



Germination and seedling growth of two varieties of rice (*Oryza sativa* L.) after presoaking in cobalt sulphate

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Abstract: In this study grains of rice varieties (Sakha 104 and Giza 178) were tested for the response to different concentrations of cobalt sulphate. There was a significant increase in percentage of germination at the low levels of the treatment solution. High cobalt levels caused a reduction in length of radicle and plumule in case of Sakha 104 (above 44.5 μm) and (above 10 μm) in case of Giza 178. The low levels of cobalt were also associated with an increase in vigor index, dry matter of root and shoot of seedlings. The results indicate that high levels of cobalt might be poisonous and have harmful effect on the germination and seedling growth in rice plants.

keywords: *Oryza sativa*, cobalt, germination, seedling vigor.

1. Introduction

Seventeen essential elements are required by plant crops for optimum growth and development. The minerals are called macronutrients when they are needed in relatively high amounts. While micronutrients for plant growth are necessary in relatively smaller amounts, they are as important as macronutrients. If there is a lack or inadequacy of any element in the soil and other nutrients, it can result in growth suppression and even full inhibition [1]. In addition to having several other vital functions in plants, micronutrients often serve as cofactors within enzyme system and participate in redox reaction. Micronutrients are mainly involved in key physiological photosynthesis and respiration processes [1],[2] which can prevent these vital physiological processes thereby limiting the yield.

For the normal metabolic functions in the plant, trace elements are essential, but these are toxic at higher concentrations and can seriously interfere with physiological and biochemical functions[3].

The seeds can be processed with micronutrients either by soaking for a specific time in a nutrient solution (seed priming) or by coating with micronutrients in a specific concentration. Seed invigoration is a relatively new term and was used for both seed treatment

methods interchangeably. [4]. Although several detailed reviews are available on seed enhancements[5], priming[6], on-farm priming[7] and invigoration [4]are available for successful crop production in optimal and stressful environments[8].

Sensitivity of metals and plant toxicities depend not only upon the concentrations and toxicants, but also upon the phase-of-life or biological process (germination, survival of seedling, vegetative growth). Compared with seed germination and the growth of the early seedling, they are more susceptible to metal pollution, given that some of the mechanisms of defense have not developed.[9].

Cobalt as a transition element is an essential component, apart from the B₁₂ vitamin synthesis required for human and animal nutrition, of several enzymes and co-enzymes. [10] [11]. Depending on the level and status of the cobalt in rhizosphere and soil, growth and metabolism of plants have shown to be affected. Co is also essential for legumes in higher plants because of its application by microorganisms in atmospheric nitrogen fixation.[10],[12]. Co has also promoted several developmental processes, such as coleoptile and stem elongation, bud growth, leaf expansion and hypocotyl hook opening. It is also necessary to maintain high yields of

cucumbers[13] and to increase the growth of wheat[14], tomatoes[15] and squash plants[16] at low levels.

Trace elements are required for plant's normal metabolic function but these metals are toxic and can seriously affect physiological and biochemical functions in higher concentrations.[3].

Rice (*Oryza sativa*), is one of the most important strategy crops grown worldwide in a variety of climates. This work, was conducted to evaluate the effects of various level of Co as cobalt sulphate on the germination and seedling vigor of two rice varieties (*Oryza sativa* L. Giza 178 and Sakha 104).

2. Materials and methods

Plant Materials

Rice (*Oryza sativa*) grains var. Sakha 104 and Giza 178 were purchased from Rice Research and Training Center, Sakha, Kafr El-Sheik, Egypt.

Seed soaking and germination assay

In the first trial, the grains of the two cultivars were surface sterilized by immersion in 4.0 % sodium hypochloride solution for 20 minutes. The sterilized seeds were thoroughly washed several times with distilled water. Each cultivar group was divided into 3 sets; every 25 seeds to determine the optimum time of soaking in distilled water to give the best growth. The time of soaking was: 24, 48, and 72 hours. These sets were soaked in distilled water. After that, each group of seeds was put in plastic boxes (22 x 17 x 9 cm) and incubated in the dark at 27 ± 2 C for 7 days. At the end of the germination period, the germination rate was calculated for both cultivars at each period (Fig. 1).

Cobalt treatments

In order to determine the most appropriate concentration of CoSO_4 for rice. The seeds of each group of the two rice cultivars were divided into 11 sets; every 25 seeds were surface sterilized as before. These sets were soaked in different concentrations of CoSO_4 (1, 3, 5, 10, 26.7, 35.6, 44.5, 53.4, 62.3, 71.2 μM) and distilled water (as control) at the optimum time of soaking (recorded from the first trial) and allowed to germinate in plastic boxes (22 x 17 x 9 cm) in the incubator in the

dark at 27 ± 2 °C for 7 days. The grains were irrigated with adequate amount of distilled water twice a day. Each treatment was replicated five times. Seeds were examined every day for germination and the percentage germination was calculated after 7 days from soaking time. For calculations of a dry weight, seedlings were stored for 48 hours in an oven at 80 ° C and electronically the dry weights were calculated. The weight of the decoated seed is generally not used for germination, which is the weight of the living portion of the seed, i.e. the embryo and endosperm, where resides the potential for growth [17].

Germination parameters

- 1. Germination percentage (GP):** Evaluations of the percentage of germination have been calculated as adopted [18]:

$$\text{GP} = \left[\frac{\text{Number of germinated seeds}}{\text{Total number of seed tested}} \right] \times 100$$
- 2. Germination rate (GR):** Germination rate has been calculated as adopted [19]:

$$\text{GR} = \sum_{i=1}^d \text{Ni}/\text{Di}$$

Where; Ni (Number of seeds used) and Di (The number of days after germination).

