. https://doi.org/10.1016/S1002-0160(17)60315-1

67. Farooq MA, Ali S, Hameed A, Ishaque W, Mahmood K, Iqbal Z. Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. Ecotoxicol Environ Saf. 2013; 96: 242–249. https://doi.org/10.1016/j.ecoenv.2013.07.006 PMID: 23911213

68. Ashraf U, Kanu AS, Mo Z, Hussain S, Anjum SA, Khan I, et al. Lead toxicity in rice: effects, mechanisms, and mitigation strategies—a mini review. Environ Sci Pollut Res. 2015; 22: 18318–18332. https://doi.org/10.1007/s11356-015-4882-z PMID: 26122572

69. Islam MT, Khan MR, Mishra SK. An updated literature-based review: phytochemistry, pharmacology and therapeutic promises of Nigella sativa L. Orient Pharm Exp Med. 2019; 19: 115–129. https://doi.org/10.1007/s13596-019-00363-3

70. Islam MT, Khan MR, Mishra SK. An updated literature-based review: phytochemistry, pharmacology and therapeutic promises of Nigella sativa L. Orient Pharm Exp Med. 2019; 19: 115–129. https://doi.org/10.1007/s13596-019-00363-3

71. Wasi F-B, Jing D, Jia G-X, Zheng S-J, Zhang G-P. Genotypic difference in the responses of seedling growth and Cd toxicity in rice (Oryza sativa L.). Agric Sci China. 2006; 5: 68–76.

72. Elshama SS, Shehab GMG, El-Kenawy AE, Osman H-EH, Farag MM. Role of Nigella sativa Seeds on modulation testicular toxicity of colchicine repeated use in adult albino rats. Life Sci J. 2013; 10. PMID: 25382966

73. Mansour MA, Nagi MN, El-Khatib AS, Al-Bekairi AM. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. Cell Biochem Funct. 2002; 20: 143–151. https://doi.org/10.1002/cbf.968 PMID: 11979510

74. Benkaci-Ali F, Bailleuammer A, Waethele JP, Marlier M. Chemical Composition of Volatile Oils from Algerian Nigella sativa L. seeds. J Essent Oil Res. 2010; 22: 318–322. https://doi.org/10.1080/10412905.2010.9700335

75. Tanvir K, Akram MS, Masood S, Chaudhary HJ, Lindberg S, Javed MT. Cadmium-induced rhizospheric pH dynamics modulated nutrient acquisition and physiological attributes of maize (Zea mays L.). Environ Sci Pollut Res. 2015; 22: 9193–9203.

76. Naeem A, Saifullah, Rehman MZ, Akhtar T, Ok YS, Rengel Z. Genetic variation in cadmium accumulation and tolerance among wheat cultivars at the seedling stage. Commun Soil Sci Plant Anal. 2016; 47: 554–562.