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Influence of Nickel Treatment on Antioxidant Responses and Secondary Metabolite Production in *Eryngium foetidium* Linn.

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ABSTRACT

The present study was carried out to explore the heavy metal nickel tolerance potential in Eryngium foetidum Linn., belongs to the Apiaceae, the plant with culinary importance, and influence of nickel on secondary metabolites production. Twenty five day old seedlings/plants of E. foetidum were treated with three different concentrations of nickel (50 ppm, 100 ppm and 200 ppm) in the form of nickel sulphate ($NiSO_4$) for five days. After nickel treatment, plant growth was monitored by determining shoot length, root length, fresh and dry weights of leaves/shoots and roots, and also quantitative determination of chlorophyll pigments and L-proline. Nickel at all tested concentrations affected the plant growth compared to control group of plants. Significant increase in L-proline concentration was observed in the Ni-treated plants compared to respective controls. Accumulation of nickel was more at rhizosphere compared to aerial parts. Nickel treatment elevated the superoxide disumutase activity and malondialdehyde content at all the concentrations tested. While catalase activity was found to be increased at 50 ppm and 100 ppm concentrations of Ni, and activity slight decrease in activity at 200 ppm. Results of leaf phytochemical analyses suggest that, nickel has increased the production and accumulation of phenolics, flavonoids and saponins. There is a direct correlation observed between accumulation of nickel and phytochemicals in leaves. Nickel might have induced the production of phytochemicals in E.foetidum leaves. The plant has metal heavy tolerance, and nickel at low concentrations can be used as an elicitor for the production of secondary metabolites in E. foetidum.

Keywords: *Eryngium foetidum Linn., Nickel treatment, Biochemical changes, Secondary metabolites.*

INTRODUCTION

Heavy metal accumulation in soil is mainly resulted due to anthropogenic activities such as agricultural practices, industrial activities, natural sources such as parent material and volcanic eruptions, further enters into the food chain (Raymond et al., 2011; Nandini et al., 2018).

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Plants and animals living on earth are affected by the heavy metals. Certain heavy metals such as Zn, Ni and Mn are accumulated in the plants being essential nutrient, hence hyperaccumulators. On the other hand, these metals at high concentrations become toxic to certain plants, i.e. non accumulating plants. Hence, all plant species have developed certain mechanisms to regulate essential metal homeostasis according to metal availability and their needs (Sylvain et al., 2018).

Nickel (Ni^{2+}) is an essential micronutrient and is the lowest of all essential elements at <0.5 mg/kg of dry weight (Marschner, 1995), and a micronutrient required by plants in very small quantities (Shafeeq et al., 2012; Zoya et al., 2016). It is also recognized as seventh micronutrient heavy metal which is essential for growth and development of plant body (Shweti & Verma, 2018). Nickel is found in several enzymes including urease, hydrogenase, carbon monoxide dehydrogenase, glyoxalase I, peptide deformylase, acetyl-S-coenzyme A synthase, methylcoenzyme M reductase and Ni-containing superoxide dismutase (NiSOD) (Mulrooney & Hausinger, 2003). Ni addition markedly enhanced the dry matter production of urea-grown plants, but its deficiency significantly reduced the urease activity in the leaves and roots of plants (Gerendas & Sattelmacher, 1999). The effect of nickel toxicity on health of human beings, aquatic ecosystems, agriculture crops and products has become world wide problem (Sharma & Dubey, 2005). In plants, nickel causes oxidative stress by producing reactive oxygen species (Dat et al., 2000). The Ni^{2+} induced antioxidant system in plants such as corn and pigeon pea was well documented (Sreekanth et al., 2013). The Ni stress resulted in reduced concentration of antioxidants such as GSH and AsA (Madhava Rao & Sresty, 2000). Ni tolerance in view of Ni exposure to the plants resulted in the high level of GSH content (Freeman et al., 2004). In addition, enzymes of antioxidant system have also been stimulated to nickel stress (Madhava Rao & Sresty, 2000; Gajewska et al., 2006; Gajewska & Sklodowska, 2008; Sreekanth et al., 2013). The Ni stress can lead to induction of ROS production which affect photosynthesis, transpiration, mineral nutrition ultimately plant phytotoxicity Babar Shahzad et al., 2018).

