

Qualitative Phytochemical Responses of *Solanum melongena* L. to Lead and Cobalt Stress at Different Growth Stages

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Abstract

The present study aimed to qualitatively assess phytochemical responses in methanolic extracts of eggplant (*Solanum melongena* L.) subjected to lead (PbCl₂) and cobalt (CoCl₂) stress. Plants were exposed to control, 50, 150, and 300 ppm concentrations of PbCl₂ and CoCl₂ under pot culture conditions. Root, stem, and leaf samples were collected at 60 and 90 days after sowing and analyzed using standard qualitative phytochemical screening tests. The presence or absence of major phytochemical groups was recorded to evaluate variations associated with metal stress, plant organ, and growth stage. The results revealed noticeable variations in phytochemical composition across treatments, with several compounds detected in control and lower metal concentrations, while higher concentrations resulted in the absence of certain phytochemicals. Leaves generally exhibited better phytochemical retention compared to roots and stems. Distinct differences between lead and cobalt treatments indicated metal-specific effects on phytochemical expression. This qualitative screening provides preliminary insight into biochemical adjustments of eggplant under heavy metal stress and establishes a basis for further quantitative investigations.

Keywords: *Solanum melongena* L.; phytochemical profiling; methanolic extraction; lead toxicity; cobalt exposure; heavy metal stress

1. Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crop cultivated worldwide and is valued not only for its nutritional importance but also for its richness in diverse bioactive compounds. Plants are known to synthesize a wide range of secondary metabolites, including flavonoids and phenolic compounds, which play a crucial role in protection against environmental stresses (Winkel-Shirley, 2002).

Previous studies on eggplant have reported the presence of alkaloids, phenolics, and flavonoids that contribute to stress tolerance and antioxidant defense mechanisms (Harborne, 1998; Michalak, 2006).

In recent years, heavy metal contamination of agricultural soils has emerged as a serious environmental concern due to rapid industrialization and intensified anthropogenic activities. Among heavy metals, lead is considered a non-essential and highly toxic element that interferes with plant physiological and biochemical processes even at low concentrations (Sharma and Dubey, 2005). In contrast, cobalt is regarded as an essential micronutrient at trace levels; however, its excess accumulation can disrupt enzyme activity and metabolic balance, leading to toxicity symptoms in plants (Pilon-Smits et al., 2009). Several researchers have demonstrated that exposure to lead and cobalt induces oxidative stress, metabolic disturbances, and alterations in biochemical composition in plants (Shahid et al., 2017). Heavy metal stress is known to significantly influence the synthesis and accumulation of secondary metabolites. Gill and Tuteja (2010) reported that plants respond to metal-induced oxidative stress by modulating antioxidant defense systems and secondary metabolite production. In this context, qualitative phytochemical screening has been widely employed as a preliminary approach to detect stress-induced biochemical changes in plants (Harborne, 1998). This method enables the identification of major phytochemical groups prior to detailed quantitative evaluation.

The efficiency of phytochemical extraction largely depends on the choice of solvent. Methanol has been reported as an effective solvent for extracting a broad range of polar and semi-polar phytochemicals from plant tissues (Sasidharan et al., 2011). Furthermore, phytochemical responses may vary among different plant organs and developmental stages, as roots, stems, and leaves often exhibit organ-specific metabolic behavior under abiotic stress conditions (Ramakrishna and Ravishankar, 2011).

Based on the above scientific evidence, the present study was designed to qualitatively evaluate phytochemical constituents in methanolic extracts of root, stem, and leaf tissues of eggplant exposed to varying concentrations of lead and cobalt at 60 and 90 days after sowing. The study aims to provide preliminary qualitative insights into metal-induced biochemical responses, which may serve as a foundation for further quantitative investigations.

