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Effect of Mercury on Seed Germination and Seedling Growth of Mungbean (*Vigna radiata* (L.) Wilczek)

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ABSTRACT: Among the toxic elements release in the environment, mercury is considered highly toxic to the growth of plants. The present studies report the effects of different concentrations (1, 3, 5 and 7 mM) of mercury on seed germination and seedling growth performance of mungbea (*Vigna radiata*) as compared to control. Mercury treatment in the form of mercuric chloride at 1 mM did not show significant reduction in seed germination of *V. radiata* as compared to control. Increase in concentration of mercury to 3 mM produced significant ($p<0.05$) reduction in seed germination. Mercury treatment at 7 mM-produced significant ($p<0.05$) reduction in seedling and root length of the plants. The increase in concentration of mercury treatment at 7 mM was found sufficient to cause significant reductions in seedling dry weight of as compared to control. Mercury treatment at all concentrations decreased seed germination, shoot, and root length and seedling dry weight. Increase in mercury concentration upto 7 mM showed highest percentage of decrease in seed germination (42%), seedling length (70%), root length (66%) and seedling dry weight (47%) of mungbean as relation to control. *V. radiata* were more sensitive to mercury stress in seedling growth and root elongation than seed germination. The seedlings of *V. radiata* showed greater tolerance to mercury at 1 mM (85.83 %) and lowest at 7 mM (34.13%). These results show that there is a negative effect towards germination and growth of mungbean by mercury treatment. Minimum use of the mercury containing compounds in fungicide, pesticide and nematicide is recommended. Special care should be taken to monitor the toxic pollutants available in the immediate environment. The accumulation of such types of toxic pollutants in larger concentrations by crop can produce harmful effects to crops and ecosystem as well. © JASEM

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Introduction

Rapid increase in the industrial and anthropogenic activities and discharge of untreated chemicals in the environment are responsible for spreading of different types of chemical compounds in the air, soil and water, affecting the environment and growth of plants. Among the toxic elements release in the environment, mercury is considered highly toxic to the growth of plants (Iqbal et al., 2014). During the past couple of decades the concentrations of heavy metals in the environment of city have appeared at dangerous level. Mercury also used for eliminating various pests causes harmful effects on agricultural plants (Simon, 2014). They produce toxic effects on the leaves where crucial functions such as photosynthesis and transpiration are carried out, cause morphological, anatomical and physiological changes, inhibit pollen germination and pollen tube formation and thus affect fruit production (Simon, 2014; Tort et al., 2005). Seed health plays an important role for successful cultivation and yield exploitation of a crop species (Rajput et al., 2005). Attention has been given, in developed countries, about the effects of metal toxicities on germination and growth of plants. Mercury (5 µM $HgCl_2$), a general blocker of aquaporins in various organisms, reduced the speed of seed germination and induced a true delay in maternal seed coat (testa) rupture and radicle emergence, by 8-9 and 25-30 h, respectively **(**Willigen et al., 2006**).** Mercury stress may result in decreased foliar chlorophyll content and/or damage to internal leaf structure (Dunagan et al., 2007). Mercury contamination is widespread in different ecological compartments such as atmosphere, soil and water. There is evidence showing bioaccumulation and biomagnification of Hg in aquatic food chains, with higher concentrations detected in carnivorous fish (Zhang and Wong, 2007). Mercury in the agricultural ecosystem is a global concern because of its high potential toxicity (Iqbal et al., 2014; Zheng et al., 2008).

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Arsenic (As) and mercury (Hg) are among the most dangerous heavy metals to humans and the environment because of their toxicity towards all living organisms and their related accumulation capability (Bini and Bech, 2014; Comino et al., 2009). Mercury is released to the atmosphere from natural and anthropogenic sources. Due to its persistence in the atmosphere, mercury is subject to long range transport and is thus a pollutant of global concern. Mercury emitted to the atmosphere enters terrestrial and aquatic ecosystems which act as sinks but also as sources of previously emitted and deposited mercury when the accumulated mercury is emitted back to the atmosphere (Baya and Van Heyst, 2010). Environmental contamination caused by mercury is a serious problem worldwide. Coal combustion, mercury and gold mining activities and industrial activities have led to an increase in the mercury concentration in soil (Wang et al., 2012).

