IJBPAS, November, 2024, 13(11): 5570-5578

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# ALTERATION IN GERMINATION, GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF *MACROTYLOMA UNIFLORUM*(LAM.) VERDC. DUE TO IRON STRESS

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**Received 24th Aug. 2024; Revised 25th Sept. 2024; Accepted 20th Oct. 2024; Available online 1st Nov. 2024**

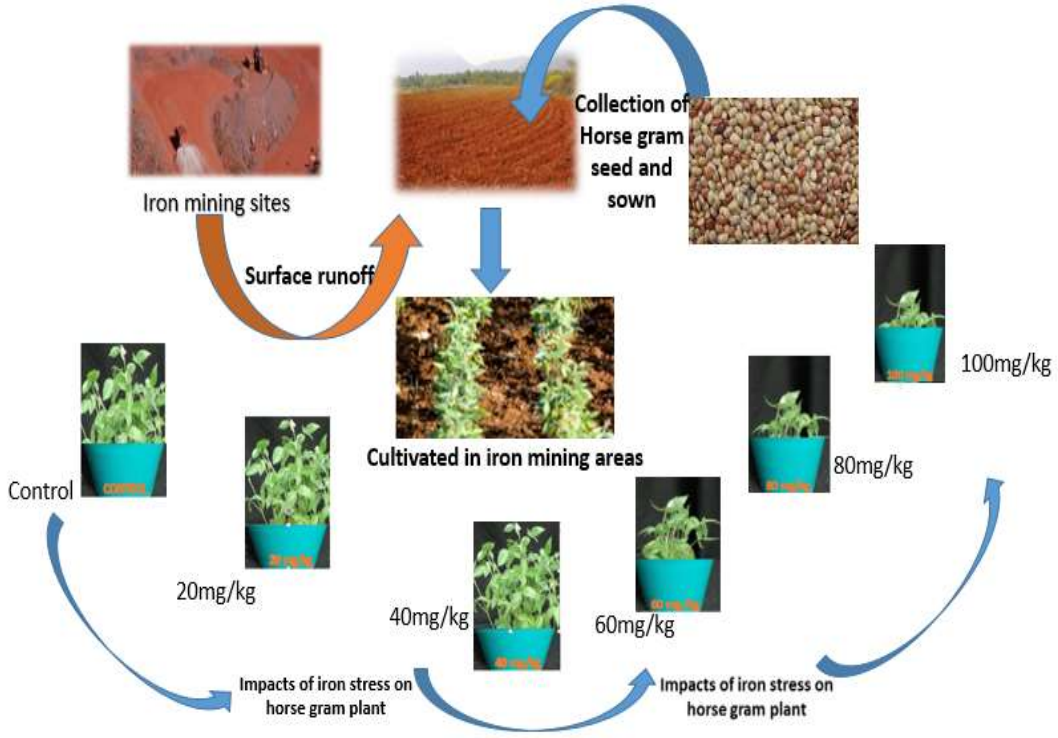
https://doi.org/10.31032/IJBPAS/2024/13.11.8442

## ABSTRACT

Iron is ubiquitously found everywhere in the earth surface. Environmental pollution due to uncontrolled discharge of iron from industrial and mining effluents has become a major threat. Odisha is a mine rich state of India and it is well known for iron production. In this study impacts of iron on germination and growth of Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] in terms of physiological and biochemical parameters were undertaken. The seeds of horse gram were germinated in six different concentrations of iron having 0-100 mg/l of iron. It was observed that different indices like Seedling vigour indices, Metal tolerance indices were found to be reduced and the percentage of phyto-toxicity was found to be increased with increasing concentration of iron. The pot culture experiment revealed that different biochemical parameters like total chlorophyll content, total soluble protein content was decreased but free proline content was increased with increasing concentration of iron. It was also observed that seeds of *Macrotyloma uniflorum* showed better result in terms of growth and different biochemical parameters upto 40 ppm of iron in soil. This study indicates that iron up to 40mg/kg could enhance the plants growth in terms of both germination and developmental stage. So, it can be recommended that plants can be grown on the soil containing 40ppm or less iron content and beyond that limit, it will be considered as toxic.

