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ORIGINAL ARTICLE

Response of Heavy Metals Stress during Seed Germination and Early Seedling Growth in Oil Crop Black Sesame (*Sesamum indicum* L.)

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During past few years, it has been globally experienced that uncontrolled urbanization and industrialization have contributed the accumulation of heavy metals in water and soil which causes physiological stress to the growing plants that finally leads to negative effects. Present study was undertaken to analyze the response of heavy metals (0.01%, 0.1%, and 1.0%) of $HgCl_2$ and equivalent concentrations of $CoCl_2$, $NiCl_2$,$CdCl_2$, $ZnSO_4$, and $CuSO_4$ (5mg/L, 10mg/L, 25mg/L, 50mg/L, and 100mg/L) during seed germination and early seedling growth in oil crop black sesame (*Sesamum indicum* L., cv. TMV3). Responses in terms of germination (partial and full) percentage mean were recorded after 3-days and 7-days of treatments respectively. Results reveal that even very low concentration (0.01%) of $HgCl_2$ treatment was seen to be significantly inhibitory leading to full seed germination (60±1.0%) after 7-days of treatments in comparison to control experiment (100±0.5%). Moreover, further higher concentration (0.1%) of $HgCl_2$ was proved to be completely lethal. Among solutions of chloride metals (Co, Ni, and Cd), $NiCl_2$ (25mg/L) was found to be the most toxic heavy metal and the rate of germination was recorded as (10±1.0%) followed by $CdCl_2$ (40±1.0%) and $CoCl_2$ (50±1.0%) after 7-days of treatments. Further, among sulfate solutions of Zn and Cu-heavy metals, $CuSO_4$ (100mg/L) proves to be strongly inhibitory (0±0.0%) for sesame seed germination in comparison to $ZnSO_4$ (30±0.5%). Significantly, very low concentration (0.01%) of $HgCl_2$ was also emerged as significantly toxic for seedling growth, hence, root-shoot length ratio (0.38±0.14cm/0.44±0.16cm) of seedlings were recorded after 10-days of treatments in comparison to control seedling (2.11±1.04cm/3.77±0.19cm). However, among chloride solutions of cobalt, nickel, and cadmium, $NiCl_2$ solution even with (10mg/L) was proved to be strongly inhibitory during early seedling growth and thus, root-shoot length ratio (0.03±0.02cm/0.33±0.22cm) was recorded. Moreover, further higher concentrations (25mg/L and above) of $NiCl_2$ treatments were proved to be strongly lethal and suppressed significantly both seed germination and seedling growth. Additionally, $CoCl_2$ and $CdCl_2$ treatments were also exhibited similar inhibitory responses in terms of inhibitions in root formation at high concentration (25mg/L). Furthermore, during sulfate solutions of Zn and Cu-treatments, $CuSO_4$ solution (100mg/L) was found to be strongly inhibitory even for seed germination while in contrast $ZnSO_4$ (100mg/L) solution was failed to suppress seed germination completely and seedling length (0.1±0.11cm/0.11±0.6cm) was recorded. Hence, present study reveals that among various heavy metals employed, Hg was found to be the strongest inhibitor for sesame seed germination and during early seedling growth followed by Ni, Cd, and Co while Zn was proved as very weak inhibitor in comparison to Cu heavy metal.

Key words: Abiotic Stress, Black Sesame, Germination, Heavy Metals, Seedling

Sesame (*Sesamum indicum* L.) is known as an economically important oil seed crop and it is widely cultivated in many parts of the world in food nutraceutical and pharmaceutical industries because of high contents of oil protein and antioxidants (Kamaraj *et al*., 2017). In fact, sesame is being grown in arid and semi-arid areas where high temperatures, high levels of solar radiation, high evaporation rate and drought climates are very common which negatively impact the productivity at large scale (Hassanzadeh *et al*., 2009; Witcombe *et al*., 2007; Dossa *et al*., 2019).

Furthermore, soil contamination in cultivated fields caused by industrial effluents mixed with toxic heavy metals has emerged as another new challenge to agriculture. Most of the effluents and wastes contain heavy metals at toxic levels to cause severe damage to crop plants (Khan *et al*., 2006). Like other organisms, plants are very sensitive to either deficiency or excess of heavy metals (El Rasafi *et al*., 2016). Hence, accumulation of toxic concentration of heavy metals in the agricultural soils is the major limitations for the cultivation of crops or failure of crops (Munzuroglu and Geckil, 2002; Mahmood *et al.*, 2007).

Literature reveals that Cu, Pb and Zn are the most commonly available heavy metals in waste amended agricultural soils (Nriagu and Pacyna, 1988; Younas and Shahzad, 1998; Jamal *et al*., 2002). However, copper is one of the most important micronutrients that are essential for plant growth (Alloway, 1995). Similarly, zinc is a non-redox micronutrient element, which has key structural and catalytic roles in many proteins and enzymes involved in energy metabolism (Sresty and Madhava, 1999; Hall and Williams, 2003). Moreover, lead is neither an essential nor a beneficial element for plant growth (Alloway, 1995).

Unfortunately, the adverse effects of heavy metals available in agricultural soil have received a very limited attention on food crops particularly sesame. So, to increase crop productivity, development of heavy metal stress tolerant genotypes of black sesame plants could be always of a meaningful approach. In general, seed germination is a crucial phase in plant life that plays important roles in seedling establishment and subsequent growth of seedlings (Bewley, 1997).

Moreover, seed germination is regulated by multiple endogenous factors, such as plant hormones, and by environmental conditions, including temperature and light (Qu *et al*., 2008; Weitbrecht *et al*., 2011; Cho *et al*., 2012; Miransari and Smith, 2014; Liu *et al*., 2018). Hence, during present study, evaluation of sesame seed response during germination and early seedling growth under heavy metals stress conditions was undertaken that could be an alternative and stable approach to cultivate heavy metals stress tolerant black sesame oil crop that is required for coastal regions as well.

MATERIALS AND METHODS

Seed Collection and Sterilization

Seeds of black sesame (*Sesamum indicum* L., cv. TMV3) cultivars were collected from Tamil Nadu Agriculture University, TNAU, Coimbatore (India). Healthy and uniform seeds were selected and washed thoroughly with teepol-20 for 15-20 min and further were surface sterilized with ethanol (70%) for 1-3 minutes followed by $HgCl_2$ (0.1%) treatments for 8-10 minutes.

Furthermore, sterilized seeds were washed 3-4 times with distilled water and were soaked in the respective stress solutions for 3hrs. The soaked seeds were then transferred to sterile petridishes (9.0cm diameter) lined with two sterile filter papers added with 5ml of distilled water or the respective heavy metals test solutions.