2. Materials and Methods

2.1 Experimental Site and Plant Material

The present study was conducted as a pot experiment during the spring season at Rajpur village, Deesa taluka, Banaskantha district, Gujarat, India. The experimental area represents a

semi-arid agro-climatic region of North Gujarat, where the average ambient temperature during the growth period ranged between 35 °C and 41 °C. Plants were maintained under natural environmental conditions with regular irrigation. The experimental plant material consisted of eggplant (*Solanum melongena* L.), cultivar MEBH-39. Certified seeds of the cultivar were procured from a local authorized seed supplier at Deesa, Gujarat. The cultivar was selected due to its uniform growth habit and suitability for physiological and biochemical stress studies.

2.2 Experimental Design and Pot Experiment

The experiment was laid out in a Completely Randomized Design (CRD) comprising seven treatments, each with three replicates. Seeds were initially sown in small polythene bags containing garden soil. After 15 days, healthy and uniform seedlings were transplanted into plastic pots, with one plant maintained per pot. Standard agronomic practices were followed throughout the experimental period to ensure normal plant growth.

2.3 Heavy Metal Treatments

Seven treatments were employed, including a control and different concentrations of lead and cobalt. Lead (Pb) was selected as a non-essential and toxic metal, while cobalt (Co) was chosen due to its conditional essentiality at low concentrations. The treatments were as follows: T₀–Control (no metal treatment), T₁–PbCl₂ at 50 ppm, T₂ – PbCl₂ at 150 ppm, T₃ – PbCl₂ at 300 ppm, T₄ – CoCl₂ at 50 ppm, T₅ – CoCl₂ at 150 ppm, T₆ – CoCl₂ at 300 ppm. The selected concentration range was based on earlier studies reporting that these levels are sufficient to induce physiological and biochemical stress responses without causing complete growth inhibition (Sharma and Dubey, 2005; Shahid et al., 2017).

2.4 Sampling and Growth Stages

Plant samples were collected at two growth stages, 60 and 90 days after sowing (DAS), to evaluate growth-stage-dependent biochemical responses. At each sampling stage, plants were carefully uprooted, and roots, stems and leaves were separated. Samples were washed thoroughly with distilled water to remove surface contaminants and processed for qualitative phytochemical analysis. Stage-specific sampling was adopted as phytochemical profiles are known to vary with plant age and duration of stress exposure (Ramakrishna and Ravishankar, 2011).

2.5 Preparation of Methanolic Extracts

Fresh plant samples were shade-dried at room temperature and finely powdered. Methanolic extraction was carried out following standard phytochemical extraction procedures with

minor modifications. Methanol was selected as the extraction solvent due to its efficiency in extracting a wide range of polar and semi-polar compounds.

The powdered samples were soaked in methanol, filtered through Whatman No. 1 filter paper, and the filtrates were collected. The extracts were stored under refrigerated conditions and used for qualitative phytochemical screening.

2.6 Qualitative Phytochemical Screening and Data Interpretation

Qualitative phytochemical screening was performed to determine the presence or absence of major phytochemical constituents in the methanolic extracts. Standard qualitative tests were conducted following the methods described by Harborne (1998) and Sasidharan et al. (2011). The phytochemicals analyzed included alkaloids, flavonoids, phenols, tannins, saponins, glycosides, carbohydrates, and proteins. Observations were recorded based on characteristic colour changes or precipitate formation.

The results were expressed as presence (+) or absence (–) of the respective phytochemicals. This semi-quantitative approach is commonly employed in preliminary phytochemical investigations to assess biochemical alterations under abiotic stress conditions (Harborne, 1998; Michalak, 2006). Comparative evaluation was carried out across treatments, plant parts, and growth stages to identify qualitative trends associated with lead and cobalt stress.

3. Results

3.1 Qualitative phytochemical responses at 60 DAS

Table 1. Qualitative phytochemical profile of methanolic extracts of leaf, stem and root of *Solanum melongena* L. under lead and cobalt stress at 60 days after sowing (DAS)

