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Effects of nickel chloride on germination and seedling growth of different wheat (*Triticum aestivum* L. em Thell.) cultivars

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Abstract
Nickel (Ni) is an essential micronutrient for seed germination and plant growth at low concentration. It becomes toxic when the concentration is high in the soil and in plants. However the efficiency of tolerance depends on the plants species. The aim of the present study is to evaluate the effect of varying concentration of nickel chloride on germination and seedling growth. A petriplates culture method was adopted to study the response of nickel chloride on nine wheat (*Triticum aestivum* L. em thell.) cultivars. The seeds of wheat cultivars were germinated in petriplates lined with whatman filter paper no-1 and treated with various concentration of nickel chloride 5,10,25,50 and 75 mg/L respectively and distilled water was used as control. The germination percentage, seedling growth and weight were recorded at ten days after soaking. The lower concentration (5 mg/L) of nickel chloride has no adverse effect on germination, shoot and root length and weight, seedling growth while the higher concentration decreased the germination, seedling growth, shoot and root length and weight. The result indicates that the low amount of nickel chloride is required for wheat cultivars. Whereas, the increasing level of nickel chloride reduced plant growth and cause toxicity. It is also cleared from findings that the cultivars HD-2985 was more tolerant. HD-3086 was moderately tolerant and PBW-343 was the most sensitive on the basis of germination and growth parameters.

Keywords: $NiCl_2$ toxicity, wheat cultivars, seed germination and Seedling growth

Introduction
Changes of climates of urban and rural areas due to anthropogenic pollution are widespread phenomenon. Increased amount of heavy metals in soil and consequently, in forage and foodstuffs produces mutagenic, carcinogenic or toxic effects upon penetration into a living organisms. Heavy metals are the elements with specific gravity more than 5.0 and atomic weight ranging from 63-200 (NCSU Water quality, 2006) [34]. There are two important facts about the heavy metals. Firstly, in low quantity heavy metals are generally non-toxic but when their amount exceeds the limit they become toxic. Secondly, some of the heavy metals are non toxic in lower concentration like as cobalt (Co), copper (Cu), iron (Fe), magnesium (Me), molybdenum (Mo), nickel (Ni) and zinc (Zn), these heavy metals are essential for plants development and plant growth in low quantity. Heavy metals including lead, nickel, cadmium, copper, cobalt, chromium and mercury play important role in environmental pollutants, they cause toxic effects to plants; thus, reducing yield and posing dangerous threats to the cultivation-ecosystem. Abiotic stress and heavy metal toxicity affect various plant processes such as germination, growth, photosynthesis, carbohydrate metabolism and osmotic homeostasis etc (Karataglis *et al*. 2008; Rosa *et al*. 2009; Panda *et al.* 2010; Sharma and Dubey 2011; Sethy and Ghosh 2013) [25, 49, 39, 54, 53]. II'yasova and Schwartz, (2005) [21] suggested that heavy metals are one of the main sources of environmental pollution and responsible for several problems, associated with industrial and agriculture activities: Heavy metals decreased the microbial activity; soil fertility and crop yield (Yang *et al.* 2005) [61]. Some heavy metals are dissolving in sewage and industrial water, when this water is used for irrigation of crops; heavy metals affect several physiological processes reducing the plant growth and yield, photosynthesis and consequently the biomass (Jamal *et al.* 2006) [22].
Nickel was first discovered by Swedish Chemist Ronstadt in 1975 as a component of enzyme urease which is present in a wide range of plant species. Two forms of urease are playing active role in seed germination and vegetative tissues (Polacco *et al.* 2013) [29, 43]. Toxic level of urea can accumulate within the tissue forming necrotic lesions on the leaf tip in the absence of nickel. In this case, nickel deficiency cause urea toxicity. Ni is the 28th elements of the periodic table and as atomic weight 58.71). The normal range of nickel is essential for plant

trace tissue in between 0.05-5ppm. Excess amount of nickel can close up the uptake the other essential Nutrients especially iron (Fe). Nickel as an essential element affects a number of biochemical and physiological processes in plants in toxic level. Nickel (Ni) is recognized as a seventh heavy metal micronutrient essential for plant development and plant growth (Chen *et al.* 2009; Mazzafera *et al.* 2013) [13, 29, 43]. Nickel is also used as a catalyst in enzymes used to help nitrogen fixation by legumes and the metabolism of urea (a nitrogen containing compound). Without nickel barley crop fail to their complete life cycle (Brown *et al.* 1987) [10]. Ni should be considered a micronutrient for cereals. Nickel is also important for bacteria and fungi, which are both important for good plant growth.

