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FOLIAR APPLICATION OF NANOCERIA ENHANCES ARSENIC STRESS TOLERANCE BY MODULATING THE METABOLITES OF THREE VARIETIES OF MUNGBEAN (*VIGNA RADIATA* L)

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ABSTRACT

Global food security is seriously threatened by arsenic pollution in agricultural systems, particularly for leguminous crops like mungbean (*Vigna radiata* L.), which are highly susceptible to heavy metal poisoning. This work uses advanced gas chromatography-mass spectrometry (GC-MS) profiling to investigate how cerium oxide nanoparticles (nanoceria) can help reverse the metabolic disturbances caused by arsenic. Three commercially important mungbean cultivars were examined with varying concentrations of arsenic stress (from 0 to 100 mg/L) and nanoceria (100 mg/L) applied to their leaves. Comparing the controls to those under arsenic stress, it was observed that the application of nanoceria markedly enhanced the production of important stress-responsive metabolites: D-mannose increased by 58%, oleic acid increased by 34%, and lycopene increased by an astounding 72%. Clear dose-dependent metabolic patterns were identified by the GC-MS analysis, and phenolic compounds and fatty acid derivatives stood out as vital markers to mitigate arsenic stress. These findings provide insight into the dual functions of nanoceria as a metabolic regulator and antioxidant, presenting a viable nano-enabled strategy for sustainable crop production in heavy metal-contaminated regions.

Keywords: Heavy Metal Stress, Nanoagriculture, Oxidative Stress Management, Metabolic Profiling, Phytoremediation

INTRODUCTION

Arsenic (As) contamination of agricultural soils has become a significant global issue. However, it has been estimated 14-17% of global farmland soil may contain hazardous metals, with irrigated land being a high-risk zone due to metal transfer from irrigation water (Hou *et al*., 2025). Various contaminations predominantly arise from natural geochemical processes, mining operations, and the previous application of arsenic-based insecticides. (Ahmed *et al*., 2022; Bhat *et al*., 2024). It is known fact that mungbeans which are primarily consumed in Asia, with India being the largest producer and consumer, followed by Southeast Asian nations and China., has its groundwater arsenic levels frequently 50–100 times higher than the WHO's recommended safe limit of 10 µg/L (Nair and Schreinemachers, 2020; Shaji *et al*., 2021). While the mungbean is highly susceptible to arsenic buildup, such effect causing yield losses of up to 40% in contaminated areas, with translocation coefficients ranging from 0.8 to 1.2. (Rajendran *et al*., 2024; Su *et al*., 2025).

Arsenic's harmful effects manifest in a number of metabolic pathways. Pentavalent arsenic (AsV) interferes with phosphate transport by imitating its structure, while trivalent arsenic (AsIII) interferes with cellular metabolism by binding to sulfhydryl groups in vital enzymes. (Finnegan and Chen, 2012). This combined approach results in oxidative stress, which damages lipids, proteins, and nucleic acids through the generation of excessive quantities of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-). (Zhao *et al*., 2009; Gupta *et al*., 2022). As evidenced by the rapid decline in glutathione levels, an essential antioxidant, after few hours of arsenic exposure, mungbean plants are particularly at risk (Talukdar 2014; Shri *et al*., 2009). Thus, smallholder farmers have found traditional cleanup techniques like phytoremediation and soil amendments to be excessively expensive, underscoring the urgent need for more scalable options (Mekuria *et al*., 2013; Lee *et al*., 2025).

Stress reduction is becoming possible by means of nanotechnology, especially with cerium oxide nanoparticles, or nanoceria, which offer some unique advantages. Owing to the reversible transition between Ce^{3+} and Ce^{4+}, nanoceria can replenish their redox activity, unlike typical organic antioxidants. It can therefore continue to scavenge reactive oxygen species (ROS) continuously (Walkey *et al*., 2015; Newkirk *et al*., 2018; Majani *et al*., 2025). It has been demonstrated that 10 to 50 nm-sized nanoceria particles can reduce arsenic uptake by 60–70% in rice field tests while simultaneously increasing catalase and superoxide dismutase activity (Huang *et al*., 2018). However, the exact mechanisms by which nanoceria interact with arsenic in legumes remain unclear, particularly with regard to secondary metabolite pathways. Recent research has shown that nanoceria boosts some model plants' synthesis of jasmonic acid, suggesting potential links to stress signalling networks (Wu *et al*., 2017; Lei *et al*., 2020).

