RESPONSE OF Citrullus colocynthis L. TO SINGLE AND COMBINED STRESSES OF
SALINITY (NaCl) AND HEAVY METAL (NiCl₂)

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ABSTRACT

Salinity and heavy metal stresses are great hindrances to germination and the early
development of plants. Nickel (Ni) is an indispensable micronutrient for plants at low while
toxic in high concentrations. Citrullus colocynthis L. is a medicinal cucurbit weed in desert
and agricultural areas. Germination and early development of seedlings determine the
future of the plant. The contemporary target of the study is to appraise the influence of
single and combined stresses by varying concentrations of NaCl and NiCl₂ on germination
and seedlings of C. colocynthis. Germination percentage, radicle length, plumule length,
fresh weight and dry weight of seedlings of C. colocynthis with three levels of salt (100mM,
200mM and 400 mM NaCl), three levels of heavy metal (50, 100, 200 uM NiCl₂) and Nine
combined levels (100 mM NaCl+50 uM NiCl₂), (200 mM NaCl+50 uM NiCl₂) (400mM
NaCl+50uM NiCl₂) (100mM NaCl+100 uM NiCl₂) (200 mM NaCl +100 uM NiCl₂) (400
mM NaCl+100 uM NiCl₂) (100 mM NaCl+200uM NiCl₂) (200 mM NaCl+200 uM NiCl₂)
(400 mM NaCl+200 uM NiCl₂) were studied. This experiment was conceded out in a
completely randomized design with four replications. The responses showed an adverse
trend with increasing stress levels. Germination and seedling growth factors declined
under high- stress levels of salt (400 mM NaCl), high HM (200 uM NiCl₂), and combined
high salt or high HM but positively improved on lower salt (100 mM NaCl), lower HM (50
uM NiCl₂) and combined lower salt with lower HM (100 mM NaCl+50 uM NiCl₂). It has been
found that desertecotype is more tolerant against single or combined stresses.

Keywords: Combined stress, Cucurbit, Germination indices, Heavy metal, Salinity.

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INTRODUCTION

Salinity and heavy metal are the most influential factors among environmental
stresses limiting plant germination. The potential of water absorption in the root
zone of plants and availability of water is reduced due to salinity and heavy metals
in the soil. Avoidable ions impose toxic properties on plant physiological as well
as biochemical procedures, which may lead to trepidation in nutrient ion
uptakes by root epidermis and root hairs reducing seed germination. The
sensitivity of the plant to salinity and heavy metal stresses varies at the
different stages of growth (Huang et al., 2016). Salinity may induce other
secondary stresses such as oxidative stress, through which active radical
accumulation may lead to the oxidation of cell’s proteins & lipids which result in
cell death (Molassiotis et al., 2006). Environmental stresses have been
studied in numerous investigations (Asgari et al., 2002). It was revealed
that onion (Allium cepa L.) exhibited different responses to salinity (Hanci and
Cebci, 2015). Germination indices of Triticum aestivum L. towards salinity
stress revealed significant effects (Soef et al., 2022). Increased salinity
significantly decreased germination indices of Agropyron desertorum (Saeedi
et al., 2013).

Heavy metals are recommended as one of the
leading cradles of ecological pollution
causing numerous complications
connected to industrial as well as
agricultural activities. The amplified extent

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of heavy metals in soil and consequently, in plants produce toxic effects in animals and humans (Bhardwaj et al., 2017). The elements with a specific gravity above 5 while atomic weight from 63-200 are classified under heavy metals (Oldham, 2011). A low quantity of heavy metals is essential for plants generally non-toxic and their amount exceeding the required limit imposes toxicity. Examples of heavy metals may be cobalt, copper, iron, magnesium, molybdenum, nickel, and zinc (Co, Cu, Fe, Mg, Mo, Ni & Zn) beneficial in low quantity and reduce germination (Khan et al., 2008; Sengupta et al., 2014; Rizvi et al., 2020).