Stress Treatments

Sesame seeds were treated with chloride solutions of heavy metals (Hg, Co, Ni, and Cd) and sulfate solutions of (Zn and Cu) metals in different concentrations. Responses of these stress treatments during seed germination and early seedling growth were recorded after 3-days and 7-days of treatments.

Moreover, chloride solutions of heavy metals (Hg, Co, Ni, and Cd), various concentrations of $HgCl_2$ (0.01%, 0.1%, and 1.0%) solutions while (5mg/L, 10mg/L, 25mg/L, 50mg/L, and 100mg/L) in equal concentrations of other heavy metals ($CoCl_2$, $NiCl_2$, and $CdCl_2$) were considered for the sesame seed treatments.

Furthermore, to understand the response of zinc and copper stress in sesame oil crop, seeds were also treated with solutions of $ZnSO_4$ and $CuSO_4$ in equal concentrations (5mg/L, 10mg/L, 25mg/L, 50mg/L, and 100mg/L).

During stress treatments, 15-20 seeds per petridish were employed and three replicates were performed in each treatment. Germination tests were conducted under dark condition at normal room temperature (25-30°C) in laboratory conditions. A seed was considered germinated when radicles were 2mm long and moreover, a control experiment was maintained with distilled water.

The germination mean percentage was determined based on counting the number of germinated seeds on the 3rd and 7th day of the treatments. Moreover, responses of heavy metals during early seedling growth were measured and calculated in terms of lengths of root and shoot of the seedlings after stress treatments.

Statistical Analysis

Statistical data were performed after first count (3rd day after treatments as partial seed germination) and final count (7th day after treatments as complete seed germination). Moreover, germination percentage (GP) and germination rate (GR) was calculated by using the following formula (Ruan *et al.,* 2002).

GP = Number of total germinated seeds/ Total number of seeds tested × 100

$$GR = \frac{\text{Number of Germinated seeds}}{3^{rd}\text{ Day of Count}} + \frac{\text{Number of Germinated Seeds}}{7^{th}\text{ Day of Count}}$$

RESULTS

Under stress conditions, observations for seed germination were recorded at the end of 3rd day and 7th day of the treatments as the incomplete and complete seed germination respectively. Moreover, seedlings lengths as root-shoot length ratio were also recorded at the end of 7th day of treatments before transferring to paper cup soil.

Response of Heavy Metals Stress during Seed Germination

Results reveal that seed germination was found to be significantly affected under heavy metals (Hg, Co, Ni, Cd, Zn, and Cu) stress conditions and responses of these heavy metals were mainly dependent on concentrations of specific heavy metals in the solutions and on durations of stress treatments.

Effect of Mercury Stress

With very low concentration (0.01%) of $HgCl_2$ treatments, seeds were seen to initiate germination at the end of 3rd day of treatments like control experiments. Furthermore, development of the complete and healthy seedlings was observed in control experiment at the end of 7th day of $HgCl_2$ treatments **(FIG.1A)** while seeds that were treated with $HgCl_2$ (0.01%) solutions, germinated seedlings were seen as reasonably suppressed **(FIG.1B)**.

Significantly, seeds that were treated with further higher concentration of $HgCl_2$ (0.1%) solution, were found to show complete inhibitions in germination **(FIG.1C)** indicating that $HgCl_2$ (0.1%) concentration proves to be toxic and lethal for sesame seed germination. Moreover, the rate of seed germination was found to be influenced with the increase in $HgCl_2$ concentrations.

In control experiment, the germination rate was recorded as (100±0.5%) at the end of 7th day of treatments while in seeds that were treated with very low concentration (0.01%) of $HgCl_2$ solution, mean germination percentage could be obtained significantly low (60±1.0%). Furthermore, higher concentrations (0.1% and 1.0%) of $HgCl_2$ solutions were proved to be completely inhibitory for sesame seed germination **(Table -1)**.

Effect of Cobalt Stress

Various concentrations of $CoCl_2$ (5mg/L, 10mg/L, 25mg/L, 50mg/L, and 100mg/L) solutions were employed during the present study. Control seeds and $CoCl_2$ (5mg/L and 10mg/L) solutions treated seeds were found to exhibit the complete and healthy germination (**FIG.1D**) within 7- days of $CoCl_2$ solution treatments. However, further higher concentrations (25mg/L, 50mg/L, and 100mg/L) of $CoCl_2$ solutions were proved to be strongly inhibitory for seed germination and seedling growth **(FIG. 1E& F)** respectively and these poorly or incompletely germinated seeds were failed to grow into complete seedlings even after 7- days of $CoCl_2$ solution treatments.

After 7-days of treatments, the maximum germination rate (100±0.5%) was recorded in control experiment. However, in lower concentrations (5mg/L and 10mg/L) of $CoCl_2$ solutions treated experiments, seeds were found to exhibit little inhibited response and thus at the end of 7th day of treatments, mean germination percentage (90±0.0% and 80±1.0%) respectively was obtained.

Significantly, with further higher concentration of $CoCl_2$ (25mg/L) solution, seed germination was found to be reasonably inhibitory and resulted (50±1.0%) of seed germination percentage. However, further higher concentrations of $CoCl_2$ (50mg/L and 100mg/L) solutions were proved to be considerably effective to suppress seed germination after 7th day of treatments **(Table–2)**.

Effect of Nickel Stress

Unlike $CoCl_2$ solution response, $NiCl_2$ (5mg/L and 10mg/L) solutions treated seeds were found to show significantly slow and inhibited germination after 3rd days of treatments. Moreover, at the end of 7th day of $NiCl_2$ solution treatments, seeds that were treated with low concentration (5mg/L) of $NiCl_2$ solution were seen to germinate and grow further into complete seedlings in comparison to control experiment (**FIG.1G)**.

However, with higher concentrations of $NiCl_2$ (25mg/L and 50mg/L) solutions, sesame seed germination was found to be completely inhibited **(FIG.1H & I**) indicating that even low concentration of $NiCl_2$ (25mg/L) solution was proved to be considerably toxic for root formation during sesame seed germination.

In general, the rate of sesame seed germination was observed to decrease with the increase in $NiCl_2$ concentrations in the treatment solutions. With lower concentrations of $NiCl_2$ (5mg/L and 10mg/L) solutions, frequency of seed germination was found to be sharply declined and recorded as (80±1.5% and 40±1.0%) respectively (**Table-3)**. However, $NiCl_2$ (25mg/L) solution was proved to be highly effective to inhibit sesame seed germination and frequency of germinated seed without root formation was obtained as low as (20±1.0%) after 7th day of $NiCl_2$ – solution treatments. Moreover, these poorly germinated seeds were failed to grow further into complete seedlings.

