

# Germination Potential of Safflower (*Carthamus tinctorius* L.) Against Nickel Heavy Metal

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#### Abstract

As a heavy metal, Nickel is also an essential element and an abiotic plant stress factor. Safflower (*Carthamus tinctorius* L.) is an oil seed plant used commercially for the different industries that belongs to the Asteraceae family. This study aims to investigate the effects of nickel concentrations ( $0, 25, 50, 100, 200, 400, 800, 1000 \text{ mg kg}^{-1}$ ) on safflower germination stages. Germination percentage, germination index and coefficient of velocity equations were calculated according to germinated seeds on days. Nickel concentrations and days after germination were effective on germination percentage, germination index and coefficient of velocity values. Especially 800 and 1000 mg kg<sup>-1</sup> nickel doses had a slightly negative effect on the investigated equations. According to the study results, it was thought that the germination ability of *C. tinctorius* seeds was quite successful in mediums with nickel-heavy metal.

**Research Article** 

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# 1. Introduction

Abiotic stress factors like drought, excessive hot or cold temperatures are dangerous for agricultural activities as they adversely affect plant growth and development (Fadiji et al., 2023). Heavy metals are elements whose density is five times greater than the density of water and affect both animals and plants adversely (Järup, 2003; Rehman et al., 2021) and are also defined as abiotic stress factors. Heavy metals in soil may originate from natural (geological parent material composition) and anthropogenic (like mining, smelting, electroplating, fuel production, power transmission, intensive agriculture, wastewater irrigation, sludge dumping, and dust) ways (Rattan et al., 2005). Heavy metals at high concentrations in soils have risks and hazards to humans and the ecosystem through their presence in the food chain (Ahmad et al., 2015). High concentrations of metals and chemicals inhibit plant germination and growth (Sethy and Ghosh, 2013); they change protein structure, disturb osmoregulation, reduce radicula growth, disturb storage food consumption, alter plant hormones like gibberellic acid (GA) and abscisic acid antioxidant (ABA), change enzyme activity, reduce proteolytic activity and cause injuries in photosystems (Seneviratne et al., 2019).

Nickel is described as heavy metal at 8.9  $g/cm^3$  density (Adriano, 2001). It is the 22nd most abundant element on Earth's crust and one of the trace metals emitted into the environment through natural and activities (Cempel and anthropogenic Nikel, 2006; Hussain et al., 2013). Although nickel is identified as an essential element for plant metabolism (at 0.05–10 mg kg<sup>-1</sup> dry weight range), at higher concentrations, its adverse effects occur (Hassan et al., 2019). Nickel deficiency causes senescence, decreases growth, and negatively affects urease activity, nitrogen

metabolism and iron uptake (Bhalerao et al., 2015; Hasssan et al., 2019). On the contrary, nickel is toxic to plants as it adversely affects plant growth, seed germination, enzyme activities (such as amylase, protease and ribonuclease enzymes), protein, phenolic, and chlorophyll content (Wang et al., 2003; Ahmad and Ashraf, 2011; Ahmed and Sardar, 2023). The excess of nickel is more common than its deficiency in plants (Alloway, 1995; Hassan et al., 2019). Safflower (Carthamus tinctorius L.) is an economically important medicinal plant in the Asteraceae family. It is also a natural dye resource, an oil seed crop (Sabzalian et al., 2009), and its oil quality shows similarity to sunflower oil due in terms of high (55-70%) linoleic acid content (Ergönül and Özbek, 2020). It is described as a cultivated crop for being grown in a wide area of usage, from medicine to industry (Iftikhar Hussain et al., 2016; Hadjadj et al., 2023).

This work aims to reveal the effect of nickel on germination in *C. tinctorius* at wide dose ranges.

# 2. Material and Methods

## 2.1. Material

*Carthamus tinctorius* (cv. Zirkon) seeds were used in this work.

## 2.2. Methods

# 2.2.1. Seed sowing and nickel concentrations

First, the seeds were surface disinfected with 5% (v/v) sodium hypochlorite (NaClO) solution for 5 minutes and rinsed with distilled water. Then, the pots were filled with 1 kg of soil containing nickel (formed NiSO<sub>4</sub>) at different concentrations (Control, 25, 50, 100, 200, 400, 800 and 1000 mg kg<sup>-1</sup>). Ten seeds were sown in each pot, and the pots were put in a greenhouse. Each application had four replications.

## 2.2.2. Equations

The germinated seeds were counted daily. After six days, germinated seed

number in pots had not been changed, so data from the first six days were used in this research. Germination percentage, germination index and coefficient of the velocity of germination equations were used for evaluating germination against nickel concentrations.

### 2.2.3. Germination percentage

Germination percentage (GP) (Ikić et al., 2012) was calculated to determine germination degrees for different concentrations with fallowing equation.