Chemical Constituents	Tests	Methanol																							
		Leaf extract 60 DAS								Stem extract 60 DAS								Root extract 60 DAS							
		T 0	T 1	T 2	T 3	T 4	T 5	T 6	T 0	T 1	T 2	T 3	T 4	T 5	T 6	T 0	T 1	T 2	T 3	T 4	T 5	T 6			
Carbohydrate	Fehling's test	+	+	+	-	+	+	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-			
Protein	Biuret test	+	+	-	-	+	-	-	+	+	-	-	-	-	-	+	+	-	-	+	+	-			
Alkaloids	Hanger test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	Wanger test	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+	+	+	-	+	+	-			
	Mayer test	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+	+	-	-	+	-	-			
	Dragendroff test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Flavonoid	Alkaline reagent	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-				
Tannin	5% FeCl3 test	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	-	+	+	-			
	Gelatin test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Phenol	Lead acetate	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+	+	-	-	+	-	-			
Amino acids	Ninhydrine	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-			
Phytosterols	Salkowski's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
	Liebermann's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-			
Anthraquinone	Anthraquinone test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Cardiac - Glycoside	Keller-Killiani	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	-	-	+	-	-			
Terpenoids	Terpenoids	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	-	+	-	-			

(+: Presence; (-): Absence.

T₀: Control; T₁-T₃: PbCl₂ (50, 150, 300 ppm); T₄-T₆: CoCl₂ (50, 150, 300 ppm).

Qualitative phytochemical screening of methanolic extracts revealed distinct variations in phytochemical composition among different plant organs and treatments at 60 days after sowing (Table 1). The presence or absence of phytochemical constituents was markedly influenced by heavy metal type, concentration, and plant part.

Carbohydrates, as detected by Fehling's test, were consistently present in leaf extracts under control and lower metal treatments; however, their absence was observed at higher concentrations, particularly at 300 ppm PbCl₂ and CoCl₂. In stem and root extracts, carbohydrate presence showed a declining trend with increasing metal concentration, with roots exhibiting the highest sensitivity to metal stress.

Proteins, detected using the Biuret test, were present in control and lower metal treatments in leaf and stem extracts but showed reduced occurrence at higher concentrations of both lead and cobalt. Root extracts showed limited protein presence, indicating greater susceptibility of root tissues to metal-induced biochemical alterations.

Alkaloid detection varied depending on the reagent used. While Hager's test showed a predominantly negative response across treatments, Wagner's, Mayer's, and Dragendorff's tests indicated the presence of alkaloids mainly in control and lower metal concentrations. A noticeable reduction in alkaloid occurrence was observed at higher metal doses, particularly in root tissues.

Flavonoids, assessed using the alkaline reagent test, were primarily detected in stem extracts under control and lower treatment conditions, while leaf and root extracts exhibited largely negative responses across treatments.

Tannins, detected by ferric chloride and gelatin tests, were prominently present in leaf extracts across control and moderate metal concentrations. However, a reduction in tannin presence was observed at higher metal levels, especially at 300 ppm PbCl₂ and CoCl₂. Stem and root extracts showed comparatively lower tannin occurrence.

Phenolic compounds, detected using the lead acetate test, showed positive responses in leaf, stem, and root extracts under control and lower stress levels. Their absence at higher concentrations suggests inhibition of phenolic synthesis under severe metal stress.

Amino acids, determined by the ninhydrin test, were detected mainly in control and lower treatments, with a decline observed at elevated metal concentrations across all plant parts.

Phytosterols showed consistent presence across most treatments as indicated by Salkowski's and Liebermann–Burchard tests, although minor reductions were noted in root extracts at higher cobalt concentrations.

Anthraquinones and terpenoids exhibited comparatively stable presence across treatments; however, reduced detection was observed in stem and root extracts under higher metal stress. Cardiac glycosides showed variable responses, with reduced presence at higher metal concentrations.

Overall, leaf extracts demonstrated greater retention of phytochemical constituents compared to stem and root extracts at 60 DAS, indicating relatively higher tolerance of aerial tissues to heavy metal stress.