Heavy metals diminished soil fertility, microbial activity, and plant yield (Sengupta et al., 2014). Heavy metals dissolved in water (from industrial sources and sewage) used for irrigation affect the plant reducing seed germination, growth as well as yield (Oguntade et al., 2015). Nickel, as a component of enzyme urease widespread plant types. An enzyme urease has a dynamic role during the germination of seeds (Mondal and Bose, 2019). In the absence of nickel, the urease enzyme is absent, so urea may be accumulated in the plants having toxic effects and forming necrotic lesions on the leaf tip. If a plant faces nickel deficiency then the urease enzyme will be deficient causing urea toxicity (Polacco et al., 2013). Nickel, with atomic number 28 and atomic weight of 58.71, is the 28th element in the periodic table. Nickel is a necessary micronutrient of plants for normal growth while its high absorption imposes adverse effects. Low concentration levels of nickel were positively affected while high concentrations of nickel chloride above 0.5mg/L showed adverse effects in various wheat (Triticum aestivum L.) cultivars (Muhammad et al., 2009; Shweti et al., 2018). Nickel is a catalyst in enzymes helping nitrogen fixation in leguminous plants and is also involved in the breakdown of urea (a nitrogenous compound). Ni may be deliberated micronutrient for cereals for example barley crop failed to complete its life cycle without nickel (Mondal and Bose, 2019). Some bacteria and fungi, friendly for plant growth also require nickel (Wang et al., 2013). Maintenance of proper cellular redox state and physiological and growth responses of the cell also require the presence of nickel (Mir et al., 2018). It has been reported that higher concentrations of nickel may stop mitosis, inhibit the transport of carbohydrates and inhibit starch accumulation in the photosynthetic zones (Du et al., 2020). Nickel toxication reduces the yield as it reduces the transport of food and nutrients from leaves toward flowers (Sachan and Lal, 2017).

Medicinal plants are important for the preparation of many drugs as they comprise active ingredients for many drugs. Production of the drug ingredients by the plant is predominantly directed by the genotype of the plant while environmental factors may have a significant influence on the magnitude and value of their constituents (Witzler et al., 2018). Citrullus colocynthis L. is an important medicinal cucurbit weed having 53% oil and 28% proteins in its seeds (Kheiry et al., 2017). The oil obtained from seeds of C. colocynthis L. is identical to Brassica oil and is being used for pharmaceutical diligence (Akusu and Emelike, 2018) due to its anti-microbial as well as anti-cancer potentials (Kapoor et al., 2020). C. colocynthis L. is also being used to treat urinary tract infections in indigenous medication by the people of the Mediterranean zone of the world (Gacem et al., 2019). The primary reason behind the harmful impact of salinity and heavy metals on the plant’s seedlings is osmotic stress which inhibits the uptake of water and ion toxification (Lutts and Lefèvre, 2015). Responses of different plants toward different saline and heavy metal stress conditions are different (Gul et al., 2016). The capacity of the plants to tolerate and rest saline and heavy metal stresses are different which may assess through germination percentage and seedling growth parameters under salinity and heavy metal stress conditions as was studied in the case of parthenium control in Punjab (Shabbir et al., 2012).

Considering the critical issues of salinity and heavy metal stress to the environment, as well as the medicinal reputation of C. colocynthis, and the deficiency of particular understanding of its response to salinity and heavy metal stresses, the current study was steered to explore the tolerance inception of C.
colocynthis to salinity and heavy metal single and combined stresses in desert and agricultural soils.

MATERIALS AND METHODS

Current study intended to evaluate the single and combined effects of sodium chloride and nickel chloride on seed germination of tumma (Citrullus colocynthis L.). For the present study, the seeds of desert weed C. colocynthis L. were obtained from the Thal desert of district Layyah Punjab, Pakistan. The experiment was conducted in the Research laboratory of the Botany department, The Islamia University of Bahawalpur, Pakistan. Selected healthy seeds with approximately uniform size were sanitized with 0.01% mercuric chloride and washed in disinfectant/distilled water, soaked in 1000ppm dilute sulphuric acid solution to break dormancy for 24 hours. Twenty seeds placed into separate Petri plates lined with the what-man filter paper no-1 accordingly; control plants (T0)=Distilled water, T1=100mMol NaCl (lower salt level), T2=200 mM NaCl (moderate salt level), T3=400 mM NaCl (High salt level), T4=50 uM NiCl₂ (lower nickel level), T5=100uM NiCl₂ (moderate nickel level) T6=200 uM NiCl₂ (higher nickel level), T7=(T1+T4 i.e. lower salt level + lower nickel level), T8=(T2T4 i.e. moderate salt level + lower nickel level), T9=(T3+T4 i.e. higher salt level + lower nickel level), T10=(T1+T5 i.e. lower salt level + moderate nickel level), T11=(T2+T5 i.e. moderate salt level + moderate nickel level), T12=(T3+T5 i.e. higher salt level + moderate nickel level), T13=(T1+T6 i.e. lower salt level + moderate nickel level), T14=(T2+T6 i.e. moderate salt level + moderate nickel level), T15=(T3+T6 i.e. higher salt level + moderate nickel level). The Petri plates were provided with distilled water and Hoagland’s nutrient solution to compensate for the evaporation. The experiment was designed completely randomized with four replicas of each treatment. Seeds were considered as germinated at attaining a minimum 1mm length by coleoptile and radicle. Germinated number of seeds was counted after ten days of soaking, and the length of radicle and plumule of germinated seeds was measured using a scale. The fresh weight and dry weight of germinated seedlings were measured by electronic balance. Germination percentage (GP) was calculated by