Higher concentration of nickel causes toxicity in plants, so called hyper-accumulators (Chen *et al*. 2009). The metabolism of nickel is essential for maintaining a proper cellular redox state and various other biological, physiological and growth responses. Photosynthesis is inhibiting by elevated level of nickel and flowers are often deformed under these condition. Presence of nickel led to a decrease in chlorophyll a and chlorophyll b contents, but did not affect the concentration of carotenoids (Gajewska *et al*. 2013) [13]. Above the 0.005mg/L^{-1} concentration of nickel cause chlorosis and necrosis in plants and host of other growth abnormalities and anatomical changes (Mishra and Kar 1974) [31]. The toxicity of nickel in plants shows different relation to the degree of sensitivity or tolerance to the metal (Kozlow 2005; Yusuf *et al*. 2011; Hussain *et al*. 2013) [25, 62, 20]. Higher concentration of nickel reduced plant height, root length, fresh and dry weight, chlorophyll content and enzyme carbonic anhydrase activity (Siddiqui *et al.* 2011) [56]. Nickel toxicity reduced maize (*Zea mays*) growth, mitotic activity or indirectly from an inhibition of carbohydrate transport and decrease starch accumulation in the leaves (Huillier *et al*. 1996) [26]. Nickel toxicity decreased supply of nutrients to the reproductive parts (Ahmad *et al*. 2007).

The most important cereals species which supply the maximum nutrition needs for human health are belong to the family *Gramineae* (*Poaceae*) such as rice, maize, wheat, barley, sorghum etc (Alloway, 2008) [4]. Wheat (*Triticum aestivum* L.) is one of most important staple food crops for about 35% world population (Shirazi *et al*. 2001) [55]. In South Asia it is cultivated in rabi (post-monsoon season) after kharif (rainy season) crops harvest (Hobbs and Morris, 1996) [19]. Globally, wheat is grown in European, China, India, United States, Russia, Canada, Pakistan, Ukraine, Australia and Turkey. It is the second most important food crop of India, which contributes nearly one-third of the total food grains production. It is consumed mostly in the most important species, occupying more than 90% of the total wheat area in the country. It is grown all over India from the sea-level up to an elevation of 3,500m in the Himalayas.

The annual rainfall in the wheat-growing regions of India ranges from 12.5 to 100 cm, but most of it is received in summer during the monsoon. In winter, when wheat is in the field, the rainfall ranges between 3-7 cm. only. As such to achieve high yield, irrigation is essential, which enables the application and utilization of required inputs. Well-drained loams and clay-loams are considered the best for growing wheat. However, good crop of wheat can be raised in sandy loams and the black soils also.

Materials and Methods

The aim of present study was to assess the effect of nickel chloride on seed germination and seedling growth of wheat (*Triticum aestivum* em thell L.) cultivars. For the present study, the pure line seeds of three (PBW-343, PBW-502 and PBW-2967) wheat cultivars were obtained from G.B. Pant University of Agriculture and Technology, Pant Nagar (UK) and six (HD-2967, HD-3086, HD-3118, HD-2985, HD-3043 and HD-3059) wheat cultivars were obtained from Indian Agriculture Research Institute (IARI) PUSA Institute, New Delhi respectively.