**Key words: Biochemical parameters, growth, Horse gram, iron, stress**

**GRAPHICAL ABSTRACT**




## INTRODUCTION

Heavy metal pollution can't be ignored due to its toxicity exert on animals, plants and human beings through food chain. Heavy metals are nonbiodegradable and hence remain present in soil and water bodies for a longer duration in the environment adding to the concern **[1]**. It was also reported that heavy metals in water bodies emerged as a major concern towards health issues, threatening both terrestrial as well as aquatic organisms **[2]**. Now a day's agricultural soils are being contaminated due to trace metal in agricultural soil are major environmental problems today **[3]** (Chand *et al*., 2012). Uptake of metals by plants can have strong adverse impact on both plants as well as animals through the food chain **[4]** (Sadon *et al*., 2012). Heavy metals create major threats towards environmental pollution. Contamination of heavy metal pollution possesses major risks to environment and human health **[5]** (Enas *et al*., 2021). Heavy metals make significant contribution to environmental pollution since the beginning of industrial revolution because of anthropogenic activities **[6]** (Arzoo *et al*., 2017). Heavy metals can include elements lighter than carbon and can exclude some of the heaviest metals **[7]** (Duffus, 2002). Industrialization and environmental pollution with technology have gone ahead to affect human health **[8]** (Yagdi *et al*., 2000). Air pollution from industries is aesthetically offensive and can be a true health hazard to humans as well as to vegetation **[9]**

(Janick, 1986). Heavy metals are commonly released into the air from industrial emissions, the use of fossil fuels for heating systems and means of transport exhausts **[10]** (Onder *et al*., 2007). Iron and steel industries are the main causes of air pollution emissions. In this study *Macrotyloma uniflorum* (Lam.) Verdc. seedling was taken as a test plant.

*Macrotyloma uniflorum* (Lam.) Verdc., commonly known as horse gram is one of the legume species. The seeds of horse gram are generally consumed by cattle. It is also consumed by human being and is considered as an excellent source of iron and molybdenum. It is also recommended as ayurvedic medicines to treat renal stones, piles and oedema etc. This plant is mainly cultivated in the states of Andhra Pradesh, Karnataka, Odisha, Tamil Nadu, Madhya Pradesh, Chhattisgarh, Bihar, West Bengal, Jharkhand, and in foothills of Uttaranchal and Himachal Pradesh in India. It is also cultivated in other countries mainly Sri Lanka, Malaysia, West Indies etc.

**MATERIALS AND METHODOLOGY**

Seeds of *Macrotyloma uniflorum* (Lam.) Verdc. were collected from Orissa University of Agriculture and Technology, Bhubaneswar to be used as the experimental material. Seeds of horse gram were surface sterilized with 0.1% mercuric chloride and washed thoroughly with tap water and then with distilled water. Hundred uniform seeds were germinated in Petri dish with different nickel concentration and it was incubated for five days and then number of germinated seeds were counted and percentage of germination were calculated, Seedling vigour indices **[11]** (Abdul Baki, 1973), Metal tolerance indices **[12]** (Turner and Marshal, 1972) and Percentage of Phytotoxicity **[13]** (Chou *et al*., 1978) were calculated. Another experiment had been carried out in pot culture in which seeds were sown in pre-treated soil which was treated with different concentration of iron ranging from 20 mg/kg to 100 mg/kg with a control soil and different growth and biochemical parameters like total chlorophyll content **[14]** (Arnon, 1949), total protein content **[15]** (Lowry *et al*., 1951) and free proline content **[16]** (Bates *et al*., 1974) were estimated in plants at 10, 20 and 30 days after treatment. All the experiments were done in triplicates and the data was statistically analyzed and standard error of mean (SEM) was calculated.