Mercury is a global environmental pollutant that is present in soil, water, air and biota. The effect of increasing rates of mercury (10, 25, 50 and 75 mg/kg) treatments in *Catharanthus roseus* was observed on plant viability. The increase in concentration of heavy metal decreases plant growth and leads to its death (Iqbal et al., 2014; Kumar et al., 2011). The effect of mercuric acetate on seedling growth, total chlorophyll, chlorophyll a and b, reducing, non-reducing and total sugar content, protein content and lipid content was studied in peanut plants (*Arachis hypogaea*) at 0.0001 mM and 0.001 mM concentrations. Reduction in length was more in roots than in shoots. Chlorophyll-a, chlorophyll-b and total chlorophyll contents decreased significantly under mercuric acetate treatment (Bhanumathi et al., 2005). In vivo cytogenetic assay in *Allium cepa* root tip cells has been carried out to detect the modifying effect of *Ocimum sanctum* aqueous leaf extract against chromium and mercury induced genotoxicity. It was observed that the root post-treated with the leaf extract showed highly significant ($p<0.001$) recovery in mitotic index (MI) and chromosomal aberrations (CA) when compared to pre-treated (Cr/Hg) samples and the lower doses of the leaf extract were found to be more effective than bigger doses (Babu and Maheshwari, 2006). Seeds of cabbage (*Brassica rapa*), cole (*B. napus*), head cabbage (*B. oleracea*), and spinach (*Spinacia oleracea*) were found significantly inhibited when treated with $HgCl_2$ concentration upto 8 mM. *B. oleracea* was the most sensitive species to mercury among the four test species, *B. campestris* was the most resist species to mercury pollution. These four vegetables were more sensitive to mercury stress in coleoptile growth and root elongation than seed germination (Ling et al., 2010). Mercury contamination of the environment is of worldwide concern because of its global presence and its potent neurotoxicity. Mining, smelting and the electronics industry are the main sources of mercury pollution (Deng et al., 2011).

The genus *Vigna* contains several species that are important in the world agriculture. Cowpeas (*V. unguiculata*), mungbeans (*V. radiata*) and urd beans (*V. mungo*) provide a significant portion of the dietary protein in many societies. All of the cultivated *Vigna* species can be grown over a wide range of environmental conditions. The mungbeans are widely grown in southern Asian countries.

In Pakistan substantial quantities of agricultural chemicals are used annually to enhance yield (Nuzhat et al., 2005). Mercuric chloride ($HgCl_2$), the main representative of mercury compounds, is the target of numerous investigations, not only because of its intrinsic toxicity but also because it accounts for the toxicity of elemental mercury since the latter is converted to Hg^{+2} by oxidation (Sobral-Souza et al., 2014). The ever increase in mercury concentration over the wide areas of Karachi and rural areas raises serious questions as to its effects on the growth and vigor of plants. A decline in the agriculture areas play an important role which can lead to certain restriction in the availability of crop for human beings. The data on the effects of mercury on mungbean crop is presently seems scanty. The increase in concentration of metals like mercury can produce toxic effects on plants growth. The response of plant growth to toxic effects of heavy metals has become the subject of great interest in recent years because of their nature of high toxicity to plants. Attention has been given in developed countries, about the effects of metal toxicities on plants growth. Therefore, a study was carried out to determine the effect of mercury on seed germination and seedling growth of an important crop mungbean (*V. radiata)*.