\[
\text{Germination percentage} = \frac{\text{number of germinated seeds}}{\text{total seeds}} \times 100
\]

Root length and shoot length were measured by using a centimeter and millimeter-scale while fresh weight and dry weight were measured by electronic balance.

Statistical analysis

The trial was repeated twice with four replicates. Data analysis for (ANOVA) and (LSD) at 0.05 probability was done by using software Statistix 8.1. For the graphical presentation of the data, MS-Excel was used.

RESULTS

Germination percentage

Citrullus colocynth both ecotypes showed a significant increase in germination percentage as compared to control at lower salt T1 (100 mM NaCl), moderate level of salt T2 (200 mM NaCl), lower heavy metal T4 (50 uM NiCl₂) and a slight increase at a moderate level of heavy metal T5(100 uM NiCl₂) while a decrease in a high level of salt T3 (400 mM NaCl) and high level of heavy metal T6 (200 uM NiCl₂) in single salt and heavy metal stress. In combined stresses of salt and heavy metal levels, a significant increase in germination was recorded in combined lower salt and lower Heavy metal T7 (100 mM NaCl+ 50 uM NiCl₂) and a slight increase where the lower salt level was provided with moderate-heavy metal like in T10 (100 mM NaCl+100 uM NiCl₂). Lower heavy metal levels embraced the negative impact of higher salt levels in combined treatments as a decrease in germination percentage was observed under combined higher levels of salt and heavy metals (Fig. 1, 2, 3). Desert ecotype showed better germination percentage than agricultural in any stress level (Table 1 & 2).
**Seedling radicle length**

*Citrullus colocynthis* desert ecotype showed better performance in case of resistance and tolerance against salt or heavy metal in seedling radicle length than agricultural ecotype at any level of single or combined salt and heavy metal stresses. Best results of increased seedling radicle as compared to control were observed in T1 (100 mM NaCl), T4 (50 μM NiCl₂) and T7 (100 mM NaCl +50 μM NiCl₂). A slight increase in radicle length was observed in T2 (200 mM NaCl), and T5 (100 μM NiCl₂). A slight decrease in seedling radicle as compared to control was observed in T8 (200 mM NaCl+50 μM NiCl₂), T10 (100 mM NaCl+100 μM NiCl₂) and T13 (100 mM NaCl+200 μM NiCl₂). A significant decrease in seedling radicle was observed in T3 (400 mM NaCl), T6 (200 μM NiCl₂), T9 (400 mM NaCl +50 μM NiCl₂), T11 (200 mM NaCl+100 μM NiCl₂), T12 (400 mM NaCl +100 μM NiCl₂), T14 (200 mM NaCl+200 μM NiCl₂) and T15 (400 mM NaCl+200 μM NiCl₂) (Fig. 4,5,6) (Table 1&2).