Before soaking the seeds, healthy and uniform sized seeds of each variety were surface sterilized with 0.01% mercuric chloride ($HgCl_2$) for one minute only and washed thoroughly in distilled water. Soaked wheat seeds were kept in BOD for 24 hours. After that, twenty seeds of each respective variety transferred into separate sterilized petriplates lined with the whatman filter paper no-1 at room temperature. Then they were treated with 10 ml of aqueous solution of nickel chloride (viz. 5, 10, 25, 50 and 75 mg/L respectively) and distilled water was used as control. The amount of water evaporated was also compensated by adding suitable quantity of distilled water to respective petriplates. The petriplates were arranged to completely randomized design with three replication of each treatment. Seeds were considered as germination when coleoptile and radicle protruded to a length of 1mm. After ten days of soaking, the germination, root-shoot length, their fresh and dry weight were evaluated. The samples were collected randomly in triplicate. The fresh yield of wheat cultivars were kept in hot air oven for 48 hours at 60ºC for dry weight sample.

Statistical analysis: Statistical analysis and plotting of graphs were done by SPSS software. Differences concentration of $NiCl_2$ treatment and control were analyzed by one way ANOVA followed by Tukey's test using SPSS software.

Results and Discussion

Seed germination: Nickel is essential micronutrient for plant growth but the high concentration of nickel causes chlorosis, necrosis and wilt in plant. The effects of varying concentration of nickel chloride on seed germination of different nine wheat (*Triticum aestivum* L. em thell) cultivars have been shown in figure-1. Germination percentage gradually decreased in all wheat cultivars as increased concentration of nickel chloride while 0.05 mg/L $NiCl_2$ concentration did not show adverse affects on seed germination. The results indicate that high concentration of $NiCl_2$ had adverse effects on seed germination of wheat cultivars. Cultivar PBW-502 showed minimum germination (80%, 85%, 70%, 65%, 65% and 60%) at varying concentration of nickel chloride (viz., control, 5, 10, 25, 50 and 75 mg/L respectively) as compared to control set, while Cultivar HD-3118 exhibited the maximum germination percentage (96%, 97%, 94%, 87%, 85% and 80%) at these concentrations. It is cleared from the present findings that HD-3118 is relatively more resistant than the other wheat cultivars to nickel chloride treatment.

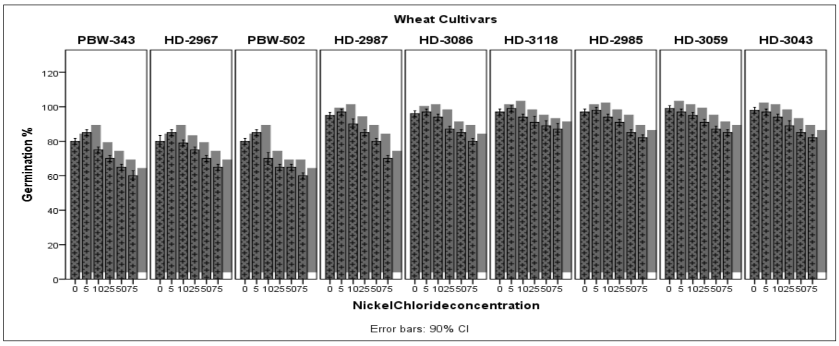

Fig 1: Effect of different concentration of $NiCl_2$ on seed germination of wheat cultivars

The germination is a very essential phase to maintain the life of living organism on earth. The germination and seedling stages are particularly important for successful subsequent development of a crop. The plant species that show heavy metal resistant power at their young stages may produce tolerant juvenile stages individuals. The present findings are similar to the, finding of Zonia *et al*. (1995) [64]; Brown *et al*. (1987, 2007) [10, 11]; Ahmad and Ashraf, (2011) [62, 1] in barley plant. High Ni concentration reduced seed germinability of many crops (Yusuf *et al*. 2010, 2011; Moosavi *et al*. 2012) [63, 62, 32]. High concentration of Nickel has direct effects on the activities, of amylases, proteases and ribonucleases, thereby affecting the digestion and mobilization of food reserve in germinating seed (Ahmad *et al*. 2011) [62, 1]. The high concentrations of nickel inhibit growth of soyabean seedling (Prasad *et al.* 2005). Low conc. of nickel promotes seed germination in rice (Das *et al*. 1978) [14] and others many crops (Welch, 1981) [60]. High Ni concentration reduced all energy requiring cellular process of seed during germination (Hall, 2000) [18].