Effect of Cadmium Stress

With lower concentrations (5mg/L and 10mg/L) of $CdCl_2$ treated solutions, sesame seeds were found to show germination initiation like control experiments while seeds that were treated with higher concentrations of $CdCl_2$ (25mg/L, 50mg/L, and 100mg/L) solutions could show symptoms of inhibited germination. At the end of 7th day of $CdCl_2$ solution treatments, the growth of germinated seedlings was found to be almost similar in seeds that were treated with $CdCl_2$ (5mg/L and 10mg/L) solutions (**FIG.2 A)** respectively while during treatments with further higher concentrations of $CdCl_2$ (25mg/L and 50mg/L) solutions, incubated seeds were found to show either slight or strong suppression in seed germination and remarkably were failed to grow into complete seedlings (**FIG.2 B & C**).

Furthermore, very high concentration (100mg/L) of $CdCl_2$ solution was proved to be completely lethal for sesame seed germination. Significantly, after 7th day of $CdCl_2$-treatments, seeds that were treated with low concentration (5mg/L) of $CdCl_2$ solution could show the complete seed germination with root and shoot while other higher concentrations of $CdCl_2$ (10mg/L and above) solutions were proved to be effective to suppress root formation completely in germinating sesame seeds (**Table–4)**.

Effect of Zinc Stress

Another heavy metal Zn in various concentrations (5mg/L, 10mg/L, 25mg/L, 50mg/L, and 100mg/L) as $ZnSO_4$ were employed. At the end of 3rd day of treatments, in control experiments and also with lower concentrations of $ZnSO_4$ (5mg/L and 10mg/L**)** treated solutions, seed germination was found to be similar without any indication of Zn- stress induced germination inhibition. However, further increase in $ZnSO_4$ concentrations (25mg/L, 50mg/L, and 100mg/L) in the solutions could show slight inhibitions in germination of seeds in comparison to lower concentrations and their growths were observed to be relatively slow.

Moreover, at the end of 7th day of $ZnSO_4$ solution treatments, the growth of seedlings particularly in seeds that were treated with low concentration of $ZnSO_4$ (10mg/L) solution was observed to be almost like control experiment **(FIG.2D)** while it was found to be little suppressed with higher concentration (50mg/L) of $ZnSO_4$ solution **(FIG.2E).** However, with very high concentration of $ZnSO_4$ (100mg/L) solution, germinated seeds were appeared to be considerably inhibited **(FIG.2F)**. Furthermore, very high concentration (100mg/L) of $ZnSO_4$ solution was proved to be significantly toxic and lethal for sesame seed germination.

The rate of germination was found to decrease with the increase in $ZnSO_4$ concentrations in the solutions. With lower concentrations of $ZnSO_4$ (5mg/L and 10mg/L) solutions, the germination frequency was found to be almost equal (100±1.5% and 90±0.5%) respectively after 7th day of treatments. However, further increase of $ZnSO_4$ concentrations in the solutions exhibited gradual inhibitions in the rate of sesame seed germination. Moreover, with very high concentration of $ZnSO_4$ (100mg/L) solution, germination was little inhibited and thus germination frequency was recorded as 30±0.5% (**Table – 5)**.

Effect of Copper Stress

Unlike $ZnSO_4$ response to sesame seed, $CuSO_4$ solution treatments with various concentrations (5mg/L, 10mg/L, 25mg/L, 50mg/L, and 100mg/L) were seen as inhibitory for seed germination and seedling growth. Moreover, in comparison to $ZnSO_4$ metal response, sesame seeds were found to exhibit the gradual inhibitions in germination with all the tested concentrations (5mg/L and 25mg/L) of $CuSO_4$ solutions after 7th day of treatments (**FIG.2G & H)** respectively.

Interestingly, seeds that were treated with high concentration (50mg/L) of $CuSO_4$ solution could show very slow germination indicating that 50mg/L of $CuSO_4$ solution could be sub-lethal concentration (**FIG.2I**) for sesame seed germination while very high (100mg/L) concentration of $CuSO_4$ solution was proved to be fully toxic.

The rate of sesame seed germination was observed to decrease with the increase in copper sulfate concentrations in the treatment solutions. During $CuSO_4$ solution treated experiments, all the concentrations were proved to be effective for seed germination, however, at the end of 3rd day of treatments, merely beginning of germination could be observed with the tested concentrations of $CuSO_4$ (5mg/L, 10mg/L, 25mg/L, and 50mg/L) solutions and complete seedling developments could be recorded after 7th day of treatments.

During this study, in comparison to $ZnSO_4$ stress response, high concentration (100mg/L) of $CuSO_4$ solution was proved to be strongly effective to inhibit completely sesame seed germination and therefore, seed germination frequency was recorded as zero after 7-days of treatments (**Table –6)**.

Response of Heavy Metals Stress during Early Seedling Growth

Responses of heavy metals stresses during growth of seedlings were also found to be affected by heavy metals treatments, moreover, responses were dependent on concentrations of specific heavy metals in the solutions and on durations of stress treatments.

Response of Hg -Stress

In comparison to seedlings growing under control condition (**FIG.3A**), seedlings that were growing with even low concentration (0.01%) of $HgCl_2$ solution were found to be strongly inhibited (**FIG.3B)**. Moreover, in general there was tendency of inhibition in root formation including lateral roots. In comparison to control, seedlings were appeared as shorter in treated solution and, thus, root-shoot length ratio of seedling was recorded as (0.38±0.14cm/0.44±0.16cm) in comparison to control seedling (2.11±1.04cm/3.77±0.19cm) (**Table-1**).

Response of Co-Stress

During $CoCl_2$ treatments in sesame seed, (5mg/L and 10mg/L) of $CoCl_2$ solutions were found to exhibit normal seedlings growth after 7 – days of treatments and responses were appeared to be similar to the control seedlings (**FIG. 3C)**. Significantly, higher concentrations of $CoCl_2$ (25mg/L and 50mg/L) solutions were proved to be strongly inhibitory and moreover, germinated seeds were failed to grow further into healthy seedlings (**FIG. 3D)**. However, very high concentration of $CoCl_2$ (100mg/L) solution was proved to be toxic.

In general, seedlings lengths were found to decline with the increase in $CoCl_2$ concentrations in treated solutions and therefore, with $CoCl_2$ (5mg/L) solution treatment, seedling length was found to be the maximum and root-shoot length ratio was recorded as (2.31±0.54cm/2.31±0.36cm) in comparison to control seedling (2.11±1.04cm/3.77±0.19cm) (**Table-2**). Moreover, seedlings that were growing with low concentration (5mg/L) of $CoCl_2$ solution were shifted to the cup soil stage followed by treatments with respective concentration of $CoCl_2$ solutions **(FIG. 4B)** were indicated poor growth in comparison to control seedlings **(FIG. 4A)**.