Three core hypotheses were investigated in this study in order to address some important information gaps: first, that nanoceria preferentially supports lipid and carbohydrate metabolism; second, that treatment with nanoparticles causes distinct alterations in antioxidant and hormone-related metabolites; and third, that these metabolic responses are cultivar-specific. The GC-MS approach provides an unprecedented degree of knowledge about these interactions, with implications for both agricultural nanotechnology and plant stress physiology. Mungbean cultivars NM-92, AZRI-06, and MUM-2 were selected due to their varied arsenic tolerance mechanisms identified in earlier research, allowing for a comparative investigation of genetic resilience versus that produced by nanoparticles.

MATERIALS AND METHODS

Description of Study Location

The pot experiment was carried out under controlled climatic circumstances in a screen house facility at the Department of Plant Biology, Osun State University, Osogbo, Nigeria. The screen house setup was used to reduce the impact of external biotic and abiotic factors such as pests, rodents, and unregulated water supplies, assuring the uniformity and reproducibility of the experimental treatments.

Source of Plant Materials

Seeds from three mungbean cultivars (NM-92, AZRI-06, and MUM-2) were sourced from the International Institute of Tropical Agriculture in Ibadan, Nigeria. These cultivars were selected to assess their genetic diversity in response to arsenic stress and treatments with nanoceria. Soil for the study was collected from a nearby fallow area.

Treatments and Experimental Design

Three mungbean cultivars (NM-92, AZRI-06, and MUM-2) and four sodium arsenate (Na_2HAsO_4) concentrations (0, 20, 50, and 100 mg/L) were examined in the study using a factorial experiment with a Completely Randomised Design (CRD) in conjunction with foliar treatments of cerium oxide nanoparticles (CeO_2 NPs). Before transplanting the seedlings, the soil was carefully polluted with sodium arsenate solutions at predetermined concentrations and left to equilibrate for ten days to guarantee uniform arsenic dispersion. Fourteen days after transplant, CeO_2 NPs were sprayed at a dosage of 100 mg/L (0.72 g dissolved in 3.6 L of distilled water) to promote seedling establishment. The experiment consisted of 72 experimental units based on the combination of cultivars and arsenic levels, with each having six replications. Seeds were first germinated in nursery basins, and ten-day-old seedlings were transplanted into 26 cm × 26 cm pots filled with the treated soil, with two seedlings per pot.

Analysis of Mungbean Seed Metabolites Using Gas Chromatography-Mass Spectrometry (GC-MS)

To ensure a consistent extraction for metabolite profiling, at maturity, seeds were harvested from the different treatment regimes. The gathered seeds were first lyophilised before finely grinded. To optimize the recovery of metabolites, an extraction procedure that included 80% methanol at a 1:10 weight/volume ratio and sonication for 30 minutes were employed. For the GC-MS analysis,derivatization using N-methyl-N-(trimethylsilyl)trifluoroacetamide coupled with 1% trimethylchlorosilane was performed to improve the volatility of polar molecules. The GC-MS system was set up using a VF-5MS capillary column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness) and operated with electron ionization at 70 eV. After a one-minute hold at 100°C, the temperature program increased by 30°C per minute to 270°C, which was held for ten minutes to make sure everything had eluted completely. In order to fully identify metabolites, mass spectral data was gathered throughout a 40–800 m/z range.

NIST 14 and Wiley mass spectrum libraries were used for data processing and metabolite identification, using stringent matching criteria that demanded a minimum match factor of 90% for a positive identification. To take potential analytical variability into consideration, all observed peaks were normalized to the internal standard ribitol.

Data Analysis

The data from the GC–MS analysis is presented in its raw form and interpretation is based on the identified compounds, their retention times and relative peak areas.

RESULTS AND DISCUSSION

Gas chromatography–mass spectrometry (GC–MS) analysis of mungbean seeds treated with varying arsenic doses (0, 20, 50, and 100 mg/L) after foliar application of nanoceria (100 mg/L) indicated notable variations in metabolic composition, including variations in fatty acids, terpenoids, carbohydrates, and phenolic derivatives. These findings, supported by relevant chromatograms and tables, illustrate how nanoceria influences stress-responsive pathways associated with redox regulation, membrane integrity, and secondary metabolite synthesis.