Effect on root and shoot length

The toxicity of heavy metals reportedly inhibitory effect on the plant growth. Nickel chloride significantly affected plant growth. The different concentrations of $NiCl_2$ on root length have been shown in Figure-2. It indicates that root length increased in all wheat cultivars except for HD-3043 and HD-3059 at 5 mg/L concentration of nickel as compare to control. The results indicate that high concentration of $NiCl_2$ drastically reduced root length in all wheat cultivars. The minimum root length was noted in cultivar PBW-343 (9.1, 9.3, 1.3, 1, 0.6 and 0.27cm respectively) at respective concentrations of nickel chloride (viz., control, 5, 10, 25, 50 and 75 mg/L), while cultivar HD-2985 showed maximum root length (20.6, 23.1, 4.5, 3, 2.1 and 1.5 cm) at the same nickel chloride concentrations. The experiment revealed that PBW-343 wheat cultivar is relatively more sensitive than the other wheat cultivars to nickel chloride treatment. The high concentration of Ni and other heavy metals inhibit the plant growth (Seregin and Ivanov 2001; Seregin *et al*. 2003; Pandolfine *et al*. 1992; Nagajyoti *et al*. 2010) [51, 52, 33], by reducing the supplying additional ion, require for wheat plant growth (Ouzounidou *et al.* 2006) [36]. High concentration of nickel chloride about 10-75 mg/L decreased the root length of wheat cultivars; as comparison to 5 mg/L $NiCl_2$ concentration. Low content of nickel is required for normal plant growth (Sreekanth *et al.* 2013) [57]. These finding were also similar with (Pandey and Sharma; 2002 and Gajewska and Sklodowsk; 2008, Uruc and Parlak; 2016, Ain *et al*. 2016) [40, 16, 58, 3]. Considerable nickel concentrations inhibit root biomass of barley seedling (Brune and Dietz, 1995) [12], inhibit cell division at root meristems in non-tolerant plant (Roberston and Meakin, 1980). The amount of Ni reduced rapidly newly formed parts of the root system in wheat plants (Page and Feller, 2005) [37]. The quantity of 100 and 200mM nickel concentration treatment were reduced 37 and 53% root growth of wheat seedling (Gajewska and Sklodowska, 2008) [16]. The same results were also recorded by Ain, *et al*., 2016 in wheat cultivars and Antonkiewicz, *et al*., 2016 [5] in maize, field bean and lettuce. The $NiCl_2$ concentration more than50µM was decreased the accumulation of nickel in root (Wang *et al.* 2009) [59]. The toxicity of heavy metal is decreased the absorption of cation and anion by plants (Pallavi and Ram Shanker 2005) [38]. The toxicity of nickel inhibited much more the root growth as comparison to shoot growth in maize (Seregin, *et al*., 2003) [52]. The effect of various level of $NiCl_2$ concentration on shoot length is showing in figure-3. The results indicate that the increasing levels of $NiCl_2$ reduced the shoot length of wheat cultivars as comparison to control. The result indicated that the low concentration of $NiCl_2$ is not affected the shoot length, while the increasing level ranging 10-75 mg/L $NiCl_2$ showed` adverse effect on wheat cultivars. Cultivar PBW-502 showed minimum length (11.7, 11, 8.2, 3, 1.3 and 0.6 cm) at different levels of $NiCl_2$ (control, 5, 10, 25, 50 and 75 mg/L respectively) where as cv. HD-3043 recorded maximum length (13.8, 13.2, 11.4, 10, 6.4 and 3.1 cm) at these concentrations of nickel chloride.

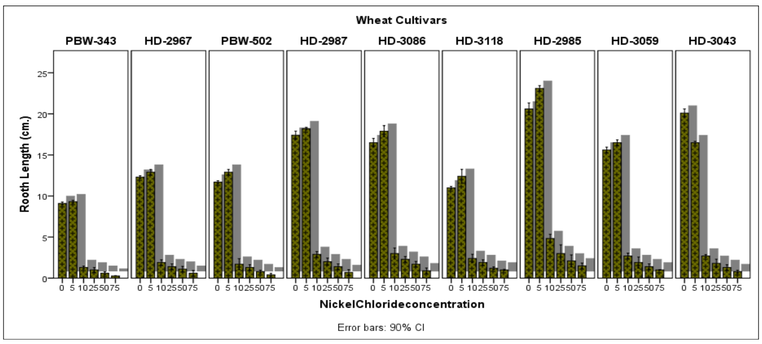

Fig 2: Effect of different concentration of $NiCl_2$ on root length (cm.) of wheat cultivars