Response of Ni-Stress

In comparison to $CoCl_2$ treated seedlings, $NiCl_2$ stress treatment was found to be more suppressive even with low concentration (5mg/L) solution and seedlings lengths were found to be little reduced while root length was found to be partially suppressed (**FIG. 3C & E)** respectively. Seedlings lengths were found to decline with the increase in $NiCl_2$ concentrations in treated solutions and therefore, with 25mg/L of $NiCl_2$ solution treatment, seedling length was found to be minimal and root formation was totally inhibited (**FIG. 3F)**, hence root-shoot length ratio was recorded as (0.0cm/0.14±0.09cm) in comparison to control seedling (2.11±1.04cm/3.77±0.19cm) (**Table-3**).

Moreover, seeds that were treated with concentration (25mg/L) of $NiCl_2$ solution were found to germinate partially without root and failed to grow further (**FIG.3F)**. Additionally, $NiCl_2$ solution (5mg/L)-treated seedlings were transferred to cup soil and were irrigated with $NiCl_2$ (5mg/L) solution **(FIG. 4C)** were found to exhibit suppressed growth.

Response of Cd-Stress

Height of differently $CdCl_2$ solution- treated sesame seedlings were observed to be slightly affected by continuous stress caused by cadmium heavy metal. For the survival tendency and growth of the seedlings, the maximum length was observed in the seedlings that were growing under control condition than the seedlings that were growing with $CdCl_2$ stress treatments.

Moreover, seedling growth was found to be slightly inhibited at lower concentrations (5mg/L and 10mg/L) of $CdCl_2$ solutions in comparison to control seedlings **(FIG. 3G)** while seedlings growing with little higher concentration (25mg/L) of $CdCl_2$ solution were found to show significant suppression in root formation (**FIG. 3H**). Furthermore, sesame seedlings growing with higher concentrations (50mg/L and 100mg/L) of $CdCl_2$ solutions were found to show inhibitory response considerably.

Results indicated that the stress caused by heavy metal $CdCl_2$ even with low concentration (25mg/L) solution could be proved completely inhibitory for root formation in germinated seeds. However, with high concentration (100mg/L) of $CdCl_2$ solution treatments, seedling development was totally restricted.

Hence, seedlings lengths were found to decline with the increase in $CdCl_2$ concentrations in the treated solutions and therefore, under stress treatments with 5mg/L of $CdCl_2$ solution, seedling length was found to be the maximum and hence, root-shoot length ratio was recorded as (0.22±0.09cm/2.05±0.28cm) in comparison to control seedling (2.11±1.04cm/3.77±0.19cm) (**Table-4**).

Moreover, seedlings growing with $CdCl_2$ solutions (5mg/L and 10mg/L) were transferred to paper cup soil and irrigated with respective $CdCl_2$ solutions were found to show gradual inhibitions in their growths **(FIG. 4D &E)** respectively.

Response of Zn- Stress

Seedling height, shoot and root elongation in sesame were found to be less affected with the increase in $ZnSO_4$ concentrations in the treatment solutions. Seedlings that were growing with concentrations of $ZnSO_4$ (5mg/L, 10mg/L, 25mg/L, and 50mg/L) solutions were found to show very least inhibitory response to heavy metal Zn stress (**FIG. 3I & J**) and seedlings lengths were appeared to be less variable.

Furthermore, seedlings growing with very high concentration (100mg/L) of $ZnSO_4$ solution were recorded as minimum in length and thus, root-shoot length ratio was recorded as (0.1±0.11cm/0.11±0.6cm) in comparison to control seedling

(2.11±1.04cm/3.77±0.19cm) (**Table-5**). After 7th day of $ZnSO_4$ solution treatments, growing seedlings were transferred to paper cup soil and further treated with respective $ZnSO_4$ solutions **(FIG. 4F & G)**.

Response of Cu-Stress

Height of various concentrations of $CuSO_4$ solutions treated sesame seedlings were significantly affected by continuous stress of $CuSO_4$ treatments. For the survival tendency and growth of the seedlings, the maximum length was observed in the seedlings that were growing under control condition than the $CuSO_4$ -treated seedlings. Moreover, seedling growth was found to be similar to the control seedlings at lower concentrations of $CuSO_4$ treatments (5mg/L and 10mg/L) **(FIG.3K)**. However, the seedlings that were growing with high concentration of $CuSO_4$ (25mg/L) solution were found to be relative inhibited (**FIG.3L)**. However, with very high concentration of $CuSO_4$ (100mg/L) solution treatment, seed germination was completely arrested.

Seedlings lengths were found to decline with the increase in $CuSO_4$ concentrations in the treated solutions. Moreover, seedlings growing with $CuSO_4$ (50mg/L) solution, seedling length was found to be minimal and therefore, root-shoot length ratio was recorded as (0.92±0.32 cm/1.16±0.45cm) in comparison to control seedling (2.11±1.04cm/3.77±0.19cm) (**Table-6**). Furthermore, $CuSO_4$ solutions treated seedlings were transferred to paper cup soil and were continuously irrigated with respective concentration of $CuSO_4$ solutions **(FIG. 4H & I)**.