**RESULTS AND DISCUSSION**

Germination of seed is the most crucial stage of plant development; the germination of seeds can be used as an indicator of early response of the plants in adverse environmental condition **[17]** (Singh *et al*., 2006). In this study, impacts of iron stress on germination of *Macrotylomauniflorum* (Lam.) Verdc. seed has been carried out. During this study it was found that the percentage of germination of seed were decreased from 96.25 ± 0.647 to 26.25 ± 0.124 and elongation of radicle length also found to be decreased from 4.4 centimetre to 1.4 centimetre **(Table-1) (Figure 1 & 2)**. Seedling vigour indices, Metal tolerance indices and percentage

of phytotoxicity were calculated and values were depicted in **Table 2**. Seedling vigoue indices and Metal tolerances were found to be increases with increasing concentration of iron whereas Percentage of phytoxicity was found to be increases with increasing concentration of iron. Increasing concentration of iron also inhibits seedling growth and also different physiological and biochemical parameters. Total chlorophyll content in leaves of horse gram were found to be decreased **(Table 3)** with increasing concentration of iron. Similarly, quantity of total soluble protein in leaves of horse gram were found to be decreased **(Table 4)** and free prolin content were found to be increased **(Table 5)** with increasing concentration of iron. Similar result was also observed that seeds of *Macrotyloma uniflorum* showed better result in terms of shoot length, root length, percentage of moisture content and different biochemical parameters in 20 ppm of Nickel at 10th, 20th and 30th days of growth thereby indicating that Nickel within 20 mg/kg could facilitate the plants growth **[18]** (Arzoo *et al*., 2014). Different concentration of nickel affected seed germination in *Cucumis sativus* L. plants. During germination Ni inhibits all cellular processes **[19]** (Hall, 2002) thus, slow down growth of plumules and radicles. Similar inhibition of germination at higher concentrations was observed by **[20]** (Mahalakshmi and Vijayarengan, 2003) with cobalt treatment in *Vigna mungo* (L.) Hepper. But the results obtained from the germination studies indicated that *Cucumis sativus* L. showed higher seedling growth and dry weight at 40 mg/kg nickel level in the soil. The values of growth parameters showed that Nickel had a significant stimulating and nutritional effect at 20mg/kg concentration which is beneficiary for plants growth and above 40 ppm it shows adverse impact on seedling growth. The growth parameters beyond this concentration indicated that a little excess of Nickel above these levels had an adverse effect. From the result of this investigation, it can be concluded that Nickel at lower concentration has a stimulating effect on the germination process and seedling growth of *Cucumis sativus* L. and will inhibit the same at higher concentrations. Similar results were reported on the effect of cadmium on *Triticum aestivum*, **[21]** (Kalita, 1993) chromium stress on*Salvia sclarea*, **[22]** (Corradi, 1993) and cobalt and zinc stress on *Pennisetum americanum* L. and nickel stress on *Macrotyloma uniflorum* (Lam.) Verdc. (Arzoo *et al*, 2014). Different physiological and biochemical changes also reported in different studies like impact of $Cr^{+6}$ toxicity in plants **[23]** (Pati *et al*., 2014) and nickel toxicity in *Arachese hypogea*L. **[24]** (Arzoo and Satapathy, 2015).

**Table 1: Impacts of iron stress on germination and radicle length of *Macrotyloma uniflorum* (Lam.) Verdc.**

| Treatments | Germination percentage | Radicle length(cm) |
|---|---|---|
| Control (00ppm) | 96.25 ± 0.647 | 4.4 ± 0.424 |
| 20 ppm | 94.5 ± 1.254 | 4.2± 0.082 |
| 40 ppm | 82.5 ± 0.842 | 3.6± 0.116 |
| 60 ppm | 76 ± 1.222 | 2.8± 0.214 |
| 80 ppm | 62.5 ± 1.326 | 2.4± 0.142 |
| 100 ppm | 26.25 ± 0.124 | 1.4± 0.325 |

Average of five replicates ± SEM

**Table 2: Impact of iron stress on Seedling vigour indices, tolerance indices and percentage of phytotoxicity**

| Treatments | Seedling vigour indices | Metal Tolerance indices | Percentage of Phytotoxicity |
|---|---|---|---|
| Control | 423.5 | 100 | 0 |
| 20 ppm | 396.9 | 95.454 | 4.761 |
| 40 ppm | 297 | 81.818 | 22.222 |
| 60 ppm | 212.8 | 63.636 | 57.143 |
| 80 ppm | 150 | 54.545 | 83.333 |
| 100 ppm | 36.75 | 31.818 | 214.286 |