The plant under the study *Eryngium foetidum* Linn., is a biennial herb and grows as naturalized weed (Cecil J Saldanha, 1996). The earlier studies have showed that, the plant *E.foetidum's* extracts and its essential oils were of analgesic (Nungki et al., 2015), anti-inflammatory (Li et al., 2012; Suwitcha et al., 2013), antioxidant (Fernandez et al., 1999), antimalarial, antibacterial (Mohammad et al., 2012; Singh et al., 2014; Sinem et al., 2015), antidiabetic, anticlastogenic (Tabarak et al., 2016), antihelminthic (Li et al., 2012; Nungki et al., 2015), anticonvulsant (Paul et al., 2008) and antiarthritic in nature. Essential oil of *E. foetidum* has been reported to contain rich amount of aldehydes, which are of perfumery importance (Nungki et al., 2015). The main constituent of essential oil is Eryngial (E-2-Dodecenal) (Singh et al., 2014). In folkloric and ethnomedicine the whole plant or its parts are used to treat stomach ache, infertility, diarrhea, seizures, fever and hypertension (Mohammad et al., 2012). The plant is a rich source of vitamins, iron and carotene and the fresh leaves are used as flavoring and seasoning agent for variety of dishes across the world (Raja Chakraverty et al., 2016).

The Triterpenoid saponin, 3-*O*-[β-D-glucopyranosyl-(1→2)-[β -D-fucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→4)- β-D-glucopyranosyl]-olean-12-en-23,28-diol was detected in this plant (Ping et al., 2012). *E. foetidum* comprises a variety of steroids namely, β-sitosterol, stigmatserol, campesterol, brassicasterol, 3α-cholesterol, (-)-clerosterol, Δ-avenosterol, Δ-avenasterol and Δ^5– stigmastadienol (Ping et al., 2012; Singh et al., 2014; Nandini et al., 2018).

E. foetidum L., being naturalized weed, putative medicinal plant with culinary importance as it is used as substitute of coriander, very well understood for its medicinal importance world wide. It is best to evaluate the plant for the capability to

withstand harsh environment condition and might be having capability and/or tolerance to the effect of toxic ion Ni by inducing the antioxidant enzyme system responses. The use of local plant species for stress studies is meaningful because these plants may survive under local environmental conditions of the area as compared to other plant species. In view of the culinary importance of *E. foetidum* and nickel as an essential micronutrient at low concentrations and a toxic element at high concentractions, the current was carried out to evaluate the the *E. foetidum*'s withstanding ability towards heavy metal nickel treatment, and influence of nickel on leaf secondary metabolite production.

MATERIALS AND METHODS

Collection of Plant Material:

Eryngium foetidum Linn., plants were collected from Hassan District of Karnataka state during the monsoon and post-monsoon season in the year 2018. The plant was taxonomically identified with the help of (Flora of Karnataka florakarnataka.ces.iisc.ac.in).

Chemicals and Reagents:

Chemicals and reagents were procured from either Sigma-Aldrich (USA) or HIMEDIA (Mumbai, India) or Sisco Research Laboratories (Mumbai, India) and were of either analytical grade. Millipore (Milli-Q) water was used throughout all the investigations. All other chemicals and reagents used unless and otherwise mentioned were of analytical grade.

Plant growth and metal stress treatments:

Seeds of *Eryngium foetidum* Linn. were collected from wild, surface sterilized with 0.5% sodium hypochlorite (v/v in sterile distilled water) solution imbibed in sterile double distilled water and germinated on the moistened Whatmann No.1 filter paper to raise the seedlings. Initially Hoagland medium was used for the acclimatization of the seedlings and plantlets for 25 days. Then, the plantlets were transferred to the plastic pots containing garden soil for further growth. Plants in the pots were categorized as control group (watered with water), treatment and metal stress groups as 0^{th} day (25^{th} day) and 5^{th} day (30^{th} day) groups and watered with distilled water containing 0 (control group), 50 ppm, 100 ppm, and 200 ppm nickel metal ($NiSO_4$) concentrations and maintained.