- 3. Main daily germination:** germination value (GV), pick value (PV) and Mean daily germination (MDG) assessments were calculated using the following equations. [20]:
 - MDG = GP / total experiment days
 - PV = Maximum germinated seed number at one day / day number
 - GV = PV \times MDG
- 4. Seedling vigor index:** It was calculated as adopted by [21]:

$$\text{SVI I} = \text{GP} \times \text{Seedling length}$$

$$\text{SVI II} = \text{GP} \times \text{Seedling weight}$$

Seedling parameters

The seedling parameters was calculated as adopted, including plumule length, radical length, seedling fresh weight and seedling dry weight [22]:

- 1. Plumule length:** The plumule length of the 10 seedlings (7-days-old) from the

seed to the tip of the plumule was recorded and expressed in centimeters.

2. **Radicle length:** The radicle length of 10 seedlings (7-days-old) from the seed to the tip of the radicle was recorded and expressed in centimeters.
3. **Seedling fresh weight:** The weight of 10 seedlings (7-days-old) was measured and expressed in grams.
4. **Seedling dry weight:** The weight of 10 seedlings (7-days-old) was recorded and expressed in grams after oven drying at 80°C for 48 h.
5. **Seedling relative water content (SRWC):** For 7-day-old seedlings, it was calculated as taken by [23] using the following relationship:

$$\text{SRWC} = [(\text{Fresh Weight} - \text{Dry Weight}) / \text{Fresh Weight}] \times 100$$

Statistical analysis

The seed germination and seedling growth data were statistically analyzed to determine the level of significance at $P \leq 0.05$ on personnel computer using one-way analysis of variance (ANOVA) with Post Hoc Duncan's test by COSTAT Version 6.3 (developed by, Cohort software, Berkeley, California, USA).

3. Results and Discussion

I. Germination parameters

(Table 1) in both cultivars the germination percentage was 100 % with 5, 10, 26.7, 35.6, and 44.5 μM CoSO_4 in Sakha 104 cultivar and with 5 and 10 μM CoSO_4 in Giza 178 cultivar as compared with control values. On the other hand, a non-significant increase in % germination was observed with 1 and 3 μM CoSO_4 in Sakha 104 and with 1, 3, 26.7, and 44.5 μM CoSO_4 in Giza 178 as compared with the control values. Furthermore, a pattern similar to control or non-significant decrease in % germination was recorded with 53.4, 62.3, and 71.2 μM CoSO_4 in Sakha 104 and with 35.6, 53.4, 62.3, and 71.2 μM CoSO_4 in Giza 178, as compared with control values. Different authors have reported that metal has a negative effect on germination [17],[24]. Many other authors described the effect of metals on seed germination as reducing the absorption and transport of water [25],[26] or causing death or

an embryonic damage at least [27]. In this context [28] a reduction in the germination percentage of paddy and ragi at higher cobalt levels could be due to cobalt ion interferences. Related germination inhibitions at higher cobalt treatment concentrations have been reported in *Vigna mungo* (L.) [29].

The data presented in table 1 showed a comparable and significant increase in mean daily germination, pick value and germination value with 5, 10, 26.7, 35.6 and 44.5 μM CoSO_4 for Sakha 104, meanwhile a significant increase in mean daily germination and pick value and germination value with 5 and 10 μM CoSO_4 and non-significant change in germination value with the rest concentrations of CoSO_4 for Giza 178 as compared with the control values. On the other hand, similar values with control were recorded in mean daily germination, pick value and germination value with 53.4 and 62.3 μM CoSO_4 in Sakha 104 cultivar and in mean daily germination and pick value at concentrations 35.6, 53.4 and 62.3 μM CoSO_4 in Giza 178 cultivar. The highest concentration 71.2 μM CoSO_4 recorded a non-significant decrease in both cultivars as compared with control values (table 1). It is possible to explain the reduced germination by the decrease in physiological and metabolic activities [30] As stated by [31] the inhibitory effect of Co on germination of wheat seed was noted from 200 ppm onwards (300 and 400 ppm). At the highest concentration of Co (500 ppm), 50 percent of the control was reduced in percentage germination. Higher concentrations of Co may cause ionic toxicity to decrease seed germination or may be due to osmotic effects. [32]. In different crops such as paddy [29], Ragi [33] and *Vigna mungo* [34], similar germination results were reported under Co exposure. From the results of the previous germination parameters (germination percentage, mean daily germination, pick value and germination value), some CoSO_4 concentrations (5, 10, 26.7, 35.6 and 44.5 μM CoSO_4) in Sakha 104 cultivar and (5 and 10 μM CoSO_4), for Giza 178 one were suitable for germination. This indicates that there were differences in the sensitivity to different varieties of cobalt concentrations. The same results have been achieved by [35] in tomato plants. As regards the vigor index I and II in the

variously treated rice seeds of both cultivars presented in table 1, there was a progressive increase in vigor index I and II at 44.5 and 10 μM CoSO_4 , for Sakha 104 and Giza 178, cultivar respectively as compared with control values (Table 1). However, the values of vigor index I and II were either non-significantly increased or decreased with the other concentrations in both cultivars as compared with controls. [36] found, in *Phaseolus vulgaris*, For 100 ppm of Co, the highest germination index was obtained, with values up to 300ppm of Co increasing. Perhaps the triggering effect of Co on *Phaseolus vulgaris* seed was attributed to the fact that Co improved the activity of the enzyme α -amylase, one of the important enzymes involved in the seed germination. [36]. Although Co is essential in minor quantities, a higher level has been shown to have drastic impact on Fe proteins, the activity of catalases, chlorophyll levels, transpiration rate, water potential and mobility of other essential components in some plants. [37].

The recorded vigor index is concentration-dependent and the concentration of Co in Sakha 104 at 44.5 μM CoSO_4 showed the highest vigor index I and II of 1006.800 and 7.095, respectively. Meanwhile, in Giza 178 cultivar at 10.0 μM CoSO_4 showed the highest vigor index I and II of 1419.333 and 4.623, respectively. These results of the vigor index indicate that the addition of Co even at these concentrations had a positive impact on the elongation of plumule and radicle as shown in table 1.