**Table 3: Impact of iron stress on total chlorophyll content in *Macrotyloma uniflorum* (Lam.) Verdc.**

| Treatments | Total Chlorophyll content (mg/g of fresh wt.) | | |
|---|---|---|---|
| | $10^{th}$ days after treatment | $20^{th}$ days after treatment | $30^{th}$ days after treatment |
| Control | 3.562± 0.354 | 3.644± 0.397 | 3.698± 0.286 |
| 20 ppm | 3.668± 0.864 | 3.846± 0.426 | 3.884± 0.454 |
| 40 ppm | 3.896± 0.268 | 3.904± 0.538 | 3.928± 0.583 |
| 60 ppm | 3.044± 0.424 | 2.988± 0.444 | 2.732± 0.274 |
| 80 ppm | 2.888± 0.968 | 2.686± 0.321 | 2.408± 0.444 |
| 100 ppm | 2.768± 0.463 | 2.482± 0.165 | 2.044± 0.254 |

**Table 4: Impact of iron stress on total soluble protein content in *Macrotyloma uniflorum* (Lam.) verdc.**

| Treatments | Total soluble protein content (mg/g of fresh wt.) | | |
|---|---|---|---|
| | $10^{th}$ days after treatment | $20^{th}$ days after treatment | $30^{th}$ days after treatment |
| Control | 4.624± 0.759 | 4.608± 0.286 | 4.432± 0.444 |
| 20 ppm | 4.888± 0.385 | 4.822± 0.756 | 4.812± 0.355 |
| 40 ppm | 5.329± 0.574 | 5.268± 0.424 | 5.204± 0.232 |
| 60 ppm | 4.086± 0.264 | 3.654± 0.648 | 3.346± 0.424 |
| 80 ppm | 2.648± 0.422 | 2.488± 0.264 | 2.148± 0.751 |
| 100 ppm | 2.432± 0.473 | 2.104± 0.222 | 1.822± 0.408 |

**Table 5: Impact of iron stress on free prolin content in *Macrotyloma uniflorum* (Lam.) Verdc.**

| Treatments | Free prolin content (µg/g of fresh wt.) | | |
|---|---|---|---|
| | $10^{th}$ days after treatment | $20^{th}$ days after treatment | $30^{th}$ days after treatment |
| Control | 6.212± 0.406 | 6.425± 0.286 | 6.444± 0.648 |
| 20 ppm | 6.104± 0.324 | 6.132± 0.584 | 6.147± 0.736 |
| 40 ppm | 6.008± 0.793 | 6.114± 0.404 | 6.136± 0.424 |
| 60 ppm | 8.968± 0.326 | 9.998± 0.266 | 10.678± 0.849 |
| 80 ppm | 9.448± 0.188 | 10.482± 1.398 | 12.462± 1.428 |
| 100 ppm | 9.888± 0.432 | 11.686± 1.424 | 12.868± 0.426 |