Plants of both control group and the metal (Ni^{2+}) treatment groups were subjected for plant growth measurements, chlorophyll determination, antioxidant enzyme assays, and membrane lipid peroxidation in terms of malondialdehyde (MDA) content. Similarly after respective treatment experiments leaves were collected, shade dried, powdered and used for nickel analysis and extracted with methanol for phytochemicals and quantitative determination of phenolics, flavonoids and saponins.

Measurement of plant growth:

Growth of plants both control and nickel-treatment groups was assessed by measuring the shoot length (SL) and root length (RL) and fresh (FW) and dry weights (DW) of leaves/shoots and roots. For dry weight determination plant samples (leaves/shoots and roots) were oven dried at 80 °C for 15 min and then vacuum-dried at 40 °C to constant weight and then dry weights (DW) were recorded.

Determination of leaf chlorophyll contents:

Leaf chlorophyll contents were estimated in leaf-acetone extracts of both control and Ni-treatment groups by spectrophotometric analysis at 645 nm and 663 nm (Daniel Arnon, 1949).

Total chlorophyll, chlorophyll a and chlorophyll b of control group and Ni-treatment group leaves determined using the following equations respectively:

Total chlorophyll = $20.2(A_{645}) + 8.02(A_{663})$

Chlorophyll a = $12.7(A_{645}) + 2.69(A_{663})$

Chlorophyll b = $22.9(A_{645}) + 4.68(A_{663})$

Total L-proline content in leaf samples was measured spectrophotometrically using the ninhydrin reaction following the method suggested by Bates et al. (1973) at 520 nm (Systronics, India) and expressed on a fresh weight (FW) basis (µmol proline/g FW).

Determination of changes in antioxidant enzyme levels:

Fresh leaf tissues (0.5g) of both control and Ni-treatment groups were collected and separately washed with double distilled water. Then ground using mortar and pestle by maintaining chilled condition in the homogenization buffer specific for each enzyme. The homogenate was filtered through muslin cloth and centrifuged at 10,000 rpm for 20 min at 4ºC, in cold centrifuge. The supernatant was used for superoxide dismutase (SOD) and catalase (CAT) enzyme assays. Total soluble protein concentrations in all the enzyme extracts of both control and Ni-treated group leaf tissue homogenates were determined by dye-binding assay method using Coomassive brilliant blue G-250 and bovine serum albumin as standard (Bradford, 1976).

Assay of Superoxide dismutase (SOD) (EC 1.15.11) activity:

Superoxide dismutase (SOD) (EC 1.15.11) activity was assayed by using the photochemical NBT method (Beauchamp and Fridovich, 1971; Rajinder Dhindsa, 1981). An inhibition curve was made against different volumes of extract that causes inhibition of the photo-reduction of NBT by 50%, that was calculated with the help of following formula,

$$\text{SOD (units)} = \frac{1}{\text{Vol. of extract (µl) required to cause 50\% of NBT inhibition X } 10^{-3}}$$

Assay of Catalase (CAT) (EC 1.11.1.6) activity:

Catalase (CAT) (EC 1.11.1.6) activity was assayed in the tissue extracts of both control and Ni-treatment groups following the method of Aebi, (1984). The activity was assayed by monitoring the decrease in absorbance due to H_2O_2 reduction at 240nm up to 3 min at 15 sec intervals, using a UV-VIS spectrophotometer. Catalase activity was expressed in terms of µM of H_2O_2 reduced/min/gm tissue fresh weight at 25±2°C.

Estimation of Malonaldehyde (MDA) content:

Fresh leaves (500mg) of both control and Ni-treatment group were separately homogenized into 2.5 ml of 0.1 TCA. Homogenate was centrifuged for 10 min at 10000xg. For every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% TBA were added. The reaction mixture was heated at 95°C for 30 min, cooled by ice bath, centrifuged for 15 min at 10000xg and absorbance of the supernatant was measured at 532 nm spectrophotometrically (Systronics, India).

Heavy metal (Nickel) analysis plant tissues:

Leaves and roots of both control group of plants and Ni-treated groups were harvested, shade dried, made into powder and subjected for nickel analysis independently. Tissue powder each of both control group and Ni-treatment groups (1 gm) was weighed, taken into a clean, dried conical flask of 250 ml capacity, to this 2.5 ml HNO_3 was added, mixed and 50 ml double distilled water was added. This mixture was digested on hot plate at a temperature of 300°C for 1 h. Then it was cooled to room temperature and filtered, and the filtrate was made up to 50 ml volume with double distilled water, this solution was used for heavy metal analysis using Atomic Absorption Spectrophotometer (GBC Avanta, Australia). Results were recorded and tabulated.