II. Seedling parameters

For both rice cultivars (Sakha 104 and Giza 178) more or less comparable results were obtained, however slight variation being apparent. As shown in table 2 and figure 2 for both (Sakha 104 and Giza 178) cultivars, there was a gradual significant increase in radicle length with 3, 5, 10, 26.7, 35.6 and 44.5 μM CoSO_4 , for Sakha 104 and with 5 and 10 μM CoSO_4 , for Giza 178. Co has been reported to have a significant impact on nitrogen fixation of legumes at lower concentrations [38]. On the other hand, the results showed a significant or non-significant decrease in radicle length with 53.4, 62.3, and 71.2 μM CoSO_4 , for Sakha 104

and with 26.7, 35.6, 44.5, 53.4, 62.3 and 71.2 μM CoSO_4 , for Giza 178 as compared with the control. Cobalt at higher concentrations may directly inhibit the growth of root through cell division or cell elongation, or combinations of the two leading to limited nutrient and water intake and translocations and induced mineral deficiencies [28]. There was a significant increase in plumule length with 1, 3, 5, 10, 26.7, 35.6, and 44.5 μM CoSO_4 , for Sakha 104 cultivar and with 5 and 10 μM CoSO_4 , for Giza 178. Plumule length showed a non-significant change at 53.4 and 62.3 for Sakha 104, meanwhile a significant decrease was detected at 71.2 μM CoSO_4 comparing with the control. For Giza 178, all concentrations above 10 μM CoSO_4 showing a significant decrease in plumule length comparing with the control. The same trend was observed in seedling length for both cultivars, as compared with control values. The magnitude of increment was most pronounced at 44.5 μM CoSO_4 for Sakha 104 cultivar and at 10 μM CoSO_4 for Giza 178 cultivar (Table 2 and figure 2). The suppression of roots and shoot elongation caused by heavy metals may be due to their effect on cell division [39] or their influence on cell wall elasticity and metabolic activity [40], [41] and [42]. The suppression of the elongation of roots and shoots caused by heavy metals may be due to their impact on cell division or their effect on elasticity and metabolic activities of the cell wall. [43] It is reported that reduced root length could be caused by the decrease in mitotic cell division in the meristematic root zone under heavy metal stress. Metal accumulation in roots decreases the mitotic rate in the meristematic zones, particularly by blocking the metaphase in meristematic cells and thus reducing their length. [44]. The decline in the growth of rice seedlings in early seedlings could also be caused by Co is strongly related to the thiol bridge and therefore affects the seedlings growth. [45].

In earlier studies, seed germination was also decreased due to effect on the selected behavior of cell membrane permeability and osmotic alteration [46][47]. Stress caused by metals decreased meristematic cell growth [48] and decreased activity of amylase and protease hydrolytic enzymes leading to lower radicle and plumule length as described by [49]. The

inhibitory effect of high level cobalt levels on radicle and plumula lengths back to Co at high levels could cause cell division or cell elongation inhibitions or combinations, leading to limited exploration of the soil volume in nutrient and water uptake and translocation of nutrients and mineral deficient. [50][51]. Thus, it can be argued that the delay in germination, growth and biomass of the pea seedling under Pb, Co and Cd stress could be caused by its accumulation in radicles and uptake to plumule, interaction with enzymes and other biomolecules and changes in physiological processes required for normal seedling growth [52]. Very little data is collected on the phytotoxic effect of excess Co. Recent studies of Co phytotoxicity in tomato (*Lycopersicon esculentum* L.), rape seed (*Brassica napus* L.) and barley (*Hordeum vulgare* L.) have shown negative effects on shoot growth and biomass [17]. Inhibiting the effect of cobalt on germination and plumule and radicle growth at high concentrations may be due to higher permeability of the embryo cover to it or because of the adverse effect of the metals on the activity of enzymes such as protease and amylase causing food supply to be inhibited by the growing plumule and radicle [53].

For seedling biomass production, careful examination of table 2, showed a significant increase in seedling fresh weight with 5, 10, 26.7, 35.6, 44.5, and 53.4 μM CoSO_4 for Sakha 104 and non-significant change at 62.3 and 71.2 μM CoSO_4 comparing with the control. For seedling dry weight and seedling relative water content (SRWC), there was a significant increase at moderate concentrations (10, 26.7, 35.6, 44.5 and 53.4 μM CoSO_4) with a pronounced increase at 44.5 μM CoSO_4 , meanwhile, there was a significant decrease at high concentrations (62.3 and 71.2 μM CoSO_4) comparing with the control. Furthermore, for Giza 178 cultivar, equal values with controls or a non-significant increase were recorded in seedling fresh weight at 1, 3, 5, 10, 26.7, 35.6, 44.5, and 53.4 μM CoSO_4 . For seedling dry weight, there was fluctuation between significant and non-significant increases at all concentrations used. The same trend was obtained in seedling relative water content (1, 3, 5, 26.7, 35.6, 44.5 and 53.4 μM CoSO_4), as compared with control values, with a

pronounced significant increase in seedling relative water content at 10 μM CoSO_4 as compared with other concentrations and control values (Table 2).

The magnitude of decrease was most pronounced in the previous parameters with concentration 62.3 and 71.2 μM CoSO_4 in both cultivars as compared with control values (Table 2). The decrease in the yield of dry phytomass at higher cobalt levels could have been caused by poor seedlings growth. Similar results have been reported in *Vigna mungo* (L.) Hepper [29]. [54] found that at improving effects on all physiological parameters of wheat plants were observed at lower concentrations (up to 200 ppm). The enhancing effect of lower concentrations of Co on plant height and leaf area has previously been reported [55]. With increasing Co concentrations, RWC decreased may be due to reduced water uptake as a result of inhibition of root growth [30]. On the other hand, [56] found that fresh weights of root, stem and leaves of cypress seedlings were significantly increased by all Co treatments compared to control treatments with the exception of 10, 40 ppm Co for fresh weight of root. The increase in seedling organs i.e. stem, roots and leaves with Co from 10 to 40 ppm was significant and decreased with 80 ppm while the fresh leaves with CO from 10 to 20 ppm were increased significantly, while Co was reduced with 40 and 80 ppm. On the other hand, with increasing Co concentration up to 40 ppm, root dry weight of cypress seedlings was gradually significantly increased, then decreased with 80 ppm, while stem and leaves dry weights were significantly increased with 10 and 20 ppm concentration then decreased with 40 and 80 ppm [56].

