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Germination and seedling growth of two canola (*Brassica napus* L.) cultivars under heavy metals

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Abstract

Heavy metals constitute significant environmental stressors, capable of impeding growth and triggering the production of reactive oxygen species. This study investigates the impact of two heavy metals, cadmium and copper sulfate, on the germination and seedling growth of two canola cultivars. The experiment employs a factorial design based on a completely randomized setup with three replications. Treatments involve cadmium and copper sulfate concentrations of 0, 10, 20, and 30 ml/l, alongside two canola cultivars (Hyola 50 and Homolious). Each petri dish contains 25 seeds, and various treatments, including a distilled water control, are applied. Seed germination percentages are recorded daily at specific intervals, and growth rates are assessed by measuring radicle length, plumule length, and seedling length on the final day of the experiment. Results indicate that different levels of cadmium do not significantly affect germination and seedling growth. In contrast, various concentrations of copper sulfate exert a significant impact on plumule length (PL), radicle length (RL), seedling length (SL), and seed vigor index (SVI) at a 1% significance level. As copper concentration increases, PL, RL, SL, and SVI decrease. Both canola cultivars exhibit significant differences in all measured germination indices at the 1% level, with Hayola 50 demonstrating superior performance and higher values than Homolious for all traits except daily germination speed. Interaction effects between all treatments do not significantly influence any of the traits examined. Therefore, it is recommended to consider Hyola 50 as a suitable plant for phytoremediation due to its superior germination characteristics.

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Introduction

Rapeseed, a significant oil plant cultivated in our country, contributes to over 90% of the nation's oil supply (Rezaei and Malekoti, 2000). The escalation of heavy metal levels poses a critical environmental and health concern. Plants accumulate substantial quantities of heavy substances such as copper, nickel, cobalt, and manganese, leading to plant poisoning (Premon et al., 2014). The toxicity of heavy metals can result in plant damage and death. Multiple sources, including industrial activities, urban areas, and fuel materials, contribute to these pollutants. The application of chemical fertilizers, particularly phosphate fertilizers, further amplifies the presence of these elements (Mohammadzadeh et al., 2010). Elevated levels of heavy metals prompt an upsurge in the production of active radicals, lipid peroxidation, and the formation of malondialdehyde, inducing oxidative stress in plants (Schutzendubel and Polle, 2002; Gouia et al., 2001). While some heavy metals like zinc (Zn), cadmium (Cd), and copper (Cu) are essential for plant growth under optimal conditions, they can impede growth and metabolism when present in excess (Riffaldi and Levi Menzi, 1989). Copper and nickel are crucial elements for plant growth, with copper participating in vital biological reactions as an enzyme cofactor and electron carrier (Sanita di Toppi and Gabbrielli, 1999). However, excessive copper concentrations can inhibit plant growth and induce various cellular changes, affecting membrane permeability, chromatin structure, and enzyme activities in respiration, photosynthesis, and senescence processes (Tewari et al., 2006).

Cadmium, identified as one of the most hazardous soil pollutants, hinders plant root and aerial part growth, substantially reduces crop yield, and disrupts nutrient absorption and biological balance. Additionally, the accumulation of cadmium in important crops and its subsequent entry into the food chain pose severe health and hygiene risks for humans and animals (Wilhelm et al., 2007). Research by Jeliazkova et al. (2003) demonstrated that copper, manganese, and cadmium exerted a more significant impact on the initial growth of fennel, black cumin, and anise roots than on germination. Mahmood et al. (2005) investigated the influence of varying copper and zinc levels on the germination and growth of corn (*Zea mays* L.) seedlings. While germination remained unaffected, the initial growth was severely limited by increasing zinc sulfate concentration. Toxicity symptoms in seedlings escalated in the presence of both elements ($ZnSO4$ and $CuSO4$), with the toxicity being more pronounced in the presence of $ZnSO4$. Given the substantial adverse effects of heavy elements on plant growth, the aim of this research is to investigate various indicators of germination and seedling growth in two canola cultivars exposed to cadmium and copper sulfate.

Materials and Methods

This study was conducted in 2020 at the Seed Technology Laboratory. Initially, the seeds underwent a disinfection process using a 5% sodium hypochlorite solution, followed by three washes with distilled water and a 20-minute disinfection in a 1:1000 benomyl solution. All equipment utilized, including Petri dishes and filter paper, underwent sterilization in an autoclave. The experiment was designed as a factorial within a completely randomized design with three replications. The experimental treatments comprised varying concentrations of heavy metals, cadmium, and copper sulfate (0, 10, 20, and 30 ml), along with two new rapeseed varieties (Hayola 50 and Hemolius). Subsequently, 25 seeds were placed inside each sterilized Petri dish, and the designated treatments, including a distilled water control, were applied. Germinated seed counts were recorded daily at a specific hour over a 12-day period, continuing until no change in the number of germinated seeds was observed in each experimental unit for three consecutive days. Germination criteria were defined as the emergence of a radicle with a length of at least one millimeter from the seed shell (Soltani et al., 2001).