Phytochemical (secondary metabolite) analysis:

Leaves of both control group and Ni-treatment groups *E. foetidum* were excised, shade dried and coarsely powdered, defatted using n-hexane. The defatted material was subjected to Soxhlet extraction using methanol as solvent. The extract was concentrated using Rotary evaporator (Medica Instruments, Mumbai) and dried in the oven at 40°C. A greasy, viscous material, semi-solid in nature and aromatic in odor, soluble in methanol was obtained, stored in the air tight bottles at 4°C till further use.

Determination of total phenolics, flavonoids and saponins:

Total phenolic content of the extract was determined using Folin–Ciocalteu reagent, absorbance was measured at 670 nm, and a calibration curve was generated using the gallic acid standard. Total phenolic content was expressed as gallic acid equivalent (GAE) mg/ml tissue extract (Kaur & Kapoor, 2002). Total flavonoids content was determined by the aluminum chloride colorimetric method, absorbance was measured at 510 nm, and a calibration curve was generated by using the catechin standard (Chang et al., 2002). Total flavonoid content was expressed as catechin equivalents (CE mg/ml tissue extract). Total saponin content of the extract was determined using vanillin-sulphuric acid reagent method (Harinder et al., 2007) *Quillaza* bark saponin was used as standard, absorbance was measured at 540 nm using UV-Visible spectrophotometer (Systronics, India). Total saponin content was expressed as *Quillaza* bark saponin equivalent (QSE) mg/ml tissue extract.

Statistical analysis of the data.

Data from 3 trials of each experiment with at least 10 plants of the same group for each trial were statistically analyzed (one way ANOVA) using MS-Excel programme. Average values (mean) and standard errors (SE) were determined, means were compared with Dunnett's test (at 5% level of significance), and data were represented as mean±SE.

RESULTS AND DISCUSSION

Seed germination:

Germination of *E. foetidum* seeds was achieved within 2-3 days, and the germination percentage was determined to be between 98% to 99%. Seedlings grown into plants and attained nearly an average of 16 –18 cms of height in 25 days on 1x Hoagland's nutrient solution.

Measurement of plant growth:

Growth of *E. foetidum* plants of both control group and Ni-groups was measured and expressed in terms of shoot lengths and root lengths, tissue (leaf/shoot and root) fresh weights and dry weights (Table 1). Shoot lengths and root lengths of the Ni-treated plants were observed to be decreased with increasing concentration of Ni compared to control group of plants (Table 1). Similar trend was observed also in case of tissue fresh weights and dry weights of the plants of both Ni-treated groups and control group. Tissue (leaf/shoot and root) fresh weights and dry weights of Ni-treated group plants were observed to be decreased compared to tissues of control group of plants (Table 1).

Table 1: Changes in growth parameters during nickel treatment in *E. foetidium*

Ni concentration (ppm)	Shoot length (cm)	Root length (cm)	Leaf/shoot Fresh weight (g) (SFW)	Root Fresh weight (g) (RFW)	Leaf/shoot Dry Weight (g) (SDW)	Root Dry weight (g) (RDW)
Control	9.5±0.62	6.2±0.48	1.52±0.82	0.89±0.78	0.28±0.08	0.12±0.008
50	10.8±0.98	5.5±0.42	1.48±0.84	0.75±0.68	0.25±0.06	0.13±0.004
100	7.9±0.86	4.3±0.64	1.28±0.62	0.69±0.54	0.16±0.08	0.11±0.006
200	6.52±0.84	4.2±0.76	1.14±0.58	0.62±0.84	0.14±0.05	0.10±0.005

The reduced growth due to Ni-treatment (Ni-stress) at the highest concentration tested (200 ppm) there was an observable response of reduced growth, that might be due to loss of turgor pressure and decreased mitotic activity under the influence of nickel. Similar findings were also reported in *Zeay mays* (Salem et al., 2006).