**Seedling fresh weight**

*Citrullus colocynthis* desert ecotype showed better performance in case of resistance and tolerance against salt or heavy metal in seedling fresh weight than agricultural ecotype at any level of single or combined salt and heavy metal stresses. Best results of increased seedling fresh weight as compared to control were observed in T1 (100 mM NaCl) T2 (200 mM NaCl), T4 (400 mM NaCl), T5 (50 μM NiCl₂) and T7 (100 mM NaCl + 50 μM NiCl₂). A slight decrease in seedling fresh weight was observed in T8 (200 mM NaCl +50 μM NiCl₂) and T10 (100 mM NaCl+100 μM NiCl₂). A significant decrease in seedling fresh weight was observed in T3 (400 mM NaCl), T6 (200 μM NiCl₂) T8 (200 mM NaCl +50 μM NiCl₂) T9 (400 mM NaCl+100 μM NiCl₂) T11 (100 mM NaCl+100 μM NiCl₂), T12 (400 mM NaCl+100 μM NiCl₂) T13 (100 mM NaCl+200 μM NiCl₂), T14 (200 mM NaCl+200 μM NiCl₂) and T15 (400 mM NaCl+200 μM NiCl₂) (Fig. 10, 11, 12; Table 1 & 2).

**Seedling dry weight**

*Citrullus colocynthis* desert ecotype showed better performance in case of resistance and tolerance against salt or heavy metal in seedling dry weight than agricultural ecotype at any level of single or combined salt and heavy metal stresses. Best results of increased seedling dry weight as compared to control were observed in T1 (100 mM NaCl), T2 (200 mM NaCl), T4 (50 μM NiCl₂), T5 (100 μM NiCl₂) and T7 (100 mM NaCl+50 μM NiCl₂). No change in seedling dry weight as compared to control was observed in T8 (200 mM NaCl +50 μM NiCl₂) and T10(100 mM NaCl +100 μM NiCl₂). A slight decrease in dry weight was observed in T4 (50 μM NiCl₂), T12 (400 mM NaCl +100 μM NiCl₂) and T13 (100 mM NaCl +200 μM NiCl₂). A significant decrease in seedling dry weight was observed in T14 (200 mM NaCl+200 μM NiCl₂) and T15 (400 mM NaCl+200 μM NiCl₂) (Fig. 13, 14, 15; Table 1 & 2).

**Fig. 1.** T0=CONTROL, T1=100 mM NaCl, T2=200 mM NaCl, T3=400 mM NaCl,

**LSD 5%=2.03**

**Fig. 2.** T0=CONTROL, T1=100 mM NaCl, T2=200 mM NaCl, T3=400 mM NaCl, T4=50 uM NiCl₂,

T5=100 uM NiCl₂, T6=200uMNiCl₂, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5,

**LSD 5%=2.09**

**Fig. 3.** ECOTYPES, E1=DESERT, E2=AGRICULTURAL

**GPE1**

**GPE2**
**Citrullus Desert Ecotype**

**LSD 5% = 0.16**

**Fig. 4.** T0=CONTROL, T1=100mMNaCl, T2=200mMNaCl, T3=400mMNaCl, T4=50uMNiCl2, T5=100uMNiCl2, T6=200uMNiCl2, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5, T12=T3+T5, T13=T1+T6, T14=T2+T6, T15=T3+T6

**Citrullus Agricultural Ecotype**

**LSD 5% = 0.16**

**Fig. 5.** T0=CONTROL, T1=100mMNaCl, T2=200mMNaCl, T3=400mMNaCl, T4=50uMNiCl2, T5=100uMNiCl2, T6=200uMNiCl2, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5, T12=T3+T5, T13=T1+T6, T14=T2+T6, T15=T3+T6

**Fig. 6.** ECOTYPES, E1=DESERT, E2=CULTIVATED
**Citrullus Desert Ecotype**

![Graph](image)

**Fig. 7.** T0=CONTROL, T1=100mMNaCl, T2=200mMNaCl, T3=400mMNaCl, T4=50uMNiCl2, T5=100uMNiCl2, T6=200uMNiCl2, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5, T12=T3+T5, T13=T1+T6, T14=T2+T6, T15=T3+T6

**Citrullus Agricultural Ecotype**

![Graph](image)

**Fig. 8.** T0=CONTROL, T1=100mMNaCl, T2=200mMNaCl, T3=400mMNaCl, T4=50uMNiCl2, T5=100uMNiCl2, T6=200uMNiCl2, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5, T12=T3+T5, T13=T1+T6, T14=T2+T6, T15=T3+T6

**Fig. 9.** ECOTYPES, E1=DESERT, E2=AGRICULTURAL

![Graph](image)
Fig. 10. T0=CONTROL, T1=100mMNaCl, T2=200mMNaCl, T3=400mMNaCl, T4=50uMNiCl2, T5=100uMNiCl2, T6=200uMNiCl2, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5, T12=T3+T5, T13=T1+T6, T14=T2+T6, T15=T3+T6