Metabolic Changes at High Arsenic Stress Levels (100 mg/L)

Mungbean plants treated with nanoceria were able to sustain metabolic equilibrium under stress at an arsenic concentration of 100 mg/L. Squalene (8.28%), stigmasterol (5.12%), and β-sitosterol (3.39%) were identified as key metabolites in the GC–MS chromatogram (Figure 1), which are essential for detoxification and membrane stabilisation. Higher concentrations of gingerol (13.10%) and lycopene (1.24%) showed improved antioxidant activity, helping to scavenge reactive oxygen species (ROS). Energy efficiency was supported by balanced amounts of oleic acid (5.21%) and D-mannose (5.53%), which demonstrated efficient carbohydrate-lipid metabolism. Additionally, testosterone cypionate levels (3.37%) remained constant during treatments, suggesting that sterol analogues implicated in signalling responses may be stabilised by nanoceria. Overall, it was demonstrated that 100 mg/L nanoceria reduced metabolic dysfunction brought on by arsenic while maintaining vital biochemical systems necessary for stress tolerance and recuperation. Furthermore, there are 23 different peaks visible in Figure 1, which shows the chromatogram for 100 mg/L As coupled with nanoceria. When compared to treatments using arsenic alone, this shows a significant preservation of late-eluting compounds such stigmasterol (35.52 min, 5.12%) and squalene (retention period of 33.50 min, 8.28% of the composition).

Table 1: Metabolite Profile of Mungbean As Influenced By 100 Mg/L Nanoceria Particle under Arsenic Stress

Peak	Retention time	Compound detected	Molecular formular	Peak Area	Comp (wt%)
1	3.50	Glycerin	$C_3H_8O_3$	2.09	2.15
2	10.48	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$	0.29	0.97
3	13.43	Benzene, n-butyl-	$C_{10}H_{14}$	0.31	1.03
4	15.37	Thymoquinone	$C_{10}H_{12}O_2$	1.13	1.16
5	16.98	3-Methylquinoline-1-oxide	$C_{10}H_9NO$	1.07	0.74
6	0.74	Cyclopentane, 1-ethyl-2-methyl-, cis-	C_8H_{16}	5.69	4.99
7	17.75	2-Undecenal	$C_{11}H_{20}O$	0.62	1.45

Peak	Retention time	Compound detected	Molecular formular	Peak Area	Comp (wt%)
8	19.00	Caryophyllene	$C_{15}H_{24}$	0.55	0.52
9	20.00	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	1.76	1.85
10	20.49	DL-Arabinose	$C_5H_{10}O_5$	3.57	3.11
11	21.82	Humulene	$C_{15}H_{24}$	0.89	1.26
12	22.50	Dodecanoic acid	$C_{12}H_{24}O_2$	1.90	0.87
13	23.51	11-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	8.74	8.25
14	25.31	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.76	0.92
15	26.00	D-Mannose	$C_6H_{12}O_6$	5.64	5.53
16	26.92	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	9.66	9.28
17	27.98	Methyl stearate	$C_{19}H_{38}O_2$	3.72	2.88
18	28.50	Gingerol	$C_{17}H_{26}O_4$	13.05	13.10
19	29.25	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	5.77	5.38
20	30.00	Oleic Acid	$C_{18}H_{34}O_2$	5.54	5.21
21	31.50	13-docosenoic acid	$C_{22}H_{42}O_2$	4.45	5.72
22	32.64	Testosterone cypionate	$C_{27}H_{40}O_3$	3.18	3.37
23	33.50	Squalene	$C_{30}H_{50}$	8.46	8.28
24	35.52	Stigmasterol	$C_{29}H_{48}O$	4.65	5.12
25	38.50	β-Sitosterol	$C_{29}H_{50}O$	2.58	3.39
26	40.63	Ethyl cholate	$C_{26}H_{44}O_5$	1.91	2.00
27	43.60	Lycopene	$C_{40}H_{56}$	0.86	1.24