Effect of Nickel Stress on photosynthetic pigments and L-proline:

Influence of nickel treatment on photosynthetic pigments of *E. foetidum* plants of both Ni-treated groups and control group were monitored by measuring the contents of chlorophyll a, chlorophyll b and total chlorophyll, results are depicted in Table 2.

Table 2: Effect of different concentrations of nickel (Ni) on chlorophyll pigments and L-proline content in *E foetidum* leaves after 05 days of Ni-treatment

Ni concentration (ppm)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total Chlorophyll (mg/g FW)	L-proline content (µmol/g FW)
0	1.32±0.09	0.42±0.03	1.74±0.06	1.56±0.18
50	1.03±0.04	0.52±0.02	1.55±0.08	2.48±0.12
100	0.88±0.06	0.31±0.04	1.19±0.04	3.33±0.21
200	0.52±0.04	0.34±0.06	0.86±0.05	3.88±0.42

Results of the investigations suggest that, chlorophyll pigments levels have been found to be decreased with increasing concentration of Ni compared to control group of plants (Table 2). Nickel has affected the chlorophyll content and growth of the plants. Similar findings of significant reduction in chlorophyll pigments also reported in *Pistia stratiotes* L. subjected to Ni stress (Kavitha & Pandey, 2011). Nickel has influenced the elevation of L-proline content in leaves of Ni-treated plants (Table 2). Total L-proline content was observed to be increased with increasing concentration of Ni in the treated groups of plants compared respective control (Table 2). Proline is an osmolyte that has been accumulated during stress conditions in plants. During the present study, nickel treatment and accumulation in leaves might have induced the accumulation of L-proline in *E. foetidum.* Similar findings of increased L-proline accumulation was also reported in *Triticum aestivum* L. seedlings (Kadiriye Uruc Parlak, 2016) under the influence of nickel stress.

Nickel accumulation in tissues:

Results of atomic absorption spectrometry (AAS) analysis tissue powder suggest that, the Ni content in roots and leaves of *E. foetidium* increased significantly with increasing nickel concentration in the medium used for treatments in the Ni-treated plants. Compared to control group of plants with exposure to increasing nickel concentrations both roots and leaves (Table 3).

Table 3: Accumulation of Nickel in roots and leaves of *E foetidum* after 5 days of treatment

Ni concentration (ppm) used to water plants	Nickel in roots (mg/gm FW)	Nickel in leaves (mg/gm FW)
0	0.026 ± 0.001	0.011 ± 0.003
50	11.54 ± 0.16	1.32 ± 0.44
100	33.19 ± 0.22	1.98 ± 0.36
200	72.21 ± 1.67	2.56 ± 0.62

Significant accumulation of nickel was observed in roots compared to leaves of *E. foetidium* plants treated with all the tested concentrations Ni. However, the accumulation of Ni is more in roots compared to leavels. These results suggest that *E. foetidium* has the ability to translocate Ni from rizhosphere (roots) to aerial parts (leaves). The results of nickel accumulation and translocations of the present study are comparable to that of the similar findings reported in other plants (Kavitha & Pandey, 2011; Kadiriye Uruc Parlak, 2016; Chandana & Joseph, 2028; Egli, 2018).

Effect of nickel treatment on enzymatic antioxidants:

Two antioxidant enzymes *i.e.*, superoxide dismutase (SOD) (EC 1.15.1.1) and catalase (CAT) (EC 1.11.1.6) were assayed in the leaves of plants treated with Ni and respective control groups (Fig. 1). Activity of SOD was significantly increased in the Ni-treated plants at all concentrations tested compared to respective control group of plants (Fig. 1).