Fig. 11. T0=CONTROL, T1=100mMNaCl, T2=200mMNaCl, T3=400mMNaCl, T4=50uMNiCl2, T5=100uMNiCl2, T6=200uMNiCl2, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5, T12=T3+T5, T13=T1+T6, T14=T2+T6, T15=T3+T6

Fig. 12. ECOTYPES, E1=DESERT, E2=AGRICULTURAL
Fig. 13. T0=CONTROL, T1=100mMNaCl, T2=200mMNaCl, T3=400mMNaCl, T4=50uMNiCl2, T5=100uMNiCl2, T6=200uMNiCl2, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5, T12=T3+T5, T13=T1+T6, T14=T2+T6, T15=T3+T6

Fig. 14. T0=CONTROL, T1=100mMNaCl, T2=200mMNaCl, T3=400mMNaCl, T4=50uMNiCl2, T5=100uMNiCl2, T6=200uMNiCl2, T7=T1+T4, T8=T2+T4, T9=T3+T4, T10=T1+T5, T11=T2+T5, T12=T3+T5, T13=T1+T6, T14=T2+T6, T15=T3+T6

Fig. 15. ECOTYPES, E1=DESERT, E2=AGRICULTURAL
Table 1. Analysis of variance for effect of salt (NaCl), Heavy metal (NiCl₂), combined Salt +Heavy metal (NaCl + NiCl₂) Stresses on germination percentage & Seedling (Radicle length, plumule length, fresh weight, and dry weight) of Citrullus colocynthis from Desert Ecotype (E1) in Petri Experiment.

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Table 2. Analysis of variance for effect of Salt (NaCl), Heavy metal (NiCl₂), combined Salt +Heavy metal (NaCl + NiCl₂) Stresses on germination percentage & Seedling (Radicle length, plumule length, fresh weight, and dry weight) of Citrullus colocynthis from Agricultural Ecotype (E2) in Petri Experiment.