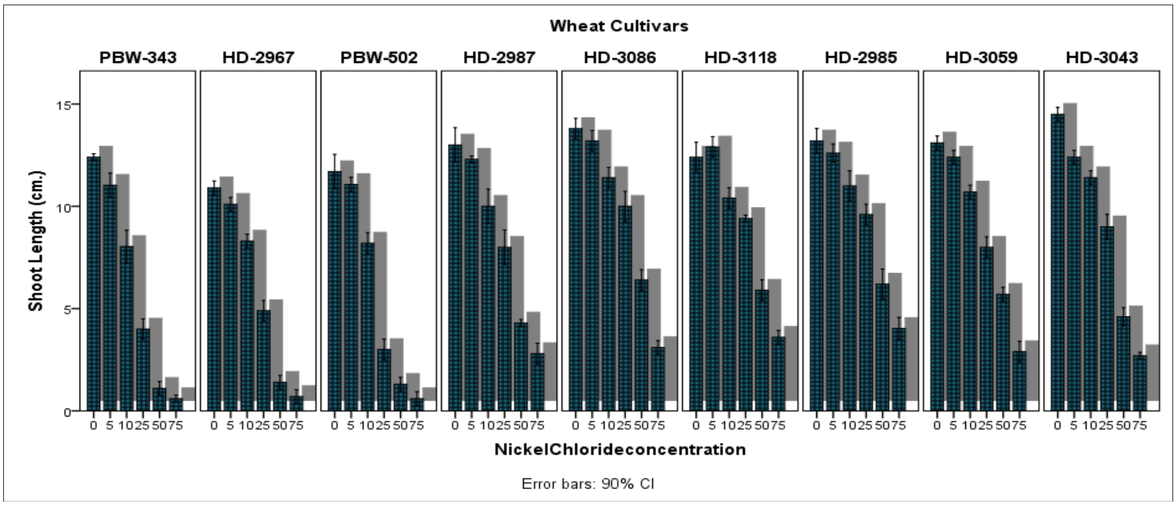

Fig 3: Effects of different concentration of $NiCl_2$ on shoot length (cm.) of wheat cultivars

The result showed that shoot length in all cultivars were reduced with increasing concentration of $NiCl_2$. Heavy metals ions inhibited the root and shoot growth of young wheat plants; nickel ions much reduced the shoot growth of young wheat plants, the least reduction observed at zinc ions (Sazanova *et al*. 2012) [50]. Ni^{2+} affects the lipid formation and plasma membrane activity of H-ATPase in shoots of *Oryza sative* (Ros *et al*. 1992) [42] and inhibits the shoot growth in *Phaseolus vulgaris* (Piccini and Malavolta, 1992) [42]. High concentration of nickel decreased the Fe content in the shoot of rye plant while the low concentration of nickel increased Fe content (Khalid and Tinsley, 1980) [24].

Effect on root and shoot yield

The effects of various level of $NiCl_2$ concentration on root and shoot fresh weight have been shown in figure-4 and figure-5 respectively. Increasing level of $NiCl_2$ reduced the yield of wheat cultivars as comparison to control set. The results indicate that the cultivar HD-2967 sowed minimum root fresh weight (79, 70, 25, 14, 9 and 6mg) with increasing concentration of nickel chloride (viz., control, 5, 10, 25, 50 and 75 mg/L respectively), while cultivar HD-2985 exhibited the maximum root fresh weight (115, 106, 48, 33, 21.8 and 12mg) at these concentration. We observed that the other seven varieties, PBW-343, PBW-502, HD-2987, HD-3086, HD-3118, HD-3043 and Hd-3059, indicated result between the both maximum reduction and least reduction cultivars. The root fresh weight of PBW-343 and HD-2967 cultivars showed highest reduction (6 and 5.9 mg) at 75 mg/L $NiCl_2$. The result indicted that the toxicity of $NiCl_2$ much reduced the root yield than the shoot yield. The increasing level of $NiCl_2$ reduced the fresh weight of shoot (figure-5). The cultivar PBW-502 showed minimum shoot fresh weight (78, 71, 40, 27, 21 and 9 mg) at as varying concentration of $NiCl_2$ (viz., control, 5, 10, 25, 50 and 75mg/L respectively), but cultivar HD-2987 exhibited the minimum shoot fresh weight (120, 110, 81, 58, 35 and 26 mg) at these concentration.