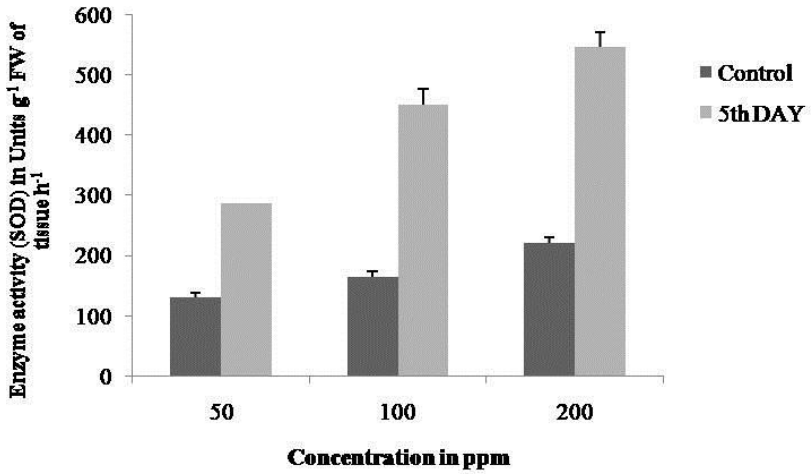

Fig. 1: Changes in superoxide dismutase (SOD) activity

Catalase (CAT) activity was significantly increased in the leaves of 50 ppm and 100 ppm Ni-treated plants compared to respective controls (Fig. 2). However, catalase activity was found to be decreased than the control group of plants in 200 ppm Ni-treated plants (Fig. 2).

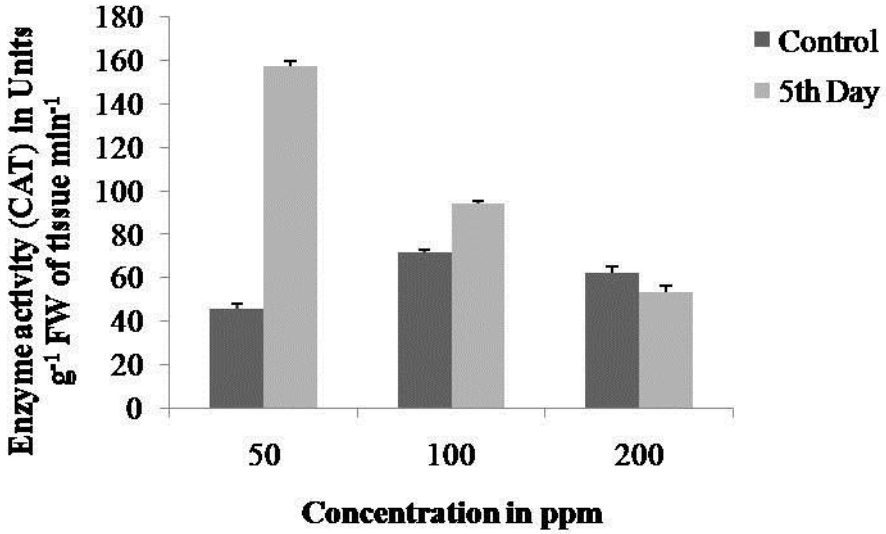

Fig. 2: Changes in catalase (CAT) activity

Oxidative stress due to environmental conditions induces the generation and ROS accumulation, which leads to the oxidation of cellular components and hindering metabolic activities (Suzuki et al., 2012).

Superoxide dismutase is a key enzyme that protects the cells from oxidative stress by converting/scavenging superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2). Catalase is the enzyme which converts H_2O_2 into H_2O and hydrogen in peroxisomes. Results of the present study reveals that SOD levels are increased at all the concentrations of Ni tested and the increase was observed to be consistent. In *E. foetidium* nickel accumulation was found to be higher in leaves than the suggested normal range of the metal in plants (<1 ppm), probably might have induced oxidative stress through the generation of superoxide radicals (O_2^-), hence elevation of superoxide dismutase levels which is a sign of metal tolerance of the plant for nickel. While there was slight fluctuation in CAT levels at 200 ppm treated plants compared to respective control group. This response of CAT activity might be due the alternative antioxidant enzymes or peroxidase present in the plant cell to scavenge H_2O_2 such as peroxidase, glutathione

peroxidase and ascorbate peroxidase. Results of the present study suggest that, nickel treatment cause oxidative stress in *E. foetidium* plants.

Membrane lipid peroxidation:

Membrane lipid peroxidation in leaves both control and Ni-treated plants was determined as malondialdehyde equivalent products (MDA) that react with thiobarbituric acid. In plants treated with Ni a significant increase in malondialdehyde content was observed. MDA concentration was increased with increase in stress intensity and duration of exposure. Maximum amount of MDA concentration was recorded in 100 ppm Ni treated plantlets compared to other Ni-treatments and respective control (Fig. 3).