MATERIALS AND METHODS

Certified and healthy seeds of mungbean {*Vigna radiata* (L.) Wilczek (Fabaceae)} were purchased from a local seed company. The seeds were surface sterilized with dilute solution of sodium hypochlorite for one minute to avoid any fungal contamination. The seeds were washed with distilled water and transferred in Petri dishes (90 mm diameter) on filter paper at room temperature. There were three replicates. Initially, 5 ml solution of mercuric chloride in different ranges 1, 3, 5 and 7 mM were applied. All solutions were changed daily. The control treatment consisted of just the distilled water. After seven days, seed germination percentage, root and seedling length were noted for *V. radiata*. The seedling dry weight was determined by drying the plant materials in an oven at 80^0C for 24 hours. The data obtained were statistically analyzed by Analysis of variance and Duncan's Multiple Range Test. A tolerance index was determined by the following formula as described by Iqbal and Rahmati (1992).

Mean root length in metal solution/Mean root length in distilled water X 100.

RESULTS AND DISCUSSION

The seed germination and seedling growth performance of *V. radiata* were tested in different concentrations (1, 3, 5 and 7 mM) of mercury as compared to control (Fig.1). Mercury treatment in the form of mercuric chloride at 1 mM did not produce any significant ($p<0.05$) reduction in seed germination of *V. radiata* as compared to control. Increase in concentration of mercury at 3 mM produced significant reduction in seed germination. Root growth is an important growth variable and found affected by mercury treatment. The treatment of mercury at 7 mM produced significant ($p<0.05$) reduction in root length of *V. radiata*. The results also showed that mercury treatment in the substrate from 1 to 5 mM did not produce any significant effect on seedling dry weight of *V. radiata* as compared to control. However, increase in concentration of mercury treatment at 7 mM was found sufficient to cause significant reductions in seedling dry weight of *V. radiata* as compared with control.

The elements have specific function all of them are known to be involved in enzyme action. However, plant under stress condition is most likely to be adversely affected by high concentrations of trace elements. In present study, the effect of mercuric chloride on seed germination, seedling growth, root length and seedling dry weight of an important crop mungbean were recorded. Mercury induced oxidative stresses in *Suaeda salsa* and also mercury at a concentration of 20 μg L^{-1} disturbed protein bio-degradation and energy metabolism in *Suaeda salsa* (Wu et al., 2011).

The seed germination of mungbean responded differently to mercuric chloride treatment as compared to control. High percentage of decrease in seed germination of mungbean provided evidence that the treatment of mercury in excess may be inhibitory to plant growth and development. Inorganic mercury has been reported to produce harmful effect at 5 μg L^{-1} in culture medium for aquatic plants (Boeing, 2000). Germination rate and root elongation, as a rapid phytotoxicity test methods, possesses several advantages such as sensitivity, simplicity, low cost and suitability for unstable chemicals or samples. Information on toxicity required for the ecological risk assessment of toxic pollutants (Wang et al., 2001).

Plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage (Kunjam et al., 2015; Nagati et al., 2015). In addition, plants accumulate metal ions that disturb cellular ionic homeostasis (Acharya and Sharma, 2014; Siddiqui et al., 2014). To minimize the detrimental effects of heavy metal exposure and their accumulation, plants have evolved detoxification mechanisms (Yadav, 2010). Metals are toxic to both plants and fungi, and elevated soil metal concentrations have been documented to change the structure of ectomycorrhizal communities and high concentrations of mercury (0-366 μg g^{-1} Hg) in soil decreased survival of *Pinus rigida* seedlings (Crane et al., 2012). The mercury uptake could produce serious damage to plants by impairment of the chlorophyll synthesis and reduction of photosynthesis as a result of substitution of Mg by Hg (Lavado et al., 2007). Metal toxicity is also an important factor governing germination and growth of plants. The permeability of metals can decreased the growth of plants. Reduction in seedling growth of *V. radiata* was observed when treated with different concentration of mercury. In field open top chambers (OTCs) and soil mercury enriched experiments when employed to study the influence of mercury concentrations in air and soil on the mercury accumulation in the organs of corn (*Zea mays*) and wheat (*Triticum aestivum*). The results showed that Hg concentrations in foliages were correlated significantly ($p < 0.05$) with air Hg concentrations but insignificantly correlated with soil Hg concentrations. The author concluded that Hg in crop foliages was found mainly from air. Hg concentrations in roots were generally correlated with soil Hg concentrations ($p < 0.05$) but insignificantly correlated with air Hg concentrations, indicating that Hg in crop roots was mainly from soil (Niu et al., 2011).