Throughout the growth period, the test units were irrigated in sterile laboratory conditions with the relevant treatments as needed. Root and stem lengths were measured using a millimeter ruler. After the optimal 12-day growth period, germination percentage (GP), daily germination speed (DGS), medium daily germination (MDG), seed vigor index (SVI), plumule length (PL), root length (RL), and seedling length (SL) were measured and calculated.

Germination percentage (GP):

(1) GP = (NG/NT) ×100

In this relation, GP is the percentage of germination, NG is the number of germinated seeds and NT is the total number of seeds. (Maguire, 1962; Nichols and Heydecker, 1986)

Medium Daily Germination (MDG):

Medium daily germination, which is an indicator of daily germination rate, was determined from the following relationship (Lexmond and Vandervorm, 1981):

(2) MDG=FGP/d

In this regard, FGP is the final germination percentage (vigor) and d is the number of days to reach the maximum final germination (experiment period).

Daily Germination Speed (DGS):

This index is the average daily germination with the following formula (Majer et al., 2002):

(3) DGS=1/MDG

Seed Germination Index (SVI):

(Copeland and McDonald, 2001)

(4) SVI = SL × FGP

In this regard, SL is the seedling length and FGP is the final germination percentage.

(5) Stem length + root length = SL

Data analysis was conducted using SAS software and averages were compared using Duncan's test at the probability level of 1%. Graphs were drawn using Excel.

Results

The results of the analysis of variance for the impact of various cadmium and copper sulfate levels on germination and seedling growth indicators (seed germination percentage, shoot length, root length, seedling length, seed stand index, average daily germination, and daily germination rate) in two canola cultivars are summarized in Table 1. As observed, the impact of different cadmium levels is not significant on any of the measured germination indicators and seedling growth. Conversely, various copper sulfate levels exhibit a significant effect at the 1% level, specifically on shoot length, root length, seedling length, and seed germination index. The influence of two canola cultivars is significant at the 1% level for all measured germination indicators and seedling growth. Interactions between treatments do not show a significant effect on any of the traits.

Table 2 presents the comparison of the average effects of different cadmium and copper sulfate levels on the measured germination indices and seedling growth in the two canola cultivars. Figure 1 illustrates that, with an increase in copper sulfate concentration, stem length decreases. The highest value corresponds to the zero-milliliter concentration with an average of 6.03, while the lowest value is at the 30-milliliter concentration with an average of 4.92 mm. Similarly, root length decreases with increasing copper sulfate concentration, with the maximum observed at zero- and 10-milliliter concentrations (averages of 7.62 and 7.30 mm, respectively) and the minimum at 30 ml concentration (average of 5.07 mm). Seedling length also decreases with increasing copper sulfate concentration, reaching the highest averages of 13.66 and 13.64 millimeters at zero and 10 milliliters, respectively, and the lowest average of 10.04 millimeters at 30 milliliters. The seed germination index follows the same trend, reaching the highest values at zero and 10 milliliters (averages of 10.13 and 9.95, respectively) and the lowest at 30 milliliters (average of 7.51). Notably, various copper sulfate levels did not have a significant effect on germination percentage, average daily germination, and daily germination rate. Figure 2 indicates that the effect of the two canola cultivars on all measured germination indices was significant at the 1% level. The Hayola 50 cultivar outperformed in terms of the measured traits, except for the daily germination rate, exhibiting higher average traits than the Hemolius variety.