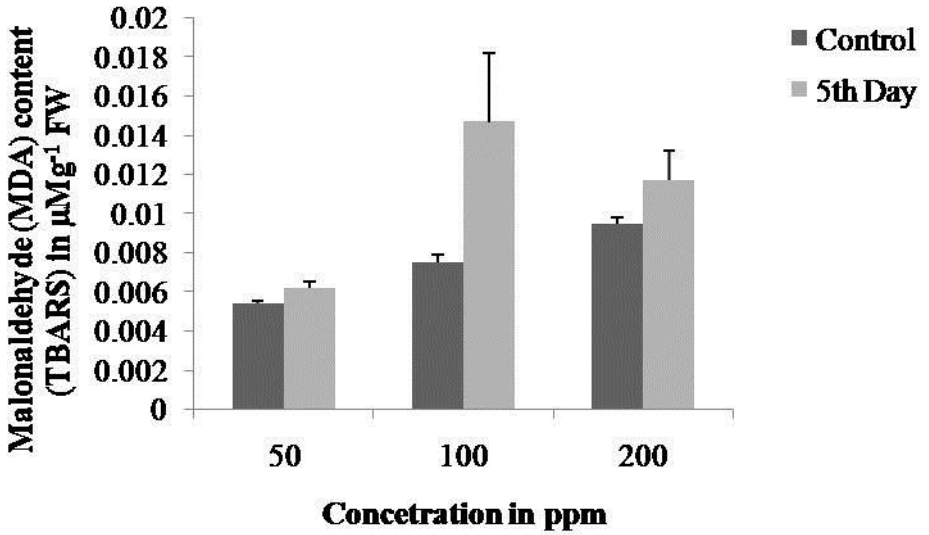

Fig. 3: Changes in membrane lipid peroxidation product malondialdehyde (MDA) content

The increased level of MDA is an indicator of oxidative stress induced by Ni in *E. foetidium* plants and probable cell membrane damage in the form of lipid peroxidation. These results are supported by the findings of Salem et al. (2006), Siddiqui et al. (2011) in *Zea mays* roots and *Triticum aestivum* respectively. However, in the present study the elevated levels of superoxide dismutase (Fig. 1), compatable solute (osmolyte) L-proline at all the concentrations of Ni treatments (Table 2) and catalase to some extent upto 100 ppm of Ni (Fig. 2) are the clear signs of tolerance shown by *E. foetidium* against imposed Ni stress treatments in spite of reduced growth (Table 1) and photosynthetic pigments (Table 2).

Influence of nickel on secondary metabolites (phytochemicals) production/accumulation:

Plant secondary metabolites such as phenolics, flavonoids and saponins were extracted from the leaves of *E. foetidium* plants treated with Ni-treated and control groups of plants and results are depicted (Table 4).

Table 4: Total phenolics, flavonoids and saponin content in *E. foetidum* leaf extract after 5 days of nickel treatment

Phytochemical in the leaf extract	Phytochemicals concentration determined			
	Control	Nickel concentration		
		50 ppm	100 ppm	200 ppm
Total phenolics (mg GAE/g DW)	4.8 ± 0.23	7.4 ± 0.48	6.2 ± 0.56	5.5 ± 0.36
Total flavonoids (mg GAE/g DW)	2.1 ± 0.14	4.9 ± 0.62	4.2 ± 0.28	2.8 ± 0.32
Total saponins (mg QSE/g DW)	3.8 ± 0.26	8.4± 0.68	7.2 ± 0.64	5.4 ± 0.44

Results revealed that, nickel has increased/induced the production and accumulation of all three secondary metabolites *i.e.*, phenolics, flavonoids and saponins in leaves at all the tested concentrations (50 ppm, 100 ppm and 200 ppm) compared to leaves of control group of plants (Table 4). However, the increase of these phytochemicals was found to be more pronounced in the leaves of 50 ppm Ni-treated plants compared to other two concentrations of Ni (100 ppm and 200 ppm) tested.

A correlation analysis between concentration Ni in the medium used to treat the *E. foetidum* plants and quantity of phytochemicals *i.e.*, phenolics, flavonoids and saponins also suggest the positive influence of metal ion (Ni) on plant secondary metabolites accumulation (Fig. 4).