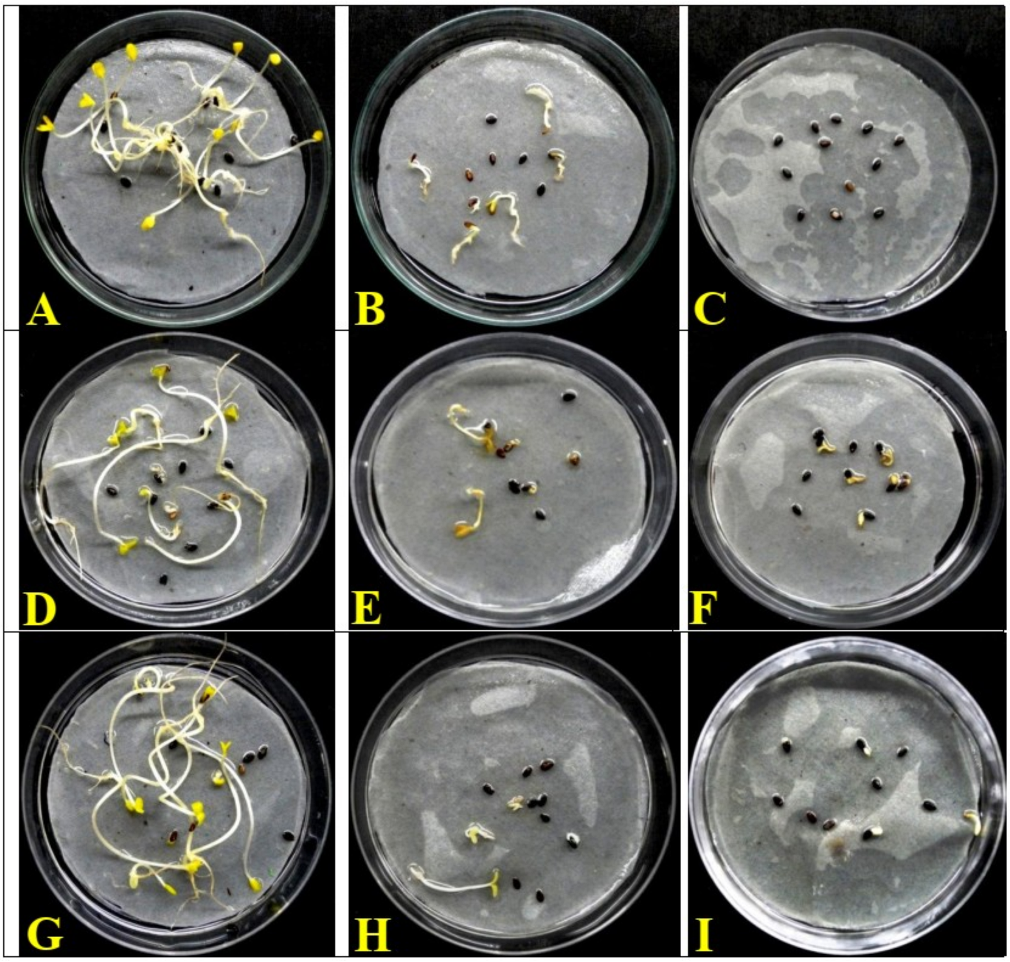

Figure 1. (A-I) *Sesamum indicum* L., response of heavy metals stress on seed germination- **(A)** Control **(B)** 0.01% of $HgCl_2$ **(C)** 0.1% of $HgCl_2$ **(D)** 5mg/L of $CoCl_2$ **(E)** 10mg/L of $CoCl_2$ **(F)** 25mg/L of $CoCl_2$ **(G)** 5mg/L of $NiCl_2$ **(H)**10mg/L of $NiCl_2$ **(I)** 25mg/L of $NiCl_2$ (after 7thday of treatments).

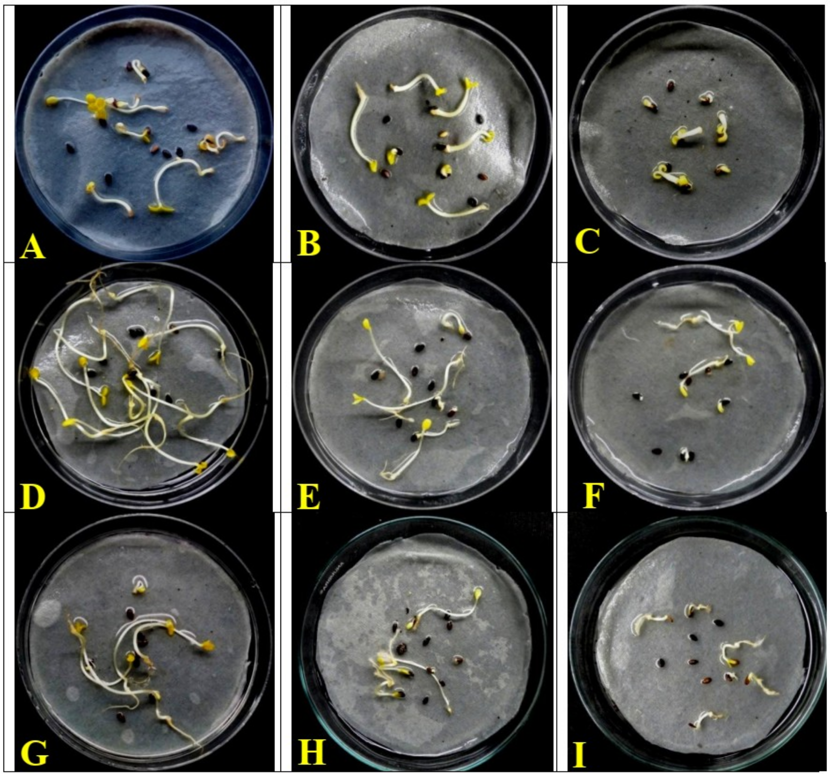

Figure 2. (A-I) *Sesamum indicum* L., response of heavy metals stress on seed germination–**(A)** 5mg/L of $CdCl_2$ **(B)** 10mg/L of $CdCl_2$ **(C)** 25mg/L of $CdCl_2$ **(D)** 10mg/L of $ZnSO_4$ **(E)** 50mg/L of $ZnSO_4$ **(F)** 100mg/L of $ZnSO_4$ **(G)** 5mg/L of $CuSO_4$ **(H)** 25mg/L of $CuSO_4$ **(I)** 50mg/L of $CuSO_4$ (after 7- days of treatments).

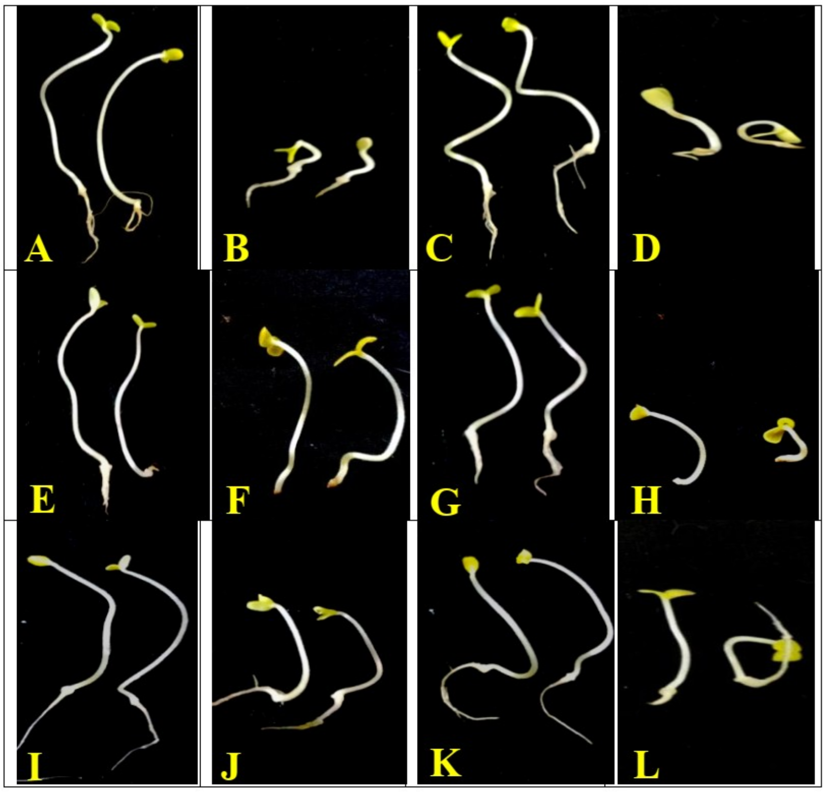

Figure 3. (A-L) *Sesamum indicum* L., Heavy metals stress- **(A)** Control **(B)** 0.01% of $HgCl_2$ **(C)** 5mg/L of $CoCl_2$ **(D)** 25mg/L of $CoCl_2$ **(E)** 5mg/L of $NiCl_2$ **(F)** 25mg/L of $NiCl_2$ **(G)** 5mg/L of $CdCl_2$ **(H)** 25mg/L of $CdCl_2$ **(I)** 10mg/L of $ZnSO_4$ **(J)** 50mg/L of $ZnSO_4$ **(K)** 5mg/L of $CuSO_4$ **(L)** 25mg/L of $CuSO_4$ (Seedlings after 10- days of treatments).