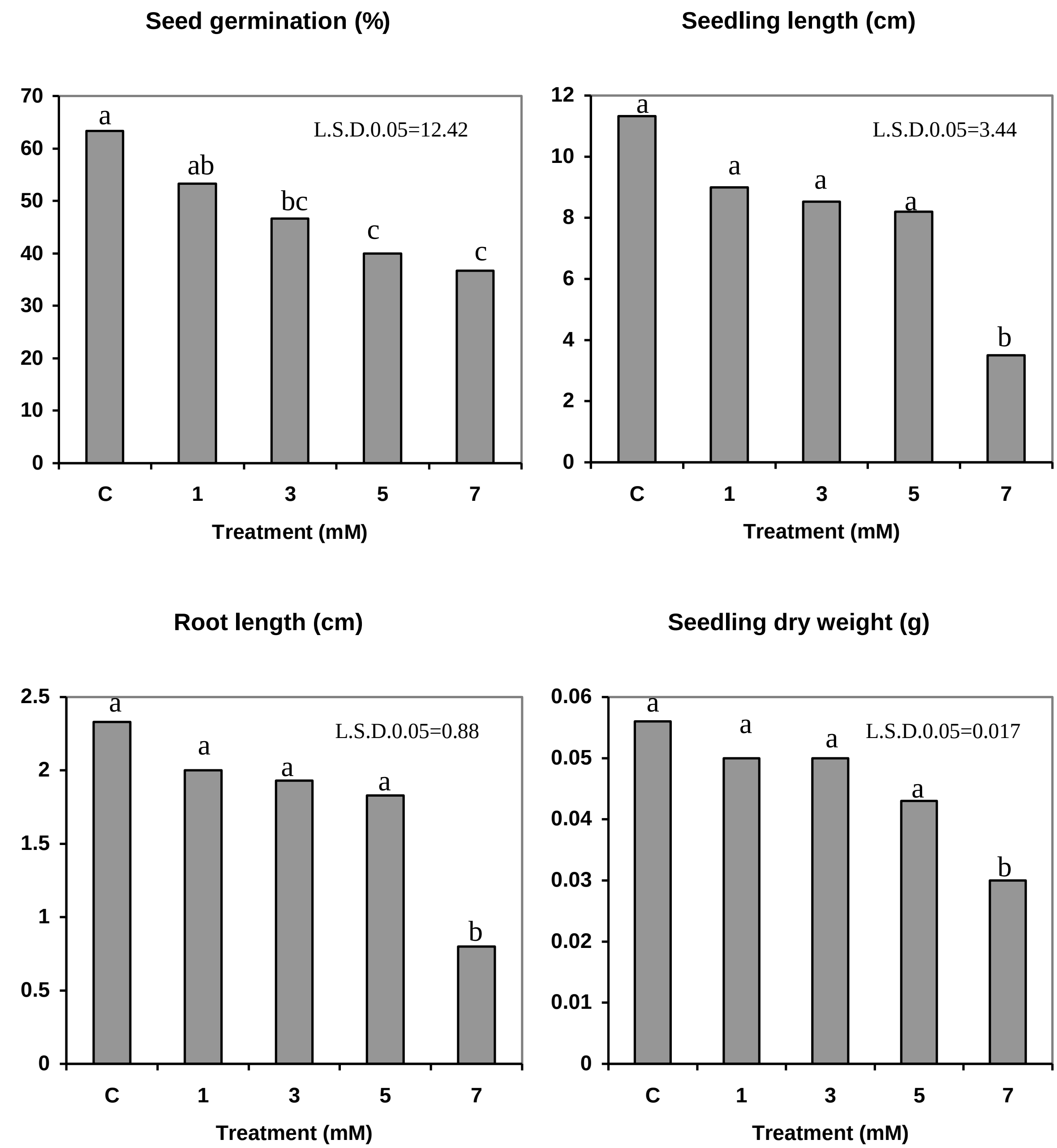

Fig. 1. Effects of different concentration of mercury treatment on seed germination (%), seedling length (cm), root length (cm) and seedling dry weight (g) for *Vigna radiata* as compared to control. Number followed by the same letters in the same bar are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

Mercury treatment at all concentration decreased seed germination, root length and seedling dry weight of *V. radiata* (Fig.2). Mercury treatment at 1 mM concentration was less toxic for decrease in seed germination (16%), seedling length (21%), root length (17%) and seedling dry weight (10%) as compared to control. Mercury treatment at 5 mM concentration showed more decrease in seed germination (37%), seedling length (27%), root length (21%) and seedling dry weight (23%) of *V. radiata*. Mercury concentration of 7 mM decrease seed germination (42%), seedling length (70%), root length (66%) and seedling dry weight (47%) of *V. radiata* as compared to control.