3.2 Qualitative phytochemical responses at 90 DAS

Table 2. Qualitative phytochemical profile of methanolic extracts of leaf, stem and root of *Solanum melongena* L. under lead and cobalt stress at 90 days after sowing (DAS)

Chemical Constituents	Tests	Methanol																									
		Leaf extract 90 DAS								Stem extract 90 DAS								Root extract 90 DAS									
		T 0	T 1	T 2	T 3	T 4	T 5	T 6	T 0	T 1	T 2	T 3	T 4	T 5	T 6	T 0	T 1	T 2	T 3	T 4	T 5	T 6					
Carbohydrate	Fehling's test	+	+	-	-	+	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Protein	Biuret test	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	Hanger test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Wanger test	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
	Mayer test	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-
	Dragendroff test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoid	Alkaline reagent	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Tannin	5% FeCl3 test	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	-	+	-
	Gelatin test	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	Lead acetate	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	
Amino acids	Ninhydrine	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+	-	-	-	-
Phytosterols	Salkowski's test	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Liebermann's test	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	+	+	-	+	-	-	+	-	-	-	-
Anthraquinone	Anthraquinone test	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac - Glycoside	Keller-Illiani	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	-	-	+	-	-	+	-	-	-	-
Terpenoids	Terpenoid test	+	+	-	-	+	-	-	+	+	+	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-

(+): Presence; (-): Absence.

T₀: Control; T₁-T₃: PbCl₂ (50, 150, 300 ppm); T₄-T₆: CoCl₂ (50, 150, 300 ppm).

Qualitative phytochemical screening of methanolic extracts at 90 days after sowing revealed pronounced variations in phytochemical composition across plant organs and heavy metal treatments (Table 2).

Compared to 60 DAS, a greater reduction in the presence of several phytochemical constituents was observed at this advanced growth stage, particularly under higher concentrations of lead and cobalt.

Carbohydrates, as indicated by Fehling's test, were detected in leaf extracts of control and lower metal treatments; however, their presence declined markedly at higher concentrations. Stem extracts retained carbohydrates under control and moderate treatments, whereas root extracts showed a predominantly negative response, indicating increased sensitivity of below-ground tissues at 90 DAS.

Proteins detected by the Biuret test were present mainly in control and lower concentrations of both metals in leaf and stem extracts. A noticeable decline in protein presence was observed at higher metal doses, especially in root tissues, suggesting progressive disruption of protein metabolism under prolonged metal stress.

Alkaloid detection exhibited reagent-dependent variation. Hager's test showed a negative response across all treatments, whereas Wagner's, Mayer's, and Dragendorff's tests indicated alkaloid presence mainly in control and lower concentrations. At elevated metal levels, alkaloid occurrence declined across all plant organs, with roots exhibiting the greatest reduction.

Flavonoids, assessed by the alkaline reagent test, were detected predominantly in stem extracts under control conditions, while leaf and root extracts exhibited largely negative responses across treatments at 90 DAS. This indicates reduced flavonoid expression at later growth stages under sustained metal exposure.

Tannins, determined by ferric chloride and gelatin tests, were consistently present in leaf extracts under control and moderate metal concentrations. However, a reduction in tannin presence was evident at higher metal levels, particularly at 300 ppm treatments. Stem and root extracts showed comparatively lower tannin occurrence, reflecting organ-specific sensitivity to metal stress.

Phenolic compounds detected by the lead acetate test showed positive responses mainly in control and lower treatments across plant parts, whereas higher concentrations of lead and cobalt resulted in their absence. This trend suggests inhibition of phenolic accumulation under prolonged metal stress.

Amino acids, as indicated by the ninhydrin test, were detected primarily in control and lower treatments in leaf, stem, and root extracts. Their reduced presence at higher metal concentrations highlights adverse effects of metal toxicity on nitrogen metabolism at 90 DAS.

Phytosterols exhibited a differential response at this stage. While stem extracts retained phytosterols under several treatments, leaf and root extracts showed reduced detection,

particularly under cobalt stress. Anthraquinones were largely absent in leaf extracts but remained consistently present in stem and root extracts across treatments.

Cardiac glycosides and terpenoids displayed variable responses, with reduced presence observed at higher metal concentrations, especially in root tissues.

Overall, at 90 DAS, leaf tissues continued to exhibit relatively higher phytochemical retention compared to stems and roots; however, the overall decline in phytochemical presence at higher metal concentrations was more pronounced than at 60 DAS, indicating cumulative effects of prolonged lead and cobalt exposure.