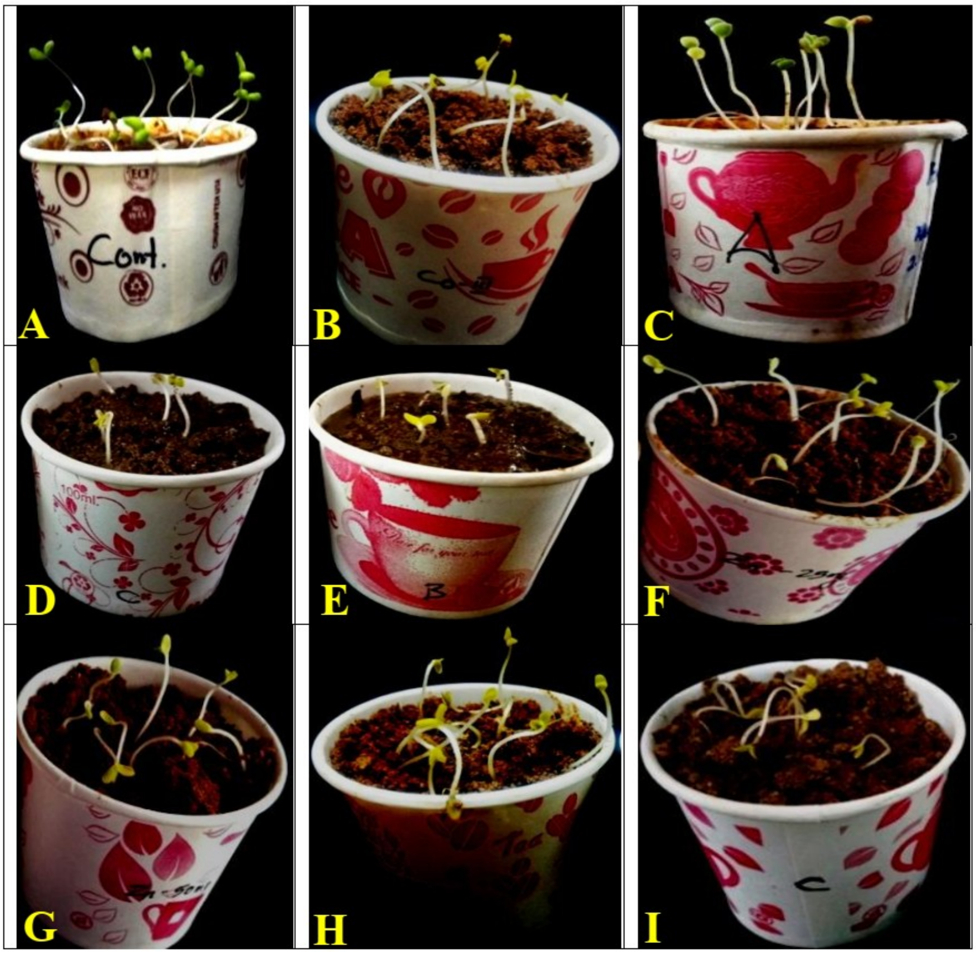

Figure 4. (A-I) *Sesamum indicum* L., Heavy metals stress - **(A)** Control **(B)** 5mg/L of $CoCl_2$ **(C)** 5mg/L of $NiCl_2$ **(D)** 5mg/L of $CdCl_2$ **(E)**10mg/L of $CdCl_2$ **(F)** 10mg/L of $ZnSO_4$ **(G)** 50mg/L of $ZnSO_4$ **(H)** 5mg/L of $CuSO_4$ **(I)** 25mg/L of $CuSO_4$ (Seedlings growing in paper cups after 10th day of treatments).

Table 1. *Sesamum indicum* L., Effects of $HgCl_2$ stress on seed germination and early seedling growth .

S. No.	Concentration of $HgCl_2$ (%)	3rd Day	7th Day		
		% Seed germination (Mean ± S.E.)	% Seed germination (Mean ± S.E.)	Root length (cm) (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
1.	Control	100±1.0	100±0.5	2.11±1.04	3.77±0.19
2.	0.01	40±1.0	60±1.0	0.38±0.14	0.44±0.16
3.	0.1	0	0	0	0
4.	1.0	0	0	0	0

Table 2. *Sesamum indicum* L., Effects of $CoCl_2$ stress on seed germination and early seedling growth .

S. No.	Concentration of $CoCl_2$(mg/L)	3rd Day	7th Day		
		% Seed germination (Mean ± S.E.)	% Seed germination (Mean ± S.E.)	Root length (cm) (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
1.	Control	100±1.0	100±0.5	2.11±1.04	3.77±0.19
2.	5	60±1.5	90±0.0	2.31±0.54	2.31±0.36
3.	10	50±0.5	80±1.0	2.12±0.76	1.67±0.60
4.	25	50±0.5	50±1.0	0	0.09±0.21
5.	50	10±0.5	0	0	0
6.	100	0	0	0	0

Table 3. *Sesamum indicum* L., Effects of $NiCl_2$ stress on seed germination and early seedling growth.

S. No.	Concentration of $NiCl_2$ (mg/L)	3rd Day	7th Day		
		% Seed germination (Mean ± S.E.)	% Seed germination (Mean ± S.E.)	Root length (cm) (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
1.	Control	100±1.0	100±0.5	2.11±1.04	3.77±0.19
2.	5	60±0.5	80±1.5	0.14±0.09	1.34±0.15
3.	10	20±1.0	40±1.0	0.03±0.02	0.33±0.22
4.	25	10±1.0	10±1.0	0	0.14±0.09
5.	50	0	0	0	0
6.	100	0	0	0	0

Table 4. *Sesamum indicum* L., Effects of $CdCl_2$ stress on seed germination and early seedling growth.