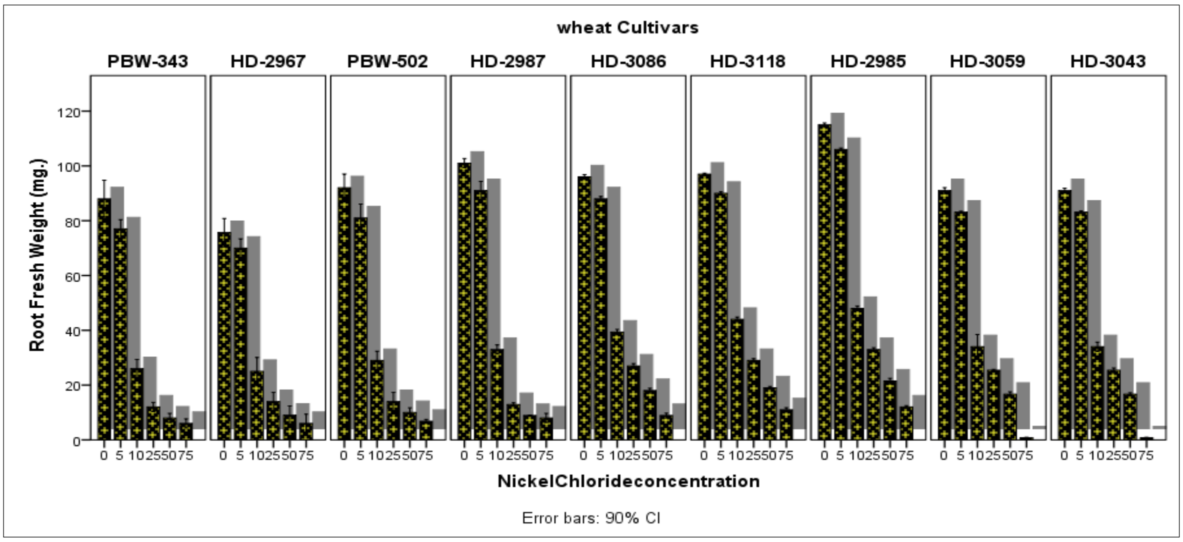

Fig 4: Effect of different concentration of $NiCl_2$ on root fresh weight (mg.)

Excess amount of $NiCl_2$ approximately 50 mg/L decreased the shoot yield than the low content. At 5 mg/L $NiCl_2$ concentration, shoot and root yield least effected in all cultivars of wheat. As the results indicate that the increased concentration of $NiCl_2$, shoot, root yield drastically reduced. An interesting point that the cultivar PBW-343 and HD-2967 were lesser affected at 5, 10, 25 and 50mg/L concentration of $NiCl_2$, but higher reduction in shoot yield was observed at 75mg/L $NiCl_2$. Our findings are similar to the finding of Bashmakov *et al.* (2006) [8] in maize and Brown *et al.* (1987) [10] in barley.

