WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Laboratory Experiments Showing the Results of Carotenoid Content in the Seedlings of Cultivar Pusa Chetki of Radish (Raphanus Sativus L.) Plant after Applying Heavy Metals

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ABSTRACT:

This study explores the effects of heavy metal exposure on carotenoid content in Raphanus sativus seedlings (cultivar Pusa chetki). Carotenoids, crucial plant pigments that protect chlorophyll from photooxidation, were analyzed after treating seeds with various metal concentrations. Results demonstrate a significant reduction in carotenoid content, particularly with higher concentrations of cadmium and lead. Nickel and copper exhibit lower inhibitory effects. Statistical analysis confirms significant differences between control and treatment groups, as well as among different metal concentrations.

The findings suggest interference with photosynthesis due to heavy metal exposure, potentially impacting carotenoid production. These results contribute to understanding the ecological implications of metal pollution on plant health and offer insights for developing strategies to mitigate its harmful effects. Further research is needed to uncover the underlying mechanisms and broader ecological consequences of heavy metal impact on carotenoid metabolism. Understanding the relationship between heavy metal exposure and carotenoid content aids in addressing the risks of metal pollution to plant growth and ecosystem well-being.

Keywords: Radish plant (Raphanus sativus L.), Cultivar Pusa chetki, Laboratory experiment, Petri dishes, Spectrophotometer, Carotenoid content, Pigment content

Objectives:

- Explore the impact of trace metal exposure on the levels of carotenoids in Raphanus sativus seedlings (cultivar Pusa chetki).
- Analyze the inhibitory effects of different heavy metals, including $NiSO_4$, $CuSO_4$, $PbNO_3$, $CdCl_2$, $ZnCl_2$ on carotenoid content.
- Determine the concentration-dependent reduction in carotenoid content caused by heavy metal exposure.
- Compare the effects of heavy metals on carotenoid content and identify the metals with the highest inhibitory impact.
- Confirm significant differences in carotenoid content between control and treatment groups using statistical analysis.
- Explore the underlying mechanisms that lead to depletion of pigment in response to heavy metal exposure.
- Offer valuable perspectives for devising approaches to alleviate the detrimental consequences of pollution caused by toxic metals on plant development and the overall well-being of ecosystems.

Introduction:

Plant pigments are naturally occurring substances produced by plants during their metabolic processes within cells. Plants contain a variety of pigments, including carotenoids, which are yellowish in colour and are always found in conjunction with chlorophylls. Unlike chlorophylls, carotenoids do not require light for their synthesis. Carotenoids have absorption peaks ranging from 425 nm to 490 nm and play a protective role by preventing the photooxidation of chlorophylls. In this particular research study, the carotenoid content in seedlings of the Raphanus sativus cultivar Pusa chetki was investigated.

The photosynthetic pigments are located in the internal membranous structures known as thylakoids, which are found within chloroplasts. The thylakoid membrane comprises a bilipid layer that encompasses both intrinsic and extrinsic proteins, constructed from carbon (50%), hydrogen (7%), nitrogen (16%), oxygen (20%), and sulphur (2%).

Proteins have important roles in metabolic reactions, storage, and transportation within plants. Among the various biochemical measurements, the assessment of photosynthetic pigments is particularly significant. The quantity of carotenoids present has a strong correlation with the rate of photosynthesis. This relationship remains consistent across different plant species, although it can be influenced by the application of mineral elements, including trace metals.

Material and methods:

In the present investigation, certified seeds of Raphanus sativus L. variety Pusa chetki were procured from National Seed Corporation, New Delhi, then kept in stoppered bottles of glass. After ensuring uniformity, the seeds underwent surface sterilization by immersing them in a 0.1% HgCl2 solution for a duration of 2 minutes. and rinsed repeatedly under distilled water. Solutions of different concentrations of Zinc chloride, Lead nitrate, Nickel sulphate, Copper sulphate and Cadmium chloride were individually prepared in distilled water. The 60-60 seeds were soaked in these different solutions for 2 hours. A control with distilled water was also being run simultaneously. The experiment was conducted with three replications for each treatment.

Then, seeds were washed thoroughly and then transferred into petri plates over wet (with distilled water), filter paper for germination and seedling growth.

After 10 days of experiment in the laboratory (at 25 degree ± 2 degree C°). The visual emergence of radicals was taken as criteria for germination.

Leaves from 10-day-old seedlings of Raphanus sativus var Pusa chetki were collected, for each treatment, fifty milligrams of leaf material were collected and homogenized with 50 ml of 80 percent acetone.

The sample was subjected to centrifugation at a speed of 2000 rpm for a duration of 10 minutes. Subsequently, the resulting volume was adjusted to 10 ml using 80% Acetone. The transparent extract was analyzed for carotenoid content using a spectrophotometer at wavelengths of 480 nm and 510 nm.

The pigment values were quantified and reported as milligram/ gram of fresh weight..