S. No.	Concentration of $CdCl_2$ (mg/L)	3rd Day	7th Day		
		% Seed germination (Mean ± S.E.)	% Seed germination (Mean ± S.E.)	Root length (cm) (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
1.	Control	100±1.0	100±0.5	2.11±1.04	3.77±0.19
2.	5	80±0.5	90±1.0	0.22±0.09	2.05±0.28
3.	10	80±0.0	80±1.0	0..13±0.07	1.37±0.34
4.	25	40±0.5	40 ±1.0	0	0.43±0.17
5.	50	10±0.5	10±0.5	0	0.17±0.7
6.	100	0	0	0	0

Table 5. *Sesamum indicum* L., Effects of $ZnSO_4$ stress on seed germination and early seedling growth.

S. No.	Concentration of $ZnSO_4$(mg/L)	3rd Day	7th Day		
		% Seed germination (Mean ± S.E.)	% Seed germination (Mean ± S.E.)	Root length (cm) (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
1.	Control	100±1.0	100±0.5	2.11±1.04	3.77±0.19
2.	5	80±1.0	100±1.5	2.23±0.82	3.27±0.57
3.	10	70±0.5	90±0.5	2.17±0.67	3.18±0.62
4.	25	40±0.0	80±2.0	1.25±0.26	1.53±0.46
5.	50	30±0.5	50±0.0	0.9±0.34	0.12±0.12
6.	100	10±0.5	30±0.5	0.1±0.11	0.11±0.6

Table 6. *Sesamum indicum* L., Effects of $CuSO_4$ stress on seed germination and early seedling growth.

S. No.	Concentration of $CuSO_4$(mg/L)	3rd Day	7th Day		
		% Seed germination (Mean ± S.E.)	% Seed germination (Mean ± S.E.)	Root length (cm) (Mean ± S.E.)	Shoot length (cm) (Mean ± S.E.)
1.	Control	100±1.0	100±0.5	2.11±1.04	3.77±0.19
2.	5	60±0.5	100±0.5	2.12±0.74	3.13±0.57
3.	10	40±0.0	90±1.0	1.63±0.26	3.08±0.72
4.	25	30±1.0	60±0.5	1.16±0.23	2.05±0.28
5.	50	10±0.5	40±0.5	0.92±0.32	1.16±0.45
6.	100	0	0	0	0

DISCUSSION

Various types of biotic and abiotic stresses have turned out a big challenge globally to the agriculture sector. These stresses cause huge damage to crop and reduce the food crop yields all over the world. Abiotic stresses such as drought, salinity, heavy metals, and high temperature have a great impact on plant development, reproduction, and production of crops. These stresses cause a major negative effect including disruption of homeostasis in plants (Kuria, 2013).

Heavy metals have been shown to affect different processes of plant functions, and clearly have a negative impact on seed germination. They can engender damage to the root system of plants (Singh and Thakur, 2014), by causing an oxidative stress by producing free radicals (Shah *et al*., 2010), or by replacing nutrient and essential metals (Henry, 2000).

It is realized that if food crops grow on widespread unregulated waste amended agricultural soils, then it may cause a food security problem because toxic concentrations of heavy metals may accumulate gradually in the agriculture soil (Munzulturoglu and Geckil, 2002) that lead to the failure of food crops (Mahmood *et al.*, 2007).

However, temporal accumulation of the heavy metals in waste amended agricultural soils at higher concentration can be toxic for plant growth due to their adverse effects on plant development and growth (Pahlsson, 1989; Ayaz and Kadioglu, 1997). In addition, crops which can tolerate may accumulate greater concentration of heavy metals and become environmental and public health issues (Stefanov *et al*., 1995; Munzuroglu and Geckil, 2002).

Several studies have reported that plant seedlings respond quickly to a higher concentration of metals in terrestrial ecosystems by changing in their growth rates and root branching patterns compared to shoot growth (Stiborava *et al*., 1986; Dinev, 1988; Breckle, 1991; Hasnian *et al*., 1993).

Moreover, the adverse effects of heavy metals especially Cu has reportedly caused structural and morphological changes of roots as well as inhibition of root hair growth of seedlings (Pahlsson, 1989; Fernandez and Henriques, 1991). It has been suggested that adverse effects of Cu on roots growth are related to severe reduction in the elongation growth of the longest root as well as root plasma membrane permeability of the seedlings (Wainwright and Woolhouse, 1977, Nriagu and Pacyna, 1988; McBride, 2001).

Significantly, SO_4^{--} and Cl^- ions from Mg^{++} and Na^+ salts didn't show negative effect on seed germination, however, a consistent inhibition of seed germination especially in rice and wheat under increased $CuSO_4$ concentrations was recorded and most possibly it could be due to consequences of greater absorption of Cu by the seeds during imbibitions and subsequent toxicity (Fernandez and Henriques, 1991; McBride, 2001). The Cu-induced oxidative stress has been also identified in severe reduction of enzyme activities involved in the seed metabolic processes related to germination (Ayaz and Kadioglu, 1997).

Furthermore, the stunted and poorly developed root systems (fibrous) have been found in most of the crops seedlings at higher Cu, Pb and Zn. Moreover, these are likely related to partial disorder of metabolic processes of the seedlings (Pahlsson, 1989; Obroucheva *et al*., 1998; Dinev, 1988; Breckle, 1991). The toxic effects of Cu often affect mitotic activity and cell division of roots with a subsequent increase in the root number of seedlings (Hall and Williams, 2003).

During present study, $HgCl_2$ treatment was found to be very toxic and lethal for sesame crop and even very low concentration of $HgCl_2$ (0.1%) was proved to be completely lethal and toxic for sesame seed germination. However, $HgCl_2$ (0.01%) was proved as little toxic and significantly inhibited sesame seed germination (60±1.0%) in comparison to control experiment (100±0.5%). Moreover, inhibitory responses of $HgCl_2$ (0.01%) stress could be also observed during early seedling growth and root-shoot length ratio was recorded as (0.38±0.14cm/0.44±0.16cm) in comparison to control seedling (2.11±1.04cm/3.77±0.19cm).

In general, the tolerance to heavy metals for plants involves exclusion and accumulation mechanisms (Hall, 2002). Cobalt (Co) has been reported to induce DNA methylation in *Vicia faba* seeds (Rancelis *et al*., 2012; Sethy and Ghosh 2014).

Moreover, $CoCl_2$ (25mg/L) solution treatment was found to show stress inhibition and further increase in $CoCl_2$ concentrations (50mg/L and 100mg/L) were seen as strong inhibitors for sesame seed germination and were turned out to be lethal. Additionally, seedling growths were also influenced with $CoCl_2$ concentrations and moreover, seedlings that were growing with low concentration of $CoCl_2$ (10mg/L) solution were found to be shorter (2.12±0.76cm/1.67±0.60cm) as root-shoot ratio in comparison to control seedlings (2.11±1.04cm/3.77±0.19cm). Significantly, poorly germinated sesame seeds with 50mg/L of $CoCl_2$ solution were failed to develop into seedlings.