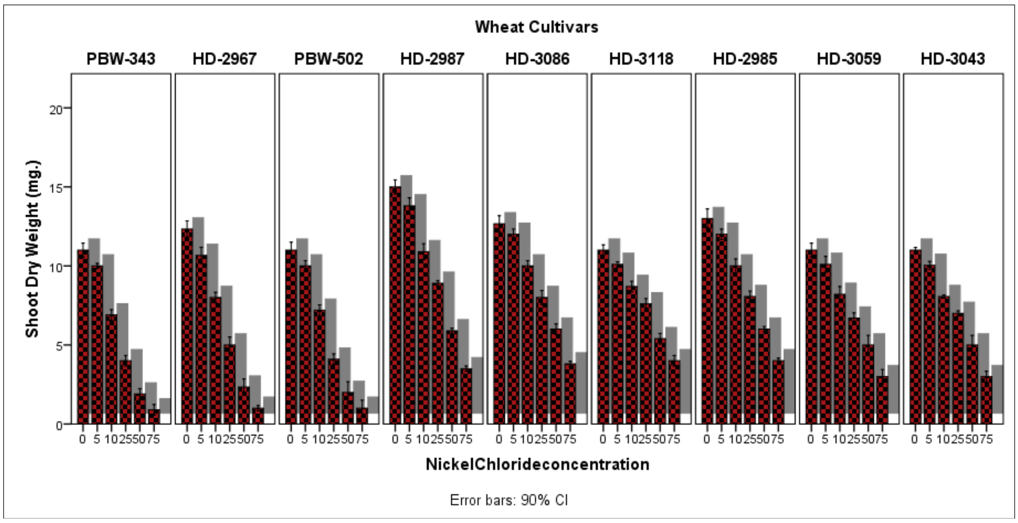

Fig 5: Effect of different concentrations of $NiCl_2$ on shoot fresh weight (mg.)

At low concentration (5mg/L) showed a significant increase in dry matter production of root and shoot. But high concentration (5mg/L) reduced the dry matter production. The effect of nickel chloride concentrations on dry weight of root and shoot of all cultivars of wheat has been depicted in the figure 6 and 7 respectively. Our findings are also similar to the, Baccouch *et al*. (2001) [7] in maize. High content of nickel significantly decreased both root and shoot dry weight of maize seedling (Bashmakov *et al.* 2006) [8].

The effect of nickel chloride concentrations on dry weight of root and shoot of all cultivars of wheat has been depicted in the figure 6 and 7 respectively. The present findings indicated that the cultivar PBW-343 showed minimum root dry weight (9, 8.2, 2.8, 1.4, 1 and. 35 mg/L respectively) at varying concentrations of nickel chloride (viz., control, 5, 10, 25, 50 and 75 mg/L respectively) than the other cultivars, while the maximum root dry weight (10, 9.58, 5, 3.5, 2.5 and 1.3mg) noted in HD-3118 cultivar. Considerable amount of $NiCl_2$ inhibited the rood dry yield in PBW-343 and HD-2967 cultivars, both these two cultivars are more sensitive against to $NiCl_2$ treatment than the others cultivars. Increasing levels of $NiCl_2$ reduced the dry matter production of shoot. Elevated amount of $NiCl_2$ decreased the dry weight of shoot in all cultivars.

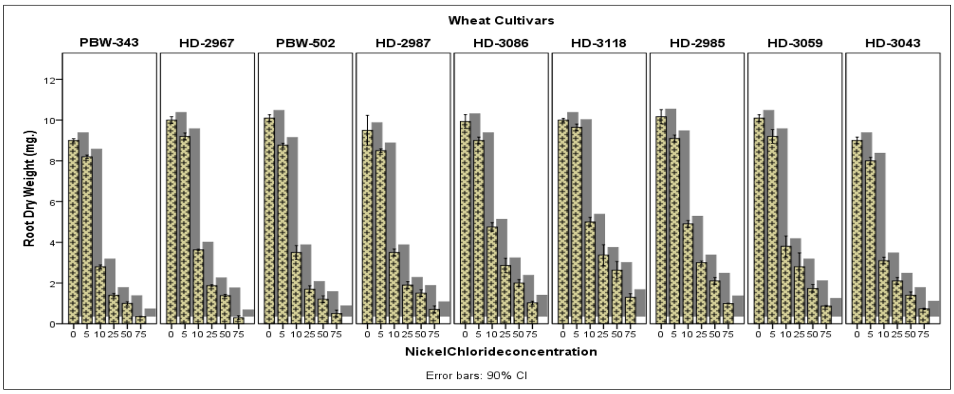

Fig 6: Effect of different concentration of $NiCl_2$ on root dry weight (mg.)