Following formula was used to find out the carotenoid content-

Carotenoid content =

$$\frac{7.6 \text{ O.D } 480 - 1.49 \times \text{O.D } 510}{a \times 100 \times W} \times V \text{ mg/g}$$

O.D. = Optical density (light absorption measured using cell of one centimeter)

V = Volume of the extract in millilitre (ml)

a = Length of light path in the cell (measured in cm)

W= Fresh weight of seedling leaves (gm)

The carotenoid content of Raphanus sativus cv Pusa chetki was recorded and tabulated. The collected data was then exposed to statistical analysis employing the F-test method.

Result and Discussion:

The recorded data, presented in tabular form, illustrates the influence of heavy metals on the levels of carotenoids in the Pusa chetki cultivar of Raphanus sativus L. Statistical analysis was performed to examine the disparities among the control group, different concentrations of heavy metals, various chemicals, and different treatments, focusing on pigment content.

It was observed that compared to other heavy metals, Ni and Cu exhibited lesser inhibitory effects on the total carotenoid content. In the control group, the total carotenoid content measured 0.17 mg/g of fresh weight, but significantly declined at concentrations of 50, 200, and 500 ppm of heavy metals (refer to table 1).

Table 1 - Showing the outcomes of carotenoid content in the seedling of cultivar Pusa chetki of radish plant after applying heavy metals.

S. No.	**Name of the chemical**	**Concentration (ppm)**					
		Control	**10**	**50**	**100**	**200**	**500**
1.	Copper sulphate	0.17	0.15	0.13	0.12	0.10	0.08
2.	Cadmium chloride	0.17	0.12	0.09	0.07	0.04	nd

S. No.	Name of the chemical	Concentration (ppm)					
		Control	10	50	100	200	500
3.	Lead nitrate	0.17	0.13	0.11	0.09	0.06	nd
4.	Nickel sulphate	0.17	0.16	0.14	0.13	0.12	0.10
5.	Zinc chloride	0.17	0.14	0.12	0.11	0.09	0.07

(The values presented are the average of three replicated measurements.)

F-ratios:-

(i) Control versus treatment = 61.00***

(ii) Among the treatments = 13.7272***

(iii) Among the chemicals = -6.121 (ins)

Ins = Insignificant

*** = Highly significant

At a concentration of 500 ppm, the carotenoid content in the Pusa chetki cultivar of Raphanus sativus showed the most substantial reduction with Cd and Pb. The levels decreased to 0.08 mg/g fresh weight for Cu, nil for Cd and Pb, 0.09 mg/g fresh weight for Ni, and 0.07 mg/g fresh weight for Zn. Statistical analysis was performed on the collected data, demonstrating a significant level of significance between the control group and treatments, as well as among the various treatment concentrations.

No discernible variations were observed among the different chemicals. Upon examining the pigment content of the Pusa chetki culture, it was evident that the application of heavy metals led to a significant decrease in carotenoid levels. Specifically, in the case of Cd and Pb, the carotenoid content in the cultivar Pusa chetki was drastically reduced at a concentration of 500 ppm. The carotenoid content in cultivar Pusa chetki decreased considerably even at 10 ppm concentration of Ni. The reduction in pigment content observed in Raphanus sativus cv Pusa chetki could potentially be attributed to the inhibition of various physiological processes, primarily photosynthesis.

The decrease in pigment content could be attributed to the disruption of protein synthesis in chloroplasts caused by the presence of heavy metals, thereby affecting their production and leading to a decline in pigment levels. A comprehensive review of available literature reveals diverse explanations offered to account for the decrease in pigment content attributed to the utilization of trace metals.

The current findings, which indicate the inhibition of pigment content in Raphanus sativus in the presence of heavy metals, align with previous reports by Root et al. (1975) in Zea mays, where reduced pigment values were observed with cadmium.

Agarwal et. al. (1977) also concluded that reduction in photosynthetic pigments of barley may be because of low Fe level, due to the presence of metallic elements. Roth et. al. (1971) observed deficiency of phosphorus and iron in oat and soybean plants caused by copper and nickel toxicity. Singh (1986) observed the inhibition of total N and P mobilisation in cotyledons of Ni-treated Pisum sativum. Crooke et al. (1954) have also shown the detrimental impacts of Ni on the accumulation of multiple mineral elements.

Conclusion:

This study yields significant findings regarding the influence of heavy metal exposure on carotenoid content in Raphanus sativus seedlings (cultivar Pusa chetki). The results indicate a significant reduction in carotenoid content, particularly at higher concentrations of cadmium and lead. Nickel and copper showed lower inhibitory effects on carotenoid production. The findings suggest that heavy metal exposure interferes with photosynthesis, potentially impacting carotenoid metabolism.

The study highlights the ecological implications of metal pollution on plant health and emphasizes the need for strategies to mitigate its harmful effects. Understanding the relationship between heavy metal exposure and carotenoid content is crucial for addressing the risks posed by metal pollution to plant growth and ecosystem well-being. Further research is necessary to uncover the underlying mechanisms and broader ecological consequences of heavy metal impact on carotenoid metabolism.

Through illuminating the suppressive impacts of heavy metals on carotenoid synthesis, this investigation enhances our understanding of plant physiology and provides valuable insights for the development of strategies aimed at mitigating the harmful consequences of heavy metal pollution.

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