Conclusions

The results of germination and seedling growth parameters of both Sakha 104 and Giza 178 rice cultivars, proved that 44.5 μM CoSO_4 was the optimum concentration for growth of Sakha 104, whereas 10 μM CoSO_4 was the optimum concentration for growth of Giza 178 cultivar. From the results of this investigation, it can be concluded that cobalt at lower concentrations has a stimulating effect on the germination process and seedling growth of rice plants. In general, rice grains treatment, by

grain soaking in the optimum concentrations of the nutrient solution of cobalt seems inexpensive and an easy method of micronutrient delivery into the plant especially

at the germination stage which reflects this positive effect on grain yield of rice as an economic and important crop

Table 1: Effects of different concentrations of CoSO₄ on the germination parameters of rice (*Oryza sativa*; Sakha 104 and Giza 178) seedlings of 7-days old. Data are the means of ten replicates ± standard error. Means in each column followed by similar letters are not significantly different at the 5% probability level, using Post Hoc Duncan's test.

Sakha 104						
Parameters Concentrations	Germination %	Mean daily germination	Pick value	Germination value	Vigor index I	Vigor index II
S Control	92.000 ^{bc} ±0.214	30.667 ^{bc} ±0.407	7.667 ^{bc} ±0.104	235.407 ^{bc} ±0.125	580.133 ^d ±0.652	4.513 ^{de} ±0.127
S 1 μM CoSO ₄	96.000 ^{ab} ±0.241	32.000 ^{ab} ±0.407	8.000 ^{ab} ±0.091	256.296 ^{ab} ±0.112	662.800 ^c ±0.184	4.735 ^{cde} ±0.082
S 3 μM CoSO ₄	96.000 ^{ab} ±0.125	32.000 ^{ab} ±0.406	8.000 ^{ab} ±0.085	256.296 ^{ab} ±0.112	736.533 ^c ±0.265	5.023 ^{cd} ±0.114
S 5 μM CoSO ₄	100.000 ^a ±0.214	33.333 ^a ±0.436	8.333 ^a ±0.084	278.074 ^a ±0.418	846.000 ^b ±0.479	5.760 ^b ±0.013
S 10 μM CoSO ₄	100.000 ^a ±0.235	33.333 ^a ±0.412	8.333 ^a ±0.063	278.074 ^a ±0.404	861.867 ^b ±0.687	6.465 ^a ±0.085
S 26.7 μM CoSO ₄	100.000 ^a ±0.123	33.333 ^a ±0.432	8.333 ^a ±0.066	278.074 ^a ±0.408	869.200 ^b ±0.469	6.567 ^a ±0.090
S 35.6 μM CoSO ₄	100.000 ^a ±0.102	33.333 ^a ±0.424	8.333 ^a ±0.088	278.074 ^a ±0.408	927.200 ^b ±0.250	6.637 ^a ±0.257
S 44.5 μM CoSO ₄	100.000 ^a ±0.132	33.333 ^a ±0.403	8.333 ^a ±0.034	278.074 ^a ±0.308	1006.800 ^a ±0.366	7.095 ^a ±0.176
S 53.4 μM CoSO ₄	92.000 ^{bc} ±0.105	30.667 ^{bc} ±0.424	7.667 ^{bc} ±0.076	235.407 ^{bc} ±0.315	524.133 ^d ±0.534	5.359 ^{bc} ±0.025
S 62.3 μM CoSO ₄	92.000 ^{bc} ±0.100	30.667 ^{bc} ±0.411	7.667 ^{bc} ±0.101	235.407 ^{bc} ±0.415	503.200 ^d ±0.563	4.332 ^{de} ±0.184
S 71.2 μM CoSO ₄	88.000 ^c ±0.111	29.333 ^c ±0.401	7.333 ^c ±0.100	215.407 ^c ±.519	299.733 ^e ±0.388	4.247 ^e ±0.108
Giza178						
Parameters Concentrations	Germination %	Mean daily germination	Pick value	Germination value	Vigor index I	Vigor index II
G Control	92.000 ^{bc} ±0.214	30.667 ^{bc} ±0.235	7.667 ^{bc} ±0.085	241.926 ^{abc} ±0.122	914.667 ^c ±0.733	3.779 ^b ±0.004
G 1 μM CoSO ₄	96.000 ^{ab} ±0.211	32.000 ^{ab} ±0.234	8.000 ^{ab} ±0.056	256.296 ^{ab} ±0.213	988.800 ^c ±0.536	4.601 ^a ±0.001
G 3 μM CoSO ₄	96.000 ^{ab} ±0.231	32.000 ^{ab} ±0.211	8.000 ^{ab} ±0.075	256.296 ^{ab} ±0.213	946.533 ^c ±0.551	3.909 ^{ab} ±0.006
G 5 μM CoSO ₄	100.000 ^a ±0.236	33.333 ^a ±0.256	8.333 ^a ±0.096	278.074 ^a ±0.222	1189.200 ^b ±0.386	4.513 ^a ±0.007
G 10 μM CoSO ₄	100.000 ^a ±0.164	33.333 ^a ±0.235	8.333 ^a ±0.063	278.074 ^a ±0.222	1419.333 ^a ±0.329	4.623 ^a ±0.003
G 26.7 μM CoSO ₄	96.000 ^{ab} ±0.145	32.000 ^{ab} ±0.201	8.000 ^{ab} ±0.088	256.296 ^{ab} ±0.213	735.333 ^d ±0.308	3.965 ^{ab} ±0.003
G 35.6 μM CoSO ₄	92.000 ^{bc} ±0.135	30.667 ^{bc} ±0.211	7.667 ^{bc} ±0.066	235.407 ^{bc} ±0.204	568.000 ^e ±0.476	3.784 ^b ±0.006
G 44.5 μM CoSO ₄	96.000 ^{ab} 0.102	32.000 ^{ab} ±0.201	8.000 ^{ab} ±0.042	256.296 ^{ab} ±0.213	534.000 ^{ef} ±0.158	3.901 ^{ab} ±0.001
G 53.4 μM CoSO ₄	92.000 ^{bc} ±0.019	30.667 ^{bc} ±0.222	7.667 ^{bc} ±0.099	235.407 ^{bc} ±0.204	465.867 ^f ±0.123	3.768 ^b ±0.001
G 62.3 μM CoSO ₄	92.000 ^{bc} ±0.184	30.667 ^{bc} ±0.236	7.667 ^{bc} ±0.101	235.407 ^{bc} ±0.204	359.600 ^g ±0.547	3.529 ^{bc} ±0.003
G 71.2 μM CoSO ₄	88.000 ^c ±0.145	29.333 ^c ±0.233	7.333 ^c ±0.111	215.407 ^c ±0.196	255.600 ^h ±0.257	3.053 ^c ±0.002