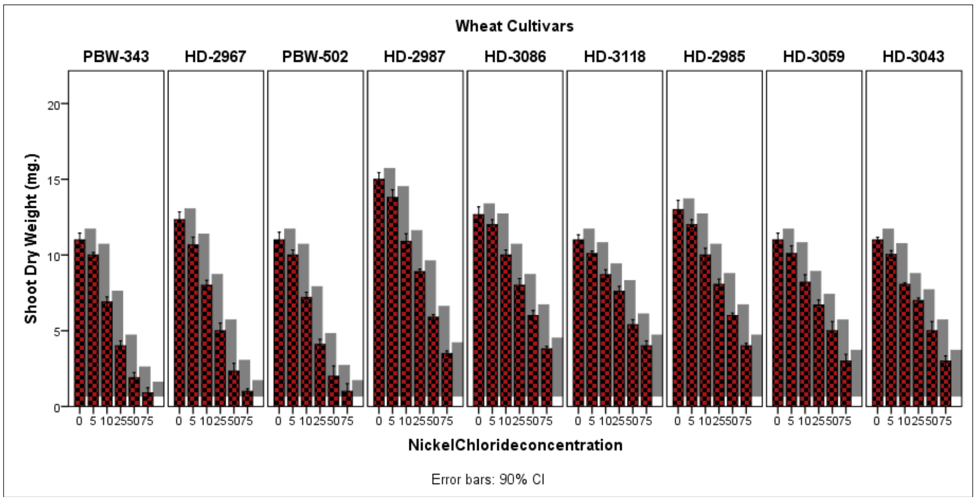

Fig 7: Effect of different concentration of $NiCl_2$ on shoot dry weight (mg.)

The increasing amount of $NiCl_2$ showed minimum shoot dry weight of PBW-343 cultivar (11, 10, 6.9, 4, 1.9, and 0.9mg), at varying concentration of $NiCl_2$ (viz., control, 5, 10, 25, 50 and 75 mg/L respectively) whereas the cultivar HD-2987 showed maximum shoot dry weight (15, 13.8, 10.9, 8.9, 5.9 and 3.5mg) at these concentration. High concentration of $NiCl_2$ showed highly reduction in root dry weight of cultivars PBW-343, HD-2967 and PBW-502 at 75mg/L concentration as comparison to other concentrations. Over finding is similar to the heavy metal toxicity reduced dry weight of wheat plants (Athar and Ahmad 2002) [6]. Increasing concentration of Ni (0.0004-0.08mM) much more decreased shoot biomass, while at the same concentration of Ni elevated wheat root biomass (Matraszek *et al*. 2016) [28]. The yield of cucumber vegetable reduced under the nickel stress (Goncalveset *et al*. 2007). Increasing amount of nickel in the nutrient solution reduced the yield of maize, and field bean (Antonkiewicz *et al.* 2016) [5]. Toxic effect of nickel significantly affected the total dry matter production and yield of plant (Sreekanth *et al*. 2013) [57]. Low accumulation of Ni increased nodulation and crop yield in soyabean (Malavolta and Moraes; 2007) [27]. Increasing concentration of nickel decreased leaf, stem and root biomass (Nie *et al*. 2015) [35]. Increasing level of Ni (40, 50 and 60 mg/L) decreased fresh and dry weight of sunflower cultivars (Ahmad *et al.* 2009) [2]. Toxicity of Ni nanoparticles decreases the fresh & dry weight of both roots and aerial parts (Miri *et al.* 2017). Higher concentration of nickel concentration showed morphological effected on wheat seedlings (Uruc and Parlak, 2016) [58] and decreased seedling growth of *Zea mays* (Bhardwaj *et al*. 2007) [9]. High Ni concentration affected growth and development of plants (Yusuf *et al.* 2010, 2011; Moosavi *et al*. 2012) [62, 63, 32].

Conclusion

From the observations it can be concluded that, low $NiCl_2$ (low 5mg/L) had stimulatory effect on seed germination, seedling growth and fresh and dry matter production of nine wheat (*Triticum aestivum* L. em tell.) cultivars. Application of $NiCl_2$ beyond 5mg/L adversely affected the germination, growth and yield. It is also concluded from findings that the cultivar HD-2985, HD-3086 and PBW-343 were recorded as more tolerant, moderately tolerant and the most sensitive respectively on the basis of germination and growth parameters.

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