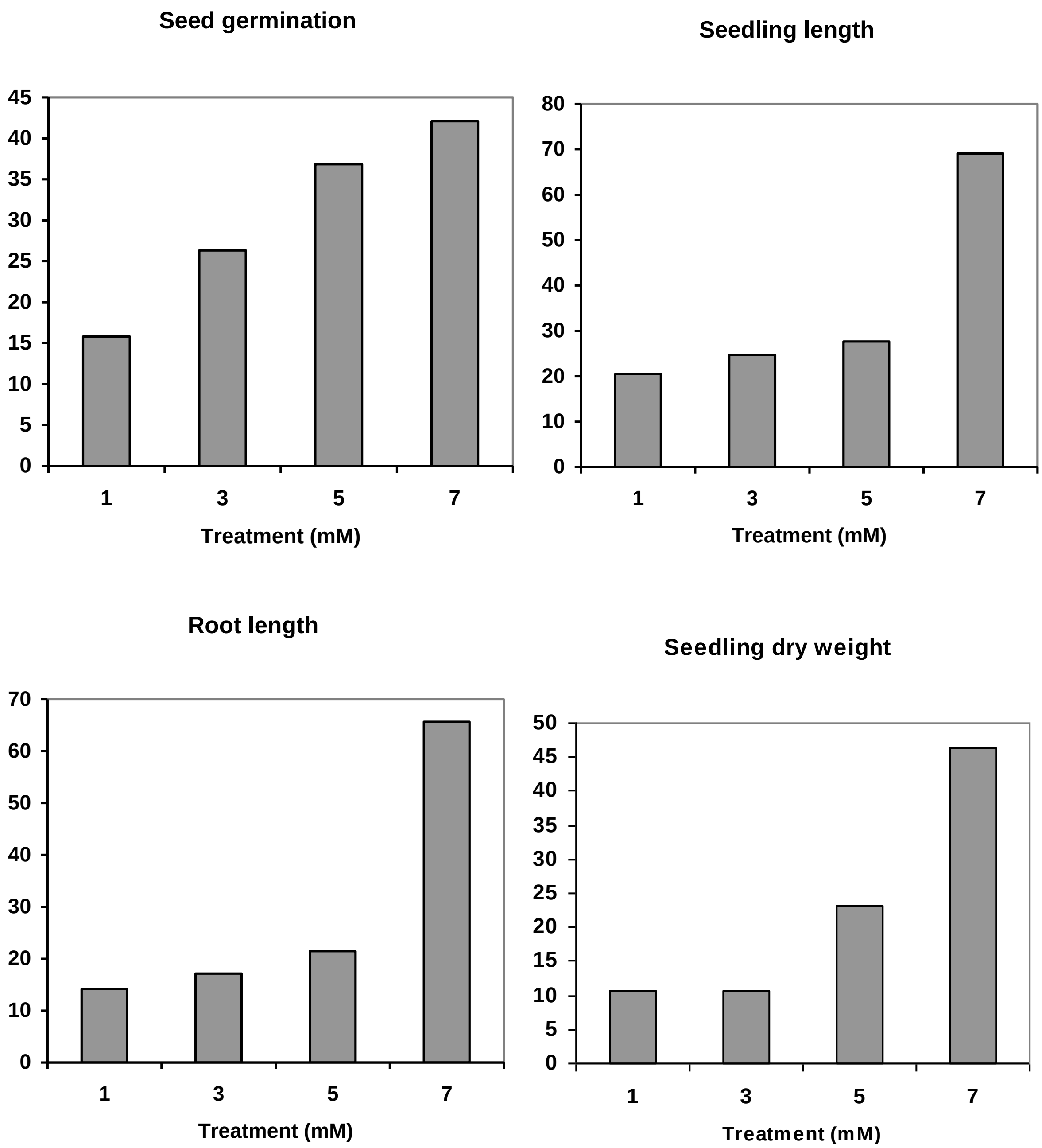

Fig. 2. Percentage decrease in seed germination, seedling length, root length and seedling dry weight of *Vigna raddiata* using different concentration of mercury.

The seedlings of *V. radiata* were also tested for percentage of tolerance to mercury. The results showed that *V. radiata* has greater tolerance to mercury at 1 mM