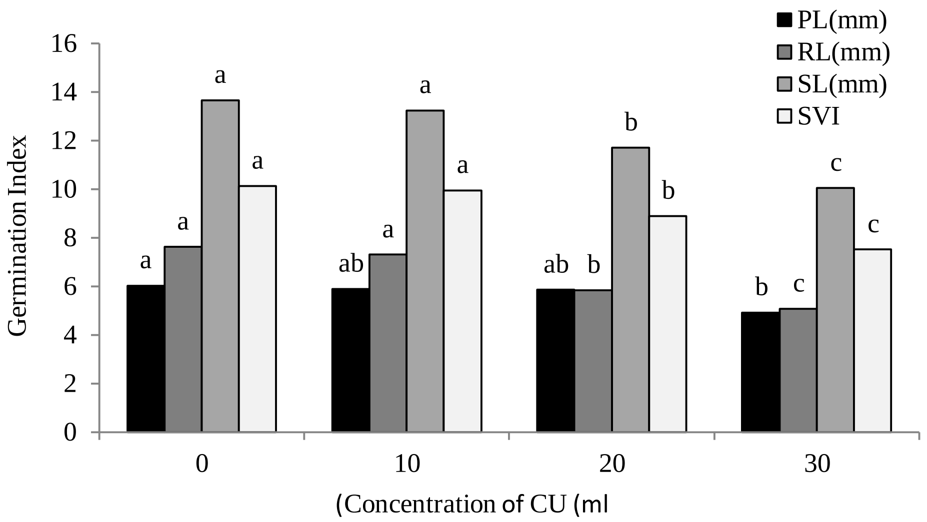

Figure 1. Effect of different concentration of Cu on canola germination indices

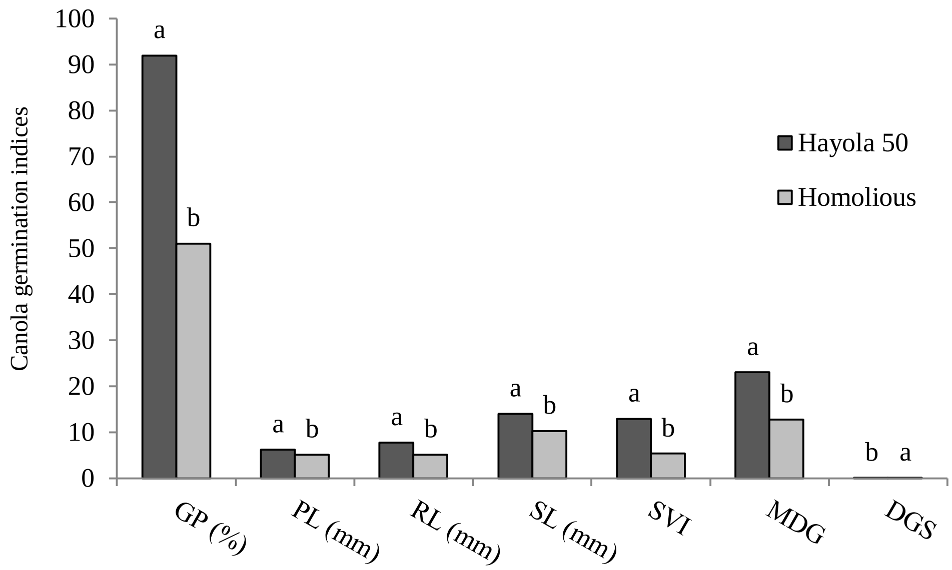

Figure 2. Effect of canola cultivars on different germination indices

Table 1. Variance analysis of different traits of two cultivars of canola as affected by cadmium and copper

S.O.V.	df	No. Germinated Seeds		Germination (%)		Plumule Length (mm)		Radicle Length (mm)		Seedling Length (mm)		Vigor Index		MDG		DGS	
Cadmium (A)	3	2.6493	ns	42.3889	ns	2.2472	ns	3.4437	ns	2.6600	ns	1.7353	ns	2.121	ns	0.0000	ns
Cu (B)	3	7.0938	ns	113.5000	ns	6.1357	**	35.0557	**	65.1815	**	34.7481	**	7.844	ns	0.0003	ns
Cultivars (C)	1	2511.26	**	40180.1667	**	29.7928	**	168.0104	**	338.3631	**	1395.8020	**	2531.76	**	0.0396	**
A x B	9	5.2604	ns	84.1667	ns	2.0166	ns	1.7064	ns	2.1511	ns	2.5009	ns	5.8993	ns	0.0003	ns
A x C	3	8.3715	ns	133.9444	ns	2.9076	ns	2.5793	ns	3.3438	ns	4.8970	ns	8.6493	ns	0.0001	ns
B x C	3	6.4826	ns	103.7222	ns	2.5458	ns	5.6307	ns	13.2884	ns	15.5387	ns	5.9271	ns	0.0003	ns
A x B x C	9	3.1863	ns	50.9815	ns	2.4034	ns	3.8262	ns	9.5363	ns	4.7302	ns	2.8530	ns	0.0003	ns
Error	64	8.6979		139.1667		1.3921		2.8098		5.5176		6.4682		8.7188		0.0004	
C.V. (%)		16.48		16.48		20.77		25.92		19.31		27.87		16.49		24.51	

ns and ** indicate non-significant and significant differences in the probability level of 1 %, respectively.