Table 2: Effects of different concentrations of CoSO₄ on the seedling parameters of rice (*Oryza sativa*; Sakha 104 and Giza 178) seedlings of 7-days old. Data are the means of ten replicates ± standard error. Means in each column followed by similar letters are not significantly different at the 5% probability level, using Post Hoc Duncan's test.

Sakha 104						
Parameters Concentrations	Radicle length(cm)	Plumule length(cm)	Seedling length(cm)	Seedling fresh weight(g)	Seedling dry weight (g)	Seedling relative watercontent(%)
S Control	3.000 ^d ±0.033	3.300 ^e ±0.088	6.300 ^{ef} ±0.088	0.035 ^d ±0.001	0.0137 ^d ±0.0002	61.122 ^{cd} ±0.407
S 1 μM CoSO ₄	3.100 ^{cd} ±0.033	3.800 ^d ±0.033	6.900 ^e ±0.058	0.035 ^d ±0.001	0.0140 ^{cd} ±0.0003	60.169 ^d ±0.624
S 3 μM CoSO ₄	3.500 ^c ±0.033	4.167 ^{cd} ±0.195	7.667 ^d ±0.204	0.037 ^{cd} ±0.001	0.0153 ^{bcd} ±0.0002	58.492 ^d ±0.478
S 5 μM CoSO ₄	4.167 ^b ±0.069	4.300 ^{cd} ±0.088	8.467 ^c ±0.150	0.042 ^c ±0.001	0.0157 ^{abc} ±0.0002	62.680 ^{bcd} ±0.293
S 10μM CoSO ₄	4.267 ^b ±0.227	4.367 ^{bc} ±0.107	8.633 ^{bc} ±0.190	0.048 ^b ±0.000	0.0167 ^{ab} ±0.0002	65.282 ^{abc} ±0.229
S 26.7μM CoSO ₄	4.300 ^b ±0.088	4.400 ^{bc} ±0.088	8.700 ^{bc} ±0.153	0.049 ^{ab} ±0.000	0.0167 ^{ab} ±0.0002	65.963 ^{ab} ±0.600
S 35.6μM CoSO ₄	4.400 ^a ±0.033	4.867 ^{ab} ±0.107	9.267 ^b ±0.117	0.049 ^{ab} ±0.002	0.0173 ^a ±0.0005	64.495 ^{abc} ±0.926
S 44.5μM CoSO ₄	4.800 ^a ±0.033	5.267 ^a ±0.069	10.067 ^a ±0.069	0.054 ^a ±0.002	0.0170 ^{ab} ±0.0003	68.472 ^a ±0.295
S 53.4μM CoSO ₄	2.700 ^d ±0.088	3.000 ^{ef} ±0.067	5.700 ^{fg} ±0.033	0.042 ^c ±0.001	0.0160 ^{ab} ±0.0003	62.218 ^{bcd} ±0.251
S 62.3μM CoSO ₄	2.233 ^e ±0.069	3.233 ^e ±0.051	5.467 ^g ±0.038	0.033 ^d ±0.001	0.0137 ^d ±0.0005	59.052 ^d ±0.286
S 71.2μM CoSO ₄	0.733 ^f ±0.051	2.667 ^f ±0.051	3.400 ^h ±0.088	0.033 ^d ±0.001	0.0153 ^{bcd} ±0.0005	53.552 ^e ±0.301
Giza178						
Parameters Concentrations	Radical length(cm)	Plumule length(cm)	Seedling length(cm)	Seedling fresh weight(g)	Seedling dry weight (g)	Seedling relativewater content (%)
G Control	5.067 ^c ±0.051	4.867 ^c ±0.126	9.933 ^d ±0.139	0.029 ^{ab} ±0.001	0.0117 ^{ab} ±0.0005	59.232 ^{bcd} ±0.623
G 1 μM CoSO ₄	5.300 ^c ±0.203	5.000 ^c ±0.167	10.300 ^c ±0.115	0.036 ^a ±0.001	0.0123 ^a ±0.0002	65.121 ^{abcd} ±0.665
G 3 μM CoSO ₄	4.967 ^c ±0.154	4.900 ^c ±0.088	10.233 ^c ±0.126	0.031 ^{ab} ±0.002	0.0097 ^{cd} ±0.0002	68.243 ^{ab} ±0.947
G 5 μM CoSO ₄	6.167 ^b ±0.135	5.733 ^b ±0.019	11.900 ^b ±0.153	0.036 ^a ±0.003	0.0113 ^{abc} ±0.0005	67.457 ^{abc} ±0.939
G 10μM CoSO ₄	7.733 ^a ±0.084	6.467 ^a ±0.171	14.200 ^a ±0.088	0.035 ^a ±0.002	0.0093 ^d ±0.0002	72.821 ^a ±0.531
G 26.7μM CoSO ₄	3.900 ^d ±0.153	3.767 ^d ±0.019	7.667 ^d ±0.135	0.031 ^{ab} ±0.001	0.0100 ^{bcd} ±0.0003	68.127 ^{ab} ±0.423
G 35.6μM CoSO ₄	3.300 ^{de} ±0.088	2.867 ^e ±0.019	6.167 ^e ±0.084	0.029 ^{ab} ±0.001	0.0117 ^{ab} ±0.0005	60.304 ^{bcd} ±0.371
G 44.5μM CoSO ₄	3.800 ^d ±0.200	2.267 ^f ±0.038	5.567 ^f ±0.069	0.030 ^{ab} ±0.000	0.0103 ^{bcd} ±0.0002	65.950 ^{abc} ±0.414
G 53.4μM CoSO ₄	2.967 ^e ±0.019	2.100 ^{fg} ±0.033	5.067 ^f ±0.038	0.030 ^{ab} ±0.000	0.0113 ^{abc} ±0.0002	61.809 ^{bcd} ±0.150
G 62.3μM CoSO ₄	2.167 ^f ±0.051	1.733 ^{gh} ±0.107	3.900 ^g ±0.153	0.027 ^b ±0.000	0.0113 ^{abc} ±0.0002	58.031 ^{cd} ±0.366
G 71.2μM CoSO ₄	1.600 ^f ±0.033	1.300 ^h ±0.033	2.900 ^h ±0.058	0.024 ^b ±0.000	0.0107 ^{abcd} ±0.0002	55.563 ^d ±0.412