and lowest at 7mM of mercury (Fig. 3). *V. radiata* showed greater tolerance (85.83 %) to mercury at 1 mM and lowest (34.33 %) at 7mM of mercury. *V. radiata* seedlings showed better percentage of tolerance 82.83 % to mercury at 3 mM.

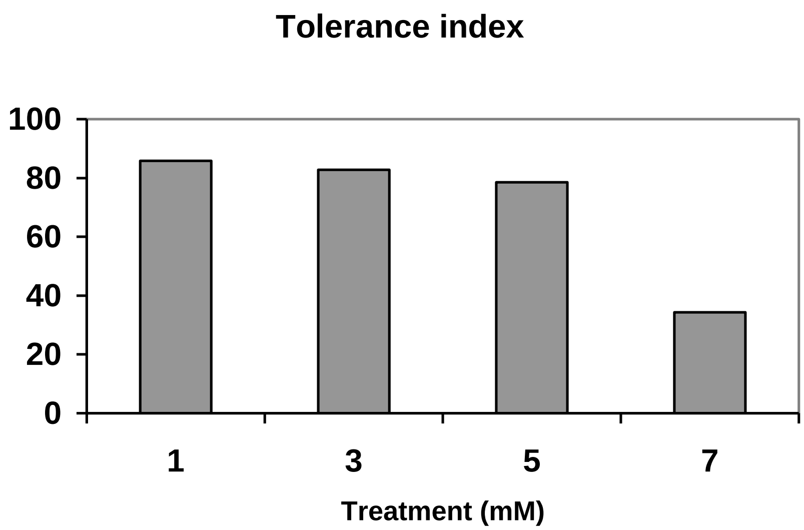

Fig. 3. Percentage of tolerance in *Vigna radiata* using different concentration of mercury