GP (%) = 
$$\frac{\text{germinated seeds}}{\text{sowed seeds}} x100$$

## 2.2.4. Germination index

The germination index (GI) (Rastegar et al., 2011) was calculated using the following equation.

 $GI = \frac{Germinated seeds on observation day}{Observation day}$ 

# **2.2.5.** Coefficient of the velocity of germination

The coefficient of the velocity of germination (CVG) (Maguire, 1962) was calculated according to the following equation to compare germination velocities.

$$CVG = \frac{G1 + G2 + \dots + Gn}{1xG1 + 2xG2 + \dots + nxGn}$$

Since the result of some equations was undefined on the days when germination was not observed, the result of the equality was accepted as zero, which is the lowest value.

## 2.3. Statistical analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) package program was used for data analysis. The ANOVA (p<0.05) test was used to find the significant difference between groups. Then the Duncan Posthoc test was performed to reveal the level of difference between groups.

## 3. Results and Discussion

Average momentary and cumulative germination values and ranges for observation days are shown in Table 1.

Table 1. Momentary and cumulative germination values of C. tinctorius for Ni doses

	1st day				2nd Day					3rd Day			
	MG		C	CG		MG		CG		MG		CG	
	Me.	Ra.	Me.	Ra.	Me.	Ra.	Me.	Ra.	Me.	Ra.	Me.	Ra.	
Control	2.50	2.00	2.50	2.00	0.25	1.00	2.75	2.00	1.00	2.00	3.75	3.00	
25	3.25	4.00	3.25	4.00	0.25	1.00	3.50	4.00	0.00	0.00	3.50	4.00	
50	3.00	3.00	3.00	3.00	0.25	1.00	3.25	4.00	1.50	2.00	4.75	4.00	
100	3.00	5.00	3.00	5.00	0.50	2.00	3.50	7.00	1.75	4.00	5.25	6.00	
200	2.75	8.00	2.75	8.00	0.50	1.00	3.25	7.00	1.00	2.00	4.25	8.00	
400	2.50	4.00	2.50	4.00	0.50	2.00	3.00	4.00	1.00	2.00	4.00	3.00	
800	1.00	3.00	1.00	3.00	1.75	1.00	2.75	3.00	0.50	1.00	3.25	4.00	
1000	0.50	1.00	0.50	1.00	0.75	2.00	1.25	2.00	0.25	1.00	1.50	3.00	
	4th Day				5tht Day				6th Day				
	MG		CG		Μ	MG		CG		MG		CG	
	Me.	Ra.	Me.	Ra.	Me.	Ra.	Me.	Ra.	Me.	Ra.	Me.	Ra.	
Control	0.75	1.00	4.50	3.00	0.00	0.00	4.50	3.00	0.25	1.00	4.75	4.00	
25	0.75	3.00	4.25	6.00	0.00	0.00	4.25	6.00	0.25	1.00	4.50	6.00	
50	0.50	2.00	5.25	6.00	0.50	1.00	5.75	5.00	0.00	0.00	5.75	5.00	
100	0.25	1.00	5.50	6.00	1.00	4.00	6.50	5.00	0.50	1.00	7.00	6.00	
200	0.00	0.00	4.25	8.00	0.00	0.00	4.25	8.00	0.25	1.00	4.50	8.00	
400	1.00	2.00	5.00	3.00	0.50	1.00	5.50	3.00	0.50	1.00	6.00	3.00	
800	0.25	1.00	3.50	4.00	1.00	2.00	4.50	3.00	0.00	0.00	4.50	3.00	
1000	1.25	1.00	2.75	4.00	1.00	2.00	3.75	3.00	0.00	0.00	3.75	3.00	

Momentary Germination (MG), Cumulative Germination (CG), Mean (Me.) and Range (Ra.)

# **3.1.** Germination percentage

Although 800 and 1000 mg kg<sup>-1</sup> concentrations are thought to affect germination adversely (Figure 1), there is

no meaningful difference. Statistical (p<0.05, ANOVA) differences do not found between doses and the control group on the germination of seeds.

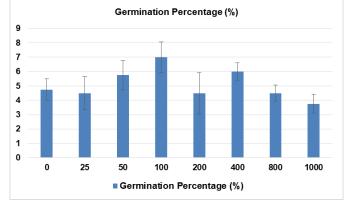


Figure 1. Germination percentage of seeds for different nickel concentrations

Germination is a complicated metabolic plant life, stage in and essential physiological reactions occur in seeds with suitable germination conditions (Afzal et al., 2022). Similar results were found in heavy metal-safflower research that only the highest metal mix concentration (180 mg kg<sup>-1</sup>) decreased the germination (p<0.01; (Houshmandfar and Moraghebi, 2011). In another research, nickel decreased the germination rate of Sorghum bicolor (L.) seeds, but no significant difference was found between nickel doses (Ertekin et al., 2020). Also, nickel applications did not affect germination percentage in Atriplex halimus (L.) and Salicornia ramosissima (J. Woods) plants (Márquez-García et al., 2013).