Nickel (Ni) is reported to be toxic to most plant species affecting amylase, protease and ribonuclease enzyme activity thus retarding seed germination and growth of many crops (Ahmad and Ashraf, 2011). Ni stress has been reported to affect photosynthetic pigments, lessen yield, and cause accumulation of Na^+, K^+ and Ca^{2+} in mung bean (Ahmad *et al*., 2007; Sethy and Ghosh, 2014).

In comparison to $CoCl_2$, $NiCl_2$ was proved to be lethal at low concentration (10mg/L) and therefore, in sesame, seed germination was found to be (40±1.0%) while 25mg/L of $NiCl_2$ was turned out to be strongly inhibitory. Seedling growth was much inhibited during $NiCl_2$ stress treatments, moreover, seedling height as root-shoot length ratio (0.14±0.09cm/1.34±0.15cm) was recorded with low concentration (5mg/L) of $NiCl_2$ solution as compared to control seedling height (2.11±1.04cm/3.77±0.19cm). Hence, present study indicated that sesame seed germination and seedling growth was strongly influenced with more negative response than $CoCl_2$ heavy metal stress.

Cd tolerance is related to the accumulation of Cd content in plants. In general, a high level of tolerance to Cd can rely on the strategy to avoid the build-up of excess metal levels in plants, and thus to prevent the onset of toxicity symptoms (Jun-Yu *et al*., 2008). During this study, $CdCl_2$ response was found to be significantly less inhibitory than $NiCl_2$ but more inhibitory than $CoCl_2$ solution. Sesame seeds were found to show germination inhibition significantly (40±1.0%) with concentration (25mg/L) of $CdCl_2$ solution. Seedling height was found to be minimal (0.13±0.07cm/1.37±0.34cm) with 10mg/L of $CdCl_2$ solution in comparison to control seedling (2.11±1.04cm/3.77±0.19cm) as root-shoot length ratio.

Seed germination under Cd stress could be decreased owing to accelerated breakdown of reserved food material in seed embryo. Present studies are in contrast with the previous results (Raziuddin *et al*., 2011; Aydinalp and Marinova, 2009; Jun-Yu *et al*., 2008; Titov *et al*., 1996) in which it was reported that Cd stress decreased seed germination, germination index and vigour index of different crops (Ahmad *et al*., 2012).

Moreover, during present study, seedlings that were growing with $CoCl_2$, $NiCl_2$, and $CdCl_2$ solutions with (25mg/L or above concentrations) were found to show strong inhibitions in root development, indicating that these heavy metals are probably involved in suppression of root development during germination that leads to failure of further growth of complete seedling.

Additionally, during $ZnSO_4$ treatments in present study, sesame seeds were proved to be little tolerant to Zn stress and even high concentration (100mg/L) of $ZnSO_4$ solution was proved to be ineffective to inhibit complete seed germination (30±0.5%) and seedling growth. In comparison to Co, Ni, and Cd heavy metals, Zn metal was turned out to be very weak stressor for sesame seed germination.

However, during seedling growth, the heights of seedlings were seen to be gradually reduced with the increase in $ZnSO_4$ concentrations in the treatment solutions and therefore, the seedlings that were growing with high concentration of $ZnSO_4$ (100mg/L) solution were found to be shortest in size (0.1±0.11cm/0.11±0.6cm) as root-shoot length ratio in comparison to control seedlings (2.11±1.04cm/3.77±0.19cm).

In contrast of zinc heavy metal that was proved to be inhibitory at high concentration (100mg/L) during present study, copper stress was proved to be strong stressor at relatively lower concentration (50mg/L) and seed germination was found to be significantly inhibited (40±0.5%). Results indicate that stress inhibition induced by Cu heavy metal for seed germination was found to decrease with the increase in $CuSO_4$ concentrations and moreover, $CuSO_4$ (100mg/L) concentration was proved to be toxic and lethal.

Inhibitory effect of Cu was also visible during early seedling growth and seedling heights were seen to be minimum (0.92±0.32cm/1.16±0.45cm) in comparison to control seedling size (2.11±1.04cm/3.77±0.19cm) as root-shoot length ratio. Hence, amongst heavy metals, used during this study, Hg was proved to be strongest inhibitor for seed germination and seedling growth followed by Ni, Co, Cd, and Cu while Zn was turned out to be weakest inhibitor for sesame seed germination and seedling growth.

Similar to present results, another study reveals that consistent change in the barley and wheat root-shoot length ratio in response to metals especially Cu and Zn is most probably related to greater inhibition of roots by metal toxicity than shoots. Metal induced changes in the structure and morphology of the roots such as absence of root hairs, stunted and fibrous root growth, and thickening or browning of roots might be involved to cause a decreased root-shoot ratio of the seedlings (Mahmood *et al*., 2007).

Additionally, a lack of consistent adverse effects demonstrated by Pb and Zn on seed germination is most probably related to interspecies differences in seed coat structures for regulating metal absorption. It is argued that plant seeds have inherent capability for selective absorption of metals in nature (Stefanov *et al*., 1995). This suggests that seed coat structures of barley, rice and wheat may have selectively reduced Pb and Zn absorption from the solution to minimize their adverse effects on germination. However, various degrees of permeability of seed coats to metals lead to a range of seed germination inhibitions (Wierzbicka and Obidziniska, 1988).

Moreover, the adverse effects of heavy metals especially Cu causes structural and morphological changes of roots as well as inhibition of root hair growth of seedlings (Pahlsson, 1989; Fernandez and Henriques, 1991). Furthermore, adverse effects of Cu on roots are related to severe reduction in the elongation growth of the longest root as well as root plasma membrane permeability of the seedlings (Wainwright and Woolhouse, 1977, Nriagu and Pacyna, 1988; McBride, 2001).

CONCLUSION

Among heavy metals (Hg, Co, Ni, Cd, Zn, and Cu) tested, stress caused by Hg was proved to be the most toxic for the seed germination, however, 0.1% of mercury chloride solution was proved to be toxic level for sesame seed germination. Additionally, cobalt and nickel both were proved to be strong stressor at concentrations (50mg/L and 25mg/L) respectively and caused toxicity for seed germination.

Moreover, 50mg/L of $CoCl_2$ and $NiCl_2$ solutions both were proved strongly effective to suppress seed germination and seedling growth. Furthermore, cadmium heavy metal stress at 100mg/L causes strong toxicity whereas 50mg/L of $CdCl_2$ solution was recorded as weak inhibitor. Furthermore, Zn response was found to be partial toxic with very high concentration (100mg/L) solution while Cu response with (100mg/L) solution was found to be completely toxic and lethal.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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