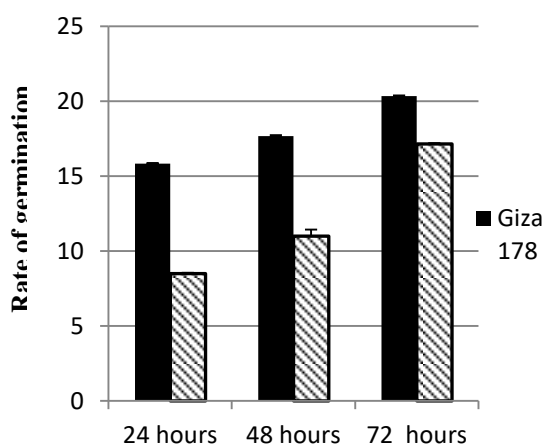


Fig. 1: Effect of different soaking periods in water on germination rate for both rice varieties in Sakha 104 and Giza 178 seedlings after 7 days germination. Vertical bars represent the standard error (\pm SE).

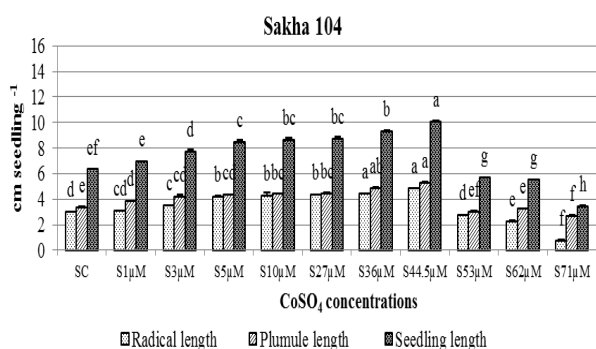


Fig. 2: Effect of different concentrations of CoSO₄ on radicle length, plumule length, and seedling length of rice (*Oryza sativa* Sakha 104) seedlings of 7-days-old. Vertical bars represent the standard error (\pm SE).

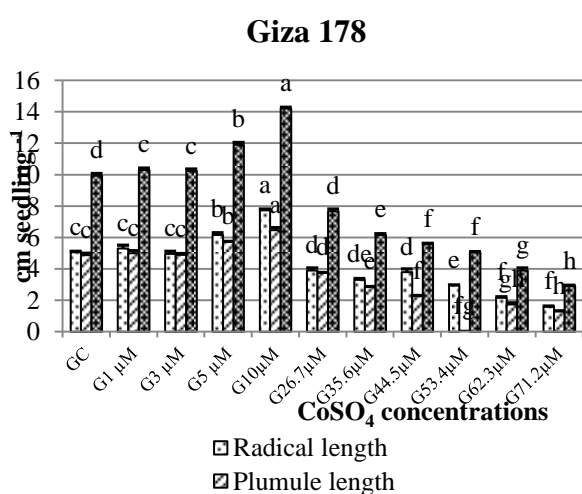


Fig. 3: Effect of different concentrations of CoSO₄ on radicle length, plumule length, and seedling length of rice (*Oryza sativa* Giza 178) seedlings of 7-days-old. Vertical bars represent the standard error (\pm SE).

4. References

- 1 K. Mengel, E. A. Kirkby, H. Kosegarten, and T. Appel, (2001). "Principles of plant nutrition.,(Kluwer Academic Publishers: Dordrecht, The Netherlands)," Princ. plant Nutr. Kluwer Acad. Publ. Dordrecht, Netherlands.,
- 2 H. Marschner, (1995) "Mineral nutrition of higher plants 2nd edition," Acad. Gt. Britain.,
- 3 K. Jayakumar, M. Rajesh, K. S. Ganesh, and P. Vijayarengan, (2013) "Cobalt alters the nodulation, leg-haemoglobin content and antioxidant potential of horse gram (*Dolichos biflorus*)," *Intl J Mod Biochem*, vol. 2, pp. 15–23.,
- 4 M. Farooq, S. M. A. Basra, A. Wahid, A. Khaliq, and N. Kobayashi, (2009) "Rice seed invigoration: a review," in *Organic farming, pest control and remediation of soil pollutants*, Springer, , pp. 137–175.
- 5 A. G. Taylor, P. S. Allen, M. A. Bennett, K. J. Bradford, J. S. Burris, and M. K. Misra, (1998) "Seed enhancements," *Seed Sci. Res.*, vol. 8, no. 2, pp. 245–256.
- 6 M. Black and J. D. Bewley, (2000) *Seed technology and its biological basis*. Crc Press.,
- 7 D. Harris, (2006) "Development and testing of 'On-Farm' seed priming," *Adv. Agron.*, vol. 90, pp. 129–178.,
- 8 M. Farooq, A. Wahid, S. M. A. Basra, and K. Siddique, (2010) "Improving crop resistance to abiotic stresses through seed invigoration," in *Handbook of Plant and Crop Stress*, CRC Press, , pp. 1031–1050.
- 9 X. Liu, S. Zhang, X. Shan, and Y.-G. Zhu, (2005) "Toxicity of arsenate and arsenite on germination, seedling growth and amyolytic activity of wheat," *Chemosphere*, vol. 61, no. 2, pp. 293–301.,
- 10 S. R. Young, (1983.) "Recent advances of cobalt in human nutrition," *Micronutr. New*, vol. 3, no. 3,
- 11 "Effect of Cobalt and Nickel on Plant Growth, Yield and Flavonoids (2007) Content of *Hibiscus sabdariffa* L.," *Australian Journal of Basic and Applied Sciences.*, pp. 73–78.,
- 12 H. J. Evans and M. Kliewer, (1964) "Vitamin B12 compounds in relation to