4. Discussion

The present study provides qualitative insights into phytochemical alterations in different organs of eggplant (*Solanum melongena* L.) under increasing heavy metal stress at two growth stages. The results clearly demonstrated that phytochemical composition was influenced by metal concentration, plant organ, and growth stage, with a general decline in phytochemical presence at higher concentrations and at the later growth stage.

At both 60 and 90 DAS, control and lower concentration treatments showed the presence of a wider range of phytochemical constituents, whereas higher concentration treatments resulted in the absence or reduced detection of several compounds. This pattern indicates that elevated heavy metal levels interfere with normal metabolic pathways responsible for the synthesis and accumulation of secondary metabolites. Sharma and Dubey (2005) reported that heavy metals disrupt cellular homeostasis and enzymatic activities, leading to metabolic imbalance and reduced biosynthesis of essential compounds.

Organ-specific variation in phytochemical retention was evident across both sampling stages. Leaves consistently exhibited higher phytochemical presence compared to stems and roots. This observation may be attributed to the fact that leaves are the primary sites of photosynthesis and secondary metabolite synthesis, and thus possess a more active antioxidant and metabolic machinery. Similar organ-dependent responses have been reported by Ramakrishna and Ravishankar (2011), who suggested that aerial tissues often show greater metabolic flexibility under stress conditions.

Roots exhibited the greatest sensitivity to heavy metal stress, as reflected by the pronounced reduction or absence of several phytochemicals, particularly at higher concentrations and at 90 DAS. Roots are the first point of contact with soil-borne metals and therefore accumulate higher metal loads, which can impair cellular integrity and enzyme function. Shahid et al. (2017) also reported that excessive metal accumulation in roots leads to oxidative stress and suppression of metabolic activities, resulting in reduced synthesis of bioactive compounds.

Growth stage-dependent differences were also apparent in the present study. Compared to 60 DAS, plants at 90 DAS showed a more pronounced reduction in phytochemical presence under higher concentration treatments. This suggests that prolonged exposure to heavy metal stress intensifies metabolic disruption over time. Gill and Tuteja (2010) explained that sustained oxidative stress under prolonged metal exposure can overwhelm antioxidant defense systems, leading to impaired secondary metabolite production.

Among the phytochemical groups analyzed, carbohydrates, proteins, phenolics, amino acids, and tannins showed noticeable sensitivity to increasing metal concentration. These compounds are closely associated with primary metabolism and stress defense, and their reduction under higher stress levels reflects disturbance in carbon and nitrogen metabolism. Michalak (2006) reported that phenolic compounds, although involved in antioxidant defense, may decline under severe metal stress due to inhibition of key biosynthetic enzymes. Flavonoids showed limited detection, particularly at later growth stages and higher concentrations, indicating suppression of flavonoid biosynthesis under sustained stress. Winkel-Shirley (2002) reported that flavonoid synthesis is tightly regulated and highly sensitive to environmental stress intensity. In contrast, phytosterols and anthraquinones exhibited relatively stable presence across treatments, suggesting that certain secondary metabolites may be less sensitive or play a protective structural role under stress conditions.

Overall, the qualitative trends observed in this study indicate that lower metal concentrations allow partial maintenance of phytochemical integrity, whereas higher concentrations lead to marked biochemical suppression. The combined effects of metal concentration, organ specificity, and growth stage highlight the complex nature of plant responses to heavy metal stress. These findings emphasize the importance of growth stage-specific and organ-specific assessment when evaluating metal-induced biochemical responses in plants.

5. Conclusion

The present study highlights growth stage- and organ-dependent qualitative alterations in phytochemical composition of methanolic extracts of eggplant (*Solanum melongena* L.) under increasing metal stress. Lower concentration treatments showed relatively greater retention of phytochemical constituents, whereas higher concentrations resulted in pronounced reductions, particularly at 90 days after sowing. Leaf tissues exhibited higher phytochemical stability compared to stems and roots. These findings provide baseline qualitative evidence of metal-induced biochemical responses and support further quantitative investigations into stress tolerance mechanisms.

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