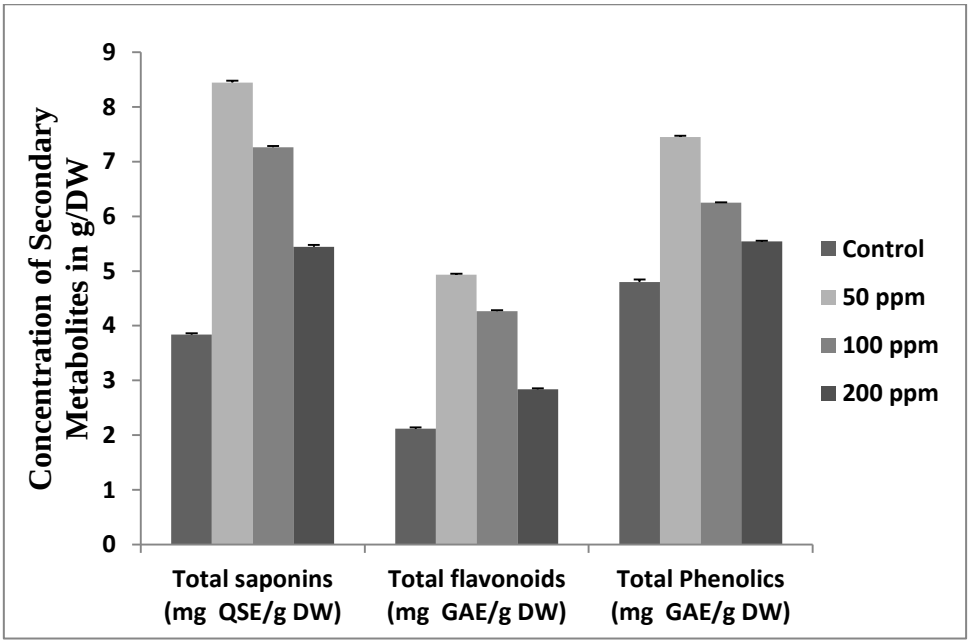

Fig. 3: Changes in membrane lipid peroxidation product malondialdehyde (MDA) content

A direct correlation has been suggested between heavy metal (including Ni) accumulation and phenyl alanine ammonia lyase (PAL) (the first enzyme of phenylpropanoid biosynthesis) gene expression in (Ma et al., 2016). Heavy metals in soil play a pivotal role in influencing a plant's morphology as well as the plant's biomass (Rabab et al., 2019). The importance of heavy metals including nickel as elicitors for the product enhancement in plant secondary metabolites has been discussed (Syed et al., 2019; Taslu, 2019). Nickel is considered an essential micronutrient for plants, plays an important role in plant growth and nitrogen uptake. Nickel deficiency disturbs plant growth, whereas high concentration of Ni is toxic for both plants and animals. Nickel concentration required for normal crop growth is from 0.05 to 10 µg/g wet weight, and a concentration above this range induces toxicity to plants (Syed et al., 2019).

CONCLUSION

Eryngium foetidum Linn., is a herb grows as naturalized weed, that has culinary importance as the plant is edible, and source pharmacologically important secondary metabolites (phytochemicals). Nickel is one of the important trace metals that is required as a cofactor for some of the important enzymes in plant metabolism at low concentrations, however the trace metal at higher concentrations is toxic to plants. In the present study an attempt has been made to evaluate metal stress tolerance of *E. foetidum* Linn. to nickel at higher concentrations than the normally required levels (50 ppm, 100 ppm, 200 ppm), and the influence of metal ion on the production of secondary metabolites.

From the results of the study, it could be concluded that, nickel affects the plant (*E. foetidum* Linn.) growth, photosynthetic pigments and membrane lipid peroxidation through MDA production. However, *E. foetidum* Linn. is tolerant to the imposed nickel stress, by increasing the osmolyte (L-proline) levels, antioxidant enzymes SOD and CAT. Further, nickel at accumulated concentrations in the leaves of *E. foetidum* Linn. elicits or induces the production of secondary metabolites, phenolics, flavonoids and saponins as there is a direct correlation elucidated between metal ion (Ni) concentration and secondary metabolite accumulation in leaves within 5 days of nickel treatment. Therefore nickel could be used as a one of the elicitors for the over production of pharmacologically active secondary metabolites in *E. foetidum* Linn., after standardizing suitable concentration.

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