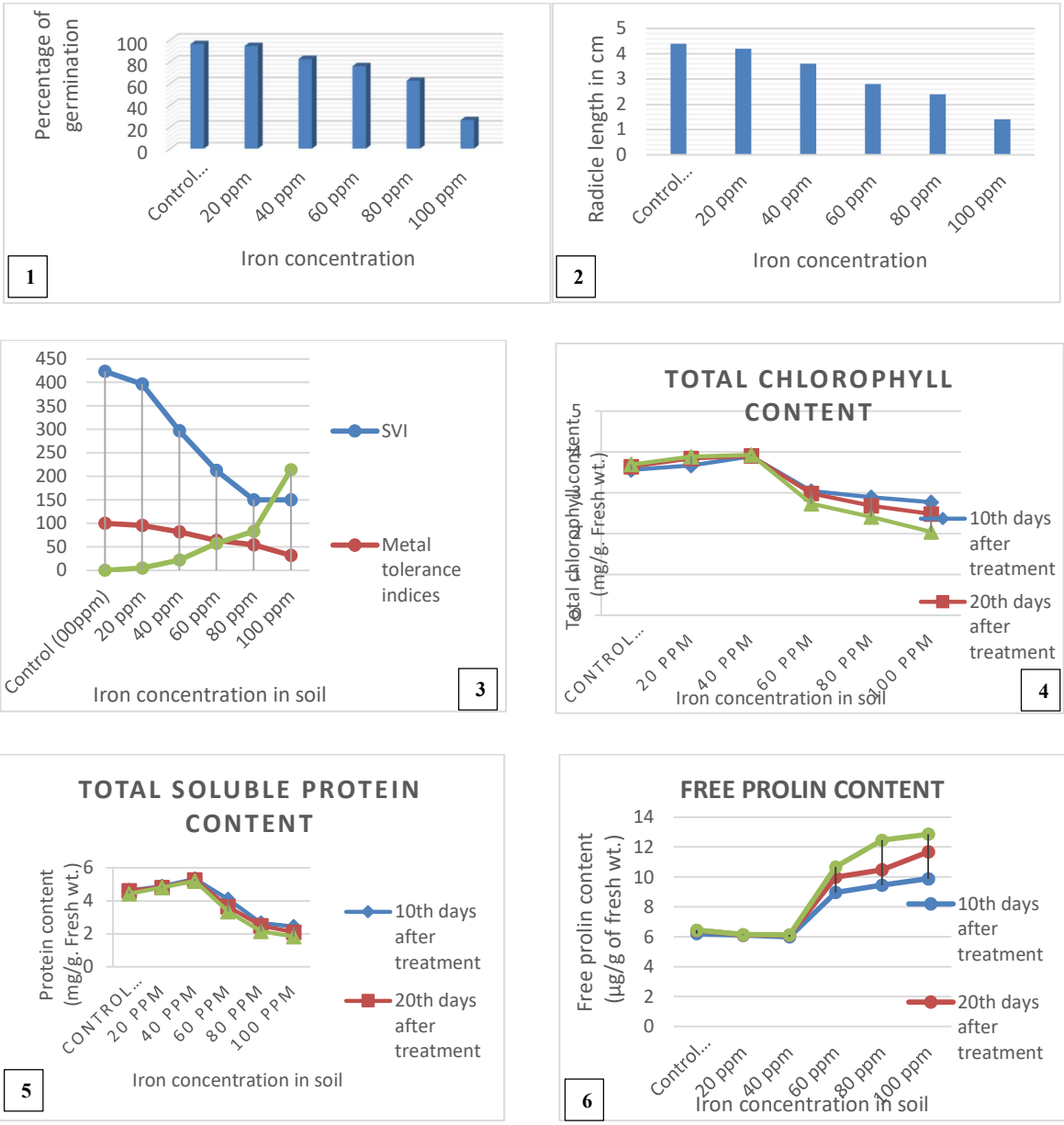



**Fig-1: Impacts of iron stress on germination of *Macrotyloma uniflorum* (Lam.) verdc.**
**Fig-2: Impacts of iron stress on radicle length of *Macrotyloma uniflorum* (Lam.) verdc.**
**Fig-3: Impact of iron stress on Seedling vigour indices, tolerance indices and percentage of phytotoxicity**
**Fig-4: Impact of iron stress on total chlorophyll content in *Macrotyloma uniflorum* (Lam.) verdc.**
**Fig-5: Impact of iron stress on total soluble protein content in *Macrotyloma uniflorum* (Lam.) verdc.**
**Fig-6: Impact of iron stress on free prolin content in *Macrotyloma uniflorum* (Lam.) verdc.**

## CONCLUSIONS

Iron is considered as one of the micronutrient because consumption of iron upto a particular level is good but excessive consumption of iron poses ill effect. In this study it was concluded that upto 40mg/kg of iron in soil is consider good for horse gram cultivation whereas more than this level poses ill impact on plant growth in terms of both qualities of plant and quantity of its yield. It can be concluded that iron at lower

concentration has a stimulating effect on the germination process and seedling growth of *Macrotyloma uniflorum* (Lam.) Verdc.

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