- the requirements of cobalt for higher plants and nitrogen-fixing organisms,” *Ann. N. Y. Acad. Sci.*, vol. **112**, no. 2, pp. 735–755,.
- 13 G. Scott and K. P. William, (1976) “Cobalt and plant development,” *Plant Physiol*, vol. **57**, pp. 886–889,.
 - 14 S. B. Wilson and D. J. D. Nicholas, (1967). “A cobalt requirement for non-nodulated legumes and for wheat,” *Phytochemistry*, vol. **6**, no. 8, pp. 1057–1066,
 - 15 A. M. A. Aly, N. G. Shehata, and T. M. Kobbia, (1991) “Effect of cobalt on tomato plant growth and mineral content,” *Ann. Agric. Sci. Ain Shams Univ.*(Egypt),.
 - 16 M. A. Atta-Aly, (1998) “Soaking summer squash seeds in low concentrations of cobalt solution before sowing increased plant growth, femaleness, and fruit yield via increasing plant ethylene level,” *J. Plant Growth Regul.*, vol. **17**, no. 1, pp. 25–32,.
 - 17 W. Li, M. A. Khan, S. Yamaguchi, and Y. Kamiya, (2005) “Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*,” *Plant Growth Regul.*, vol. **46**, no. 1, pp. 45–50,.
 - 18 F. Rezaie, M. Yarnia, and B. Mirshekari, (2008) “Allopathic effects of *Chenopodium album*, *Amaranthus retroflexus* and *Cinodon dactylon* on germination and growth of rapeseed,”.
 - 19 M. A. Ranal and D. G. de Santana, (2006) “How and why to measure the germination process?,” *Brazilian J. Bot.*, vol. **29**, no. 1, pp. 1–11,.
 - 20 R. H. Ellis and E. H. Roberts, (1981) “The quantification of ageing and survival in orthodox seeds,” *Seed Sci. Technol.*,.
 - 21 K. K. Kalsa and B. Abebie, (2012) “Influence of seed priming on seed germination and vigor traits of *Vicia villosa* ssp. *dasycarpa* (Ten.),” *African J. Agric. Res.*, vol. **7**, no. 21, pp. 3202–3208,.
 - 22 A. A. Kandil, A. E. Shareif, and M. A. Gad, (2017) “Effect of salinity on germination and seeding parameters of forage cowpea seed,” *Res. J. Seed Sci*, vol. **10**, pp. 17–26,.
 - 23 B. B. M. Sridhar, F. X. Han, S. V Diehl, D. L. Monts, and Y. Su, (2007.) “Effects of Zn and Cd accumulation on structural and physiological characteristics of barley plants,” *Brazilian J. Plant Physiol.*, vol. **19**, no. 1, pp. 15–22,
 - 24 J. K. Datta, A. Bandhyopadhyay, A. Banerjee, and N. K. Mondal, (2011) “Phytotoxic effect of chromium on the germination, seedling growth of some wheat (*Triticum aestivum* L.) cultivars under laboratory condition,” *J. Agric. Technol.*, vol. **7**, no. 2, pp. 395–402,.
 - 25 M. Black and J. D. Bewley, (1983) “Physiology and biochemistry of seeds in relation to germination.,”.
 - 26 J. M. Becerril *et al.*, “Changes induced by cadmium and lead in gas exchange and water relations,” *Plant Physiol. Biochem*, vol. **27**, no. 6, pp. 913–918, (1989).
 - 27 M. Wierzbicka and J. Obidzińska, (1998). “The effect of lead on seed imbibition and germination in different plant species,” *Plant Sci.*, vol. **137**, no. 2, pp. 155–171,
 - 28 K. Jayakumar, C. A. Jaleel, and M. M. Azooz, (2008) “Impact of cobalt on germination and seedling growth of *Eleusine coracana* L. and *Oryza sativa* L. under hydroponic culture,” *Glob. J. Mol. Sci.*, vol. **3**, no. 1, pp. 18–20,.
 - 29 K. Jayakumar and P. Vijayarengan, (2006) “Influence of cobalt on seed germination and seedling growth of *Vigna mungo* (L.) Hepper.,” *Plant Arch.*, vol. **6**, no. 2, pp. 681–682,.
 - 30 S. V Kuriakose and M. N. V Prasad, (2008). “Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* (L.) Moench by changing the activities of hydrolyzing enzymes,” *Plant Growth Regul.*, vol. **54**, no. 2, pp. 143–156,
 - 31 B. Sarma, P. Devi, N. Gogoi, and Y. M. Devi, (2014) “Effects of cobalt induced stress on *Triticum aestivum* l. crop,” *Asian J. Agric. Biol.*, vol. **2**, no. 2, pp. 137–147,.
 - 32 S. S. Shaukat, M. Mushtaq, and Z. S. Siddiqui, (1999) “Effect of cadmium, chromium and lead on seed germination, early seedling growth and phenolic contents of *Parkinsonia aculeata* L. and *Pennisetum americanum* (L.) Schumann,”