The seeds under the soil are the first targets of the heavy metals, so seed germination is one of the essential resistant stages to heavy metals (Seregin and Kozhevnikova, 2005; Akıncı and Çalışkan, 2010). In the present study, there is no statistically significant difference between the control group and nickel concentrations (Figure 1). However, it was reported that the inhibitory effects of heavy metals, ion toxicity, oxidative stress and reduced plant growth regulators as a result of auxin degradation (Hameed et al., 2001; Akıncı and Çalışkan, 2010). As a result of these effects, germination may also be reduced.

## 3.2. Germination index

Although the 800 and 1000 mg kg<sup>-1</sup> doses seemed to have a weakly negative effect on germination (Figure 2), there is no significant level difference between the doses and control group at all days according to the multiple comparisons (p<0.05, ANOVA, Duncan Posthoc Test). When the effects of doses are examined on a day-to-day basis, excluding 200, 800 and 1000 mg kg<sup>-1</sup> doses, although a statistically significant difference is found between the doses and the control group (First Day<sup>a</sup>, Other Days<sup>ab</sup>) on the germination index on the first day, the effects of the doses on the germination index on other days are not found statistically significant.

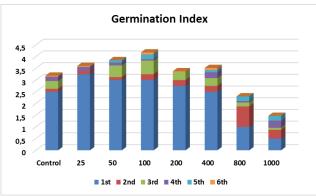


Figure 2. Germination index for different nickel concentrations

Increasing nickel concentrations may affect the germination rate, index, and time. While low nickel doses stimulate germination positively (25 ppm), 50 ppm and higher doses negatively affect spinach. (Akıncı and Akıncı, 2011). Akar (2017) reported that the germination index of heavy metal applicated seeds was higher than the control in the Festuca plant. The inhibitory effect of 200, 800 and 1000 mg kg<sup>-1</sup> doses on the germination index was observed, but other nickel concentrations increased the germination index similarly to Akar's study (2017). The highest GI values were obtained on the first day. It is known that the toxic effects of nickel heavy metal on germination are due to its effects on amylase, protease and ribonuclease enzyme activities (Ahmad and Ashraf, 2011; Sethy and Ghosh, 2013).

### 3.3. Coefficient of velocity of germination

On the 1st, 3rd and 4th days, there is no significant difference between the doses and control group on germination for CVG values (p<0.05, ANOVA, Duncan Posthoc Test). However, excluding 25, 200, 800 and 1000 mg kg<sup>-1</sup> doses, statistically significant differences (p<0.05, ANOVA) are found between the doses and the control group (For Control, 50 and 100 mg kg<sup>-1</sup> doses, 1st<sup>a</sup>, 2nd<sup>a</sup>, Other Days<sup>b</sup>- For 400 mg kg<sup>-1</sup> dose, 1st<sup>a</sup>, 2nd<sup>ab</sup>, 3rd<sup>bc</sup> and 4th<sup>c</sup>, 5th<sup>c</sup>, 6th<sup>c</sup>).

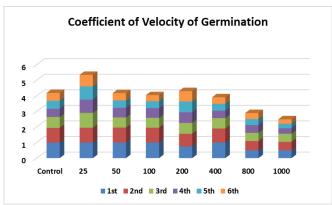


Figure 3. Coefficient of velocity of germination for different nickel concentrations

The coefficient of velocity shows how many seeds were germinated in a certain germination time (Scott et al., 1984). It indicates the rapidity of germination, which rises when the count of germinated seeds increases (Kheloufi et al., 2020; Afzal et al., 2022). This factor reaches a maximum value when all seeds germinate on the first day (Iosob et al., 2019). In the present study, the highest CVG values were obtained on the first day in control applications

# 4. Conclusions

Considering the effects of nickel concentrations on germination percentages, no statistically significant decrease is observed depending on the increase in nickel concentration. However, it is seen that the 1000 mg kg<sup>-1</sup> concentration is slightly effective. However, it is clear from the graphs that increasing concentration positively affects germination up to a certain level of nickel concentration. When the germination index values are examined, it is seen that the increase in nickel concentration, especially on the first day of germination, affects the germination index. When the data obtained from the coefficient of velocity equation are examined, it is possible to say that the germination rate did not statistically change much after the second day. In addition, when looking at the graph related to the coefficient of velocity, it is possible to see the decrease in germination rate caused by the increase in concentration, especially for the first day. It is thought that it is necessary to investigate the effects of nickel heavy metal at higher concentrations on the germination of safflower plants.

## **Declaration of Author Contributions**

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

## **Declaration of Conflicts of Interest**

All authors declare that there is no conflict of interest related to this article.

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