The reduction in the seedling and root growth of *V. radiata* provides further evidence that the mercury in excess may be inhibitory to plant growth and development. Mercury is one of the most toxic heavy metals to living organisms and its conspicuous effect is the inhibition of root growth (Wang et al., 2013). The root elongation tests have been used as simple, rapid, reliable and reproducible techniques to evaluate the damage caused by toxic compounds present in various composts (US-EPA, 1982). Many species including cabbage, lettuce, carrot, cucumber, tomato and oats have been recommended for the phytotoxicity test (F.D.A., 1982). The roots are normally considered in relation to their ability to supply water and nutrients to the plants. The root growth of *V. radiata* was found decreased by 66% at 7 mM mercury concentration. The results of this investigation have shown that mercury treatment is more toxic for *V. radiata* for root development. Mercury content and distribution as well as its effects on growth were investigated in 30 day-old tomato (*Lycopersicon esculentum*) seedlings (Chao and Park, 2000). The results showed that excess of mercury suppressed biomass production of both roots and shoots and reduced chlorophyll content in tomato leaves. The seedlings of alfalfa (*Medicago sativa*) pretreated with 0.2 mM salycylic acid for 12 h and subsequently exposed to 10 μM Hg^{2+} for 24 h displayed attenuated toxicity to the root (Zhou et al., 2009). In another investigation, plants of *Chilopsis linearis* grown with 0, 50, 100, and 200 μM Hg [as $Hg(CH_3COO)_2$] and 0 and 50 μM Au (as $KAuCl_4$) in hydroponics showed that seedling grown with 50 μM Au + 50 μM Hg and 50 μM Au + 100 μM Hg had roots 25 and 55% shorter than control roots, respectively (Rodríguez et al., 2009).

Toxicants accumulate in the plant when soluble forms are present in high quantities (Acharya and Sharma, 2014; Nagati et al., 2015). The exact amount of accumulation depends upon the solubility of the pollutants in the soil (Kunjam et al., 2015; Siddiqui et al., 2014). Under certain conditions, sufficient arsenic may be absorbed to injure or kill sensitive plants, thus altering the community structure, or arsenic may enter the leave sand thus is hazardous to any organisms feeding on them. The solubility of toxicants in the soil is greatly affected by the soil acidity. Thus greater amounts of metals such as zinc and aluminium become soluble and absorbable in acid soils. This has raised the question of whether sulphur, deposited in precipitation as sulphuric acid, and later forming an acid reaction, could change the soil acidity to the extent that certain elements such as aluminium, manganese, or zinc become toxic (Treshow, 2010).

Conclusion: According to tolerance test it could be seen that tolerance to mercury was higher at low concentration of mercury in the seedlings of *V. ratiata*. These results showed that the reason of tolerance against heavy metals might be a physiological association of the tolerance mechanism to these metals. The seedling growth of *V. radiata* showed high percentage of tolerance to mercury at 1 mM concentration. 7 mM concentration of mercury produced lowest percentage of tolerance in seedling of mungbean.

The concentration of mercury like other metals in the environment has been increased due to industrial and anthropogenic activities. Such constant increase of mercury in the environment is producing toxic effects on plant growth and can severely limit the yield. The wealth of information from the treatment of the mercury through seedling growth studies would be helpful in controlling the mercury pollution problem. Reduction in seedling growth of *V. radiata* can be considered as an over all indicator of plant vigor. The findings may contribute to ecological fragility, the potential of crop in coordinating in land management programmes. They also stabilize the soil and in some cases improve it. The cultivation management of mungbean in mercury-polluted area will help in reducing the burden of pollution to some extent. Heavy reliance on mercury containing fungicides can be discouraged to certain level and subsequently continuous release of mercury into the immediate environment may endanger freshwater life, drinking water reservoirs and use of crop for humans. Current research shows that mercury treatment at different concentration has produced an important effect on seed germination and seedling growth of *V. radiata*. Increase in the concentration of mercury in the medium, brought up certain toxic changes in germination of *V. radiata*. These results show that there is a negative effect towards germination and growth of mungbean by mercury treatment. Minimum use of the mercury containing compounds in fungicide, pesticide and nematicide is recommended. Special care should be taken to monitor the toxic pollutants available in the immediate environment. The accumulation of such types of toxic pollutants in larger concentrations by crop can produce harmful effects to crops and ecosystems. Special efforts need to be made to identify sources of mercury toxicity and there is also a need to carry out an ongoing effort to develop tolerance indices. Better sources of resistances to metal are badly needed.

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