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DISCUSSION

The outcomes of our present work revealed that both ecotypes of C. colocynthis showed different responses against different salinity and heavy metal treatments. The influence of salinity and heavy metal stress was significant on germination percentage and other growth parameters of seedlings at high levels of salt and heavy metals either single or combined stresses. Single or combined higher salt and higher heavy metal have a significant negative impact on germination of seeds, radicle protrusion,
plumule projection, fresh weight, and dry weight of seedlings. Correspondingly, numerous works confirmed that high-stress levels of salinity and heavy metal might generate a punitive situation for seed germination (Sharma et al., 2020). It may be due to a drop in interior osmotic potential due to salt and water stress already been declared (Maucieri et al., 2018), which had been recognized to variation between organic and mineral ions balance present in the cell sap and absorption of ions (Ahmad et al., 2021). The disturbance of nutrients absorption and uptake also has been described as the loss of germination percentage and growth parameters (Muhammad et al., 2006; Zahra et al., 2021). Diminution of germination percentage under salinity stress already observed (Onen et al., 2018), also may be the same under heavy metal stress and loss of water (AbdElgawad et al., 2020). The obstructive water content of seeds due to salinity and heavy metal stress leads to a decline in the activity of enzymes especially hydrolytic enzymes which have a key role to hydrolyze cotyledonous reserve food required for supplying energy by respiration during the early stages of seed growth. Limitation of water uptake, transfer of seed stocks, and direct influence on gene expression and organic structure is related to salinity and heavy metal stresses (Schillaci et al., 2019). An important reason behind the reduced seed germination may be the inhibited nutrient delivery from seed storage tissues to the developing embryo (Joshi, 2018). The minerals and heavy metals accumulation in the plant cells may lead to negative effects like ion toxicity induced mainly by salinity and drought stress (Kumar, 2020). Detaining absorbable water by seeds due to salinity and heavy metal stress can lead to reduced germination (Sukmarani et al., 2021). Decreased seed germination under salinity and heavy metal stress may be illuminated by turgor pressure limitation or the dry material storage (Khosropour et al., 2022). High levels of salinity suppress seedling growth, imposing a negative impact of NaCl on cellular membranes, and destructing cytoplasmic membranes due to ion toxicity (Shehzad et al., 2019; Shahid et al., 2020). The main factor which limits the establishment of the plant under salinity and heavy metals is impaired germination (Ali et al., 2021). The adverse impact of salinity on germination and seedling parameters of different plants has been testified previously (Li et al., 2020). The debility in germination under salinity and heavy metals has been endorsed as a collective outcome of the toxicity of salts and osmotic pressure (Kumar et al., 2022). In our experiment, combined stresses showed more adverse effects which may be due to the addition of increased chloride ions from sodium chloride as well as nickel chloride resulting in a rise in osmotic stress inhibiting seed germination (Shahzad et al., 2019). Altered water relations due to high salt stress significantly delay germination (Zhu et al., 2019) because extra salts are accumulated in intercellular spaces (Oi et al., 2020). Sometimes embryo may be damaged due to higher levels of Na+ /Cl- ions or exosmosis of water (Hakimi et al., 2022). In the present study, the desert ecotype of Citrullus colocynthis proved it more tolerant than the agricultural ecotype. All other attributes of the desert ecotype of C. colocynthis are better than the agricultural ecotype. Regarding germination and seedlings attributes under salinity and heavy metals of desert ecotype of C. colocynthis suggests dominance and greater capacity to manage with salinity levels over agricultural ecotype. Previously it is also known that Salt-tolerant plant ecotypes accumulate Na+ at a lower rate than the salt-sensitive plant ecotypes as seen in Hordeum spp. (Hmidi et al., 2019). Still, early germination is strongly correlated and coordinated with seedling growth parameters like radicle protrusion, plumule projection; fresh and dry biomass production in both ecotypes subjected to salinity, heavy metals stresses either single or combined stress. The most essential phase for the future life of a plant is its germination. The subsequent development of the plant depends upon germination and early seedling attributes. The resistance of plants to heavy metals and salinity at the seedling stage produces tolerant juvenile plants. The current discoveries are similar to the verdict of Akhtar et al., 2021. In the present study high level of NiCl₂ (200uM)
reduced the germination percentage of both ecotypes of *C. colocynthis* L. same as described earlier and a high level of Ni stress reduced seed germination of many plant types (AbdElgawad *et al.*, 2020). The reason behind this justifies that high-stress levels of nickel have straight possessions on the events related to enzymes like amylases, urease, proteases, and ribonucleases, thus affecting the breakdown and utilization of food backup in sprouting seed (Tlhajoane, 2018). High-stress levels of Nickel chloride in our experiment reduced other attributes of seedlings of *C. colocynthis* like seedling's radicle projection, and plumule protuberance; fresh and dry biomass similarly to the high levels of nickel inhibited the growth of soybean in an experiment (Aqeel *et al.*, 2021). In present studies, low concentrations of Nickel (50μM) positively promoted germination percentage and other parameters of seedlings of *C. colocynthis* like seedlings radicle extension, plumule projection, fresh and dry biomass similarly to low levels of nickel promoted germination in wheat (Sofo *et al.*, 2022).

**CONCLUSION**

Our experiment indicated that the desert ecotype of *C. colocynthis* performed better and tolerate the examined stress better than the irrigated ecotype. Higher levels of single stress i.e. 400 mM NaCl and 200 μM NiCl₂ harmed germination of both ecotypes and showed a significant decrease in germination. In combined stress conditions, better germination at combined lower salt and lower heavy metal (100 mM NaCl+50 μM NiCl₂) was observed, tolerable at lower salt and moderate metal (100 mM NaCl +100 μM NiCl₂) acceptable at moderate salt and lower metal (200 mM NaCl+50 μM NiCl₂), a slight decrease in moderate salt and moderate metal (200mM NaCl+100μM NiCl₂), a significant decrease in moderate salt and high metal (20 0mM NaCl+200 μM NiCl₂) and combined high salt and moderate metal (400mM NaCl+ 100μM NiCl₂) and combined high salt and high metal (400mM NaCl+200 μM NiCl₂). Consequently, as *C. colocynthis* is a medicinal weed and soil conserving plant, it can be recommended for the restoration of rangelands indulged in salinity and heavy metal conditions. This research will open a gateway for future researchers to study its allelopathic effects, and genome and explore it as a plant of multidimensional values.

**CONFLICT OF INTEREST**

We have no conflict of interest.

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This research work is part of my Ph. D thesis supervised by coauthor.

**AUTHOR’S CONTRIBUTIONS**

Both authors contributed comprehensively.

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