Table 2. Mean comparisons of different traits of two cultivars of canola as affected by cadmium and copper

Cultivars	No. Germinated Seeds		Germination (%)		Plumule Length (mm)		Radicle Length (mm)		Seedling Length (mm)		Seed Vigor Index		MDG		DGS	
Hayola 50	23.000	a	92.000	a	6.236	a	7.788	a	14.041	a	12.936	a	23.042	a	0.044	b
Homolious	12.771	b	51.083	b	5.122	b	5.142	b	10.286	b	5.310	b	12.771	b	0.084	a
Cu																
0 ml	17.250	a	69.000	a	6.033	a	7.629	a	13.663	a	10.130	a	18.000	a	0.067	a
10 ml	18.250	a	73.000	a	5.899	ab	7.308	a	13.249	a	9.954	a	17.833	a	0.060	a
20 ml	18.417	a	73.667	a	5.854	ab	5.846	b	11.704	b	8.892	b	18.250	a	0.061	a
30 ml	17.625	a	70.500	a	4.929	b	5.075	c	10.040	c	7.515	c	17.542	a	0.066	a
Cadmium																
0 ml	18.000	a	72.000	a	5.817	a	6.813	a	12.629	a	9.520	a	17.250	a	0.063	a
10 ml	17.833	a	71.333	a	5.422	a	6.721	a	12.143	a	9.014	a	18.250	a	0.062	a
20 ml	18.250	a	73.000	a	5.431	a	6.338	a	11.850	a	9.030	a	18.500	a	0.064	a
30 ml	17.458	a	69.833	a	6.046	a	5.988	a	12.033	a	8.927	a	17.625	a	0.065	a

ns and ** indicate non-significant and significant differences in the probability level of 1 %, respectively.

Discussion

Heavy metals exert their inhibitory effects on seed germination through various mechanisms. Some heavy metals hinder germination and initial seedling growth by inhibiting endosperm starch hydrolysis, while others impede germination by damaging the embryos (Mishra and Choudhuri, 1997). At low concentrations, heavy metals may slightly stimulate seed germination, attributed to the excessive production of reactive oxygen species (ROS) and nitrogen (RNS), such as nitric oxide (NO). This stimulates seed germination as oxidative stress gradually increases (Kranner and Colville, 2011). The presence of heavy metals in the germination medium leads to their rapid penetration into the seed, affecting vital physiological processes such as respiration and hindering cell division. This disturbance in seedling growth is attributed to disrupted processes such as membrane permeability, chromatin structure, enzyme activities in respiration and photosynthesis, and induction of senescence (Kranner and Colville, 2011; Márquez García et al., 2013). The seed structure index and the energy required for germination serve as indicators of seed strength, with higher values suggesting a greater ability to germinate (Abdul Baki and Anderson, 1973). Under stress conditions induced by heavy elements, such as copper sulfate and cadmium, a reduction or lack of nutrient transfer from cotyledons to the embryo can result in a decrease in stem length. Additionally, reduced water absorption by seeds under stress conditions decreases hormone secretion and enzyme activity, disrupting seedling growth, both in roots and stems (Chaoui and El Ferjani, 2005).

Saberi et al. (2010) demonstrated the impact of heavy metals, specifically cadmium and copper sulfate, on Saltbush (*Atriplex halimus* L.) seedlings. While cadmium did not significantly affect germination percentage and germination speed, copper sulfate led to a decrease in seedling growth. Premon et al. (2014) explored the effects of heavy metals, including bismuth nitrate, cadmium nitrate, and lead nitrate, on germination and vigor indicators in corn seeds. The study revealed that low concentrations of cadmium did not significantly affect germination percentage but caused a decrease in seed strength and alterations in germination rate indicators with increasing concentrations. Cadmium, nickel, and copper were found to negatively impact root and shoot length, except for germination percentage, while lead, at specific concentrations, showed a positive effect on these traits (Mohammadzadeh et al., 2010). Peralta et al. (2000) investigated the effects of manganese, nickel, copper, chromium, and cadmium on the growth and survival of alfalfa plants. Chromium and cadmium significantly affected seed germination and plant growth at 10 ppm, while copper and nickel impacted at 20 ppm and higher concentrations. In conclusion, the negative effects of heavy metals, particularly cadmium, on germination may result from their accumulation in cells and their binding with protein sulfhydryl groups. This interaction can lead to a decrease in protein synthesis and production, which are essential for growth, cell division, and germination processes (Siddhu and Ali Khan, 2012). Compared to other heavy metals, cadmium has a more pronounced impact on germination and seed strength (Gouia et al., 2001; Das et al., 1997).

Conclusion

The findings of this study indicate that varying concentrations of copper sulfate did not significantly impact the germination percentage of rapeseed cultivars. However, a negative effect was observed on seedling growth and, consequently, on the seed germination index of rapeseed cultivars. On the other hand, the different concentrations of cadmium in this experiment did not significantly affect the germination percentage and seedling growth of rapeseed cultivars. This suggests that these rapeseed cultivars can be considered for plant breeding purposes. Nevertheless, it is noteworthy that the Hyola 50 variety appears to exhibit greater resistance. In conclusion, future research could explore higher concentrations of cadmium on rapeseed. Consequently, the use of Hyola 50 is recommended as a suitable plant for phytoremediation due to its superior germination characteristics.

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