- Pakistan J. Biol. Sci.*,.
- 33 C. A. Jaleel, Z. Changxing, K. Jayakumar, and M. Iqbal, (2009). "Low concentration of cobalt increases growth, biochemical constituents, mineral status and yield in *Zea mays*," *J. Sci. Res.*, vol. **1**, no. 1, pp. 128–137,
- 34 O. Munzuroglu and H. Geckil, (2002) "Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*," *Arch. Environ. Contam. Toxicol.*, vol. **43**, no. 2, pp. 203–213,.
- 35 S. A. Hasan, S. Hayat, A. S. Wani, and A. Ahmad, (2011) "Establishment of sensitive and resistant variety of tomato on the basis of photosynthesis and antioxidative enzymes in the presence of cobalt applied as shotgun approach," *Brazilian J. Plant Physiol.*, vol. **23**, no. 3, pp. 175–185, , doi: 10.1590/S1677-04202011000300001.
- 36 I. M. Zeid (2001), "Responses of *Phaseolus vulgaris* to chromium and cobalt treatments," *Biologia Plantarum*, vol. **44**, no. 1. pp. 111–115,.
- 37 P. C. Nagajyoti, K. D. Lee, and T. V. M. Sreekanth, (2010). "Heavy metals, occurrence and toxicity for plants: A review," *Environmental Chemistry Letters*, vol. **8**, no. 3. pp. 199–216,
- 38 S. Palit, A. Sharma, and G. Talukder, (1994). "Effects of cobalt on plants," *The Botanical Review*, vol. **60**, no. 2. pp. 149–181,
- 39 J. Hagemeyer and S.-W. Breckle, (1996). *Growth under trace element stress*. New York: Marcel Dekker,
- 40 S. Naseer, A. Nisar, and M. Ashraf, (2001). "Effect of salt stress on germination and seedling growth of barley (*Hordeum vulgare* L.)," *Pak. J. Biol. Sci.*, vol. **4**, no. 3, pp. 359–360,
- 41 M. Kabir, M. Z. Iqbal, M. Shafiq, and Z. R. Farooqi, (2008) "Reduction in germination and seedling growth of *Thespesia populnea* L., caused by lead and cadmium treatments," *Pak. J. Bot.*, vol. **40**, no. 6, pp. 2419–2426,
- 42 M. Vijayaragavan, C. Prabhakar, J. Sureshkumar, A. Natarajan, P. Vijayarengan, and S. Sharavanan, (2011) "Toxic effect of cadmium on seed germination, growth and biochemical contents of cowpea (*Vigna unguiculata* L.) plants," *Int. Multidiscip. Res. J.*, vol. **1**, no. 5,.
- 43 D. Lerda, (1992) "The effect of lead on *Allium cepa* L.," *Mutat. Res. Lett.*, vol. **281**, no. 2, pp. 89–92,.
- 44 B. A. Sharifah and O. Hishashi, (1992) "Effect of lead, cadmium and zinc on the cell elongation of *Impatiens balsamina*," *Environ. Experi. Bot.*, vol. **32**, pp. 439–448,.
- 45 F. Lieten, (2001). "Iron nutrition of strawberries grown in peat bags," *Small Fruits Rev.*, vol. **1**, no. 2, pp. 103–112,
- 46 M. Shafiq, M. Z. Iqbal, and A. Mohammad, (2008) "Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*," *J. Appl. Sci. Environ. Manag.*, vol. **12**, no. 3,.
- 47 S. Baruah and J. Dutta, (2019) "Zinc stannate nanostructures: hydrothermal synthesis," *Sci. Technol. Adv. Mater.*,.
- 48 M. Kabir, M. Z. Iqbal, and M. Shafiq, (2009) "Effects of lead on seedling growth of *Thespesia populnea* L.," *Adv. Environ. Biol.*, pp. 184–191,.
- 49 M. Y. Ashraf, R. Sadiq, M. Hussain, M. Ashraf, and M. S. A. Ahmad, (2011) "Toxic effect of nickel (Ni) on growth and metabolism in germinating seeds of sunflower (*Helianthus annuus* L.)," *Biol. Trace Elem. Res.*, vol. **143**, no. 3, pp. 1695–1703,.
- 50 M. Stiborová, M. Doubravová, and S. Leblová (1986), "A comparative study of the effect of heavy metal ions on ribulose-1, 5-bisphosphate carboxylase and phosphoenolpyruvate carboxylase," *Biochem. und Physiol. der Pflanz.*, vol. **181**, no. 6, pp. 373–379.
- 51 K. Jayakumar, C. A. Jaleel, M. M. Azooz, P. Vijayarengan, M. Gomathinayagam, and R. Panneerselvam (2009), "Effect of different concentrations of cobalt on morphological parameters and yield components of soybean," *Glob. J. Mol. Sci.*, vol. **4**, no. 1, pp. 10–14,
- 52 S. Wang *et al.*, (2014) "Variations in

- metal tolerance and accumulation in three hydroponically cultivated varieties of *Salix integra* treated with lead,” *PLoS One*, vol. **9**, no. 9, p. e108568,.
- 53 N. Baruah, S. C. Mondal, M. Farooq, and N. Gogoi, (2019). “Influence of heavy metals on seed germination and seedling growth of wheat, pea, and tomato,” *Water, Air, Soil Pollut.*, vol. **230**, no. 12, p. 273,
- 54 B. Sarma, P. Devi, N. Gogoi, and Y. M. Devi, (2014) “Effects of cobalt induced stress on *Triticum aestivum* L. crop,” *Asian J Agri Biol*, vol. **2**, pp. 137–147,.
- 55 R. E. Kadhim, (2011) “Effect of Pb, Ni and Co in growth parameters and metabolism of *Phaseolus aureus* Roxb,” *Euphratej J. Agric. Sci.*, vol. **3**, no. 3, pp. 10–14,.
- 56 R. A. Eid, A. M. A. Mazher, R. K. M. Khalifa, and S. H. A. Shaaban, (2015) “Growth, chemical constituents and mineral content of Cypress (*Cupressus Sempervirens*, L) seedling grown on sandy soil as influenced by Nickel and Cobalt in irrigation water,” *Int. J. ChemTech Res.*, vol. **8**, no. 12, pp. 104–110,.