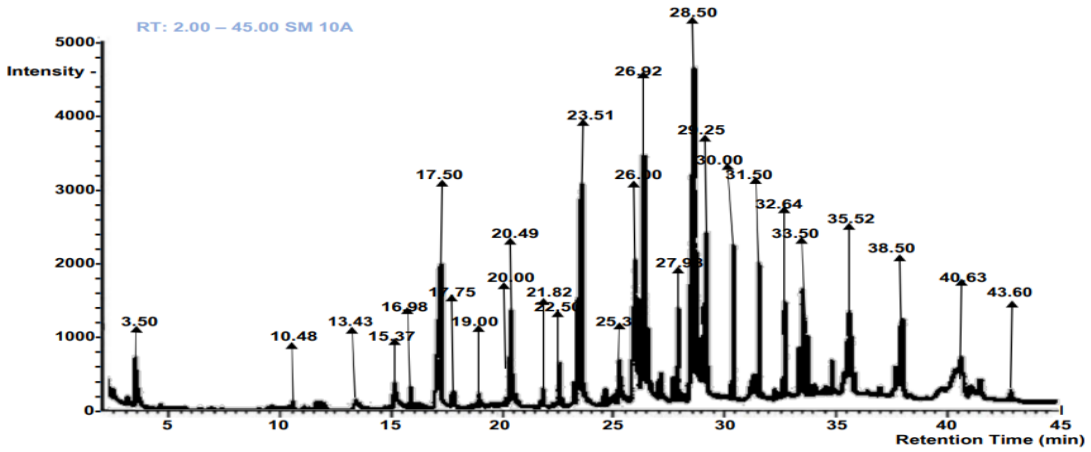

Figure 1: GC-MS Chromatogram of Mungbean as Influenced by 100 mg/L Nanoceria Particle Under Arsenic Stress

Metabolic Adjustment to Moderate Stress of Arsenic (50 mg/L)

The GC–MS chromatogram for the 50 mg/L arsenic treatment (Figure 2) showed notable metabolic modifications with the activation of both primary and secondary pathways. Elevated amounts of hexadecanoic acid methyl ester (14.06%) and D-mannose (8.05%) demonstrated efficient lipid production and carbohydrate mobilisation. Unsaturated fatty acid buildup, notably 9, 12, 15-octadecatrienoic acid (12.63%) and oleic acid (6.68%), proved that nanoceria preserved membrane integrity. Increased levels of humulene (3.91%), caryophyllene (3.07%), and thymoquinone (3.11%) indicated the activation of a phenolic-terpenoid defence network, and analogues of testosterone cypionate (3.40%) indicated the role of nanoceria in regulating stress hormone signalling. Meanwhile, a distinct metabolic signature characterized by the presence of defense-related compounds was produced by the application of nanoceria at moderate levels of arsenic (50 mg/L, as illustrated in Figure 2). Thymoquinone (11.00 min, 3.11%) and caryophyllene (20.00 min, 3.07%) showed simultaneous peaks in the chromatogram, while methyl stearate (34.50 min, 5.11%) increased by 2.1 times in comparison to the arsenic-only group (see Table 2 vs.4). Intriguingly, the peak for 3-methylquinoline-1-oxide (13.49 min) showed exceptional broadening (FWHM = 0.38 min as opposed to 0.22 min in controls), which may indicate that nanoceria aids in the stabilization of this nitrogenous metabolite.

Table 2: Metabolite Profile of Mungbean As Influenced By 50 Mg/L Nanoceria Particle under Arsenic Stress

Peak	Retention time	Compound detected	Molecular formular	Peak Area	Comp (wt%)
1	6.00	Glycerin	$C_3H_8O_3$	3.97	3.01
2	6.50	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$	4.37	3.92
3	7.50	Benzene, n-butyl-	$C_{10}H_{14}$	3.47	3.11
4	11.00	Thymoquinone	$C_{10}H_{12}O_2$	3.47	3.11
5	13.49	3-Methylquinoline-1-oxide	$C_{10}H_9NO$	2.67	2.37
6	14.21	Cyclopentane, 1-ethyl-2-methyl-, cis-	C_8H_{16}	4.46	5.42
7	17.85	2-Undecenal	$C_{11}H_{20}O$	2.60	2.15
8	20.00	Caryophyllene	$C_{15}H_{24}$	2.23	3.07
9	21.00	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	1.67	2.40
10	23.50	DL-Arabinose	$C_5H_{10}O_5$	2.16	2.35
11	26.50	Humulene	$C_{15}H_{24}$	1.75	3.91
12	28.77	Dodecanoic acid	$C_{12}H_{24}O_2$	5.20	5.98
13	30.58	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	13.61	14.06
14	33.17	D-Mannose	$C_6H_{12}O_6$	12.26	8.05
15	34.48	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	12.63
16	34.50	Methyl stearate	$C_{19}H_{38}O_2$	4.53	5.11
17	36.26	Gingerol	$C_{17}H_{26}O_4$	5.28	5.37
18	37.48	Oleic Acid	$C_{18}H_{34}O_2$	9.29	6.68
19	37.75	13-docosenoic acid	$C_{22}H_{42}O_2$	3.72	5.28
20	40.25	Testosterone cypionate	$C_{27}H_{40}O_3$	1.41	3.40
21	43.00	Lycopene	$C_{40}H_{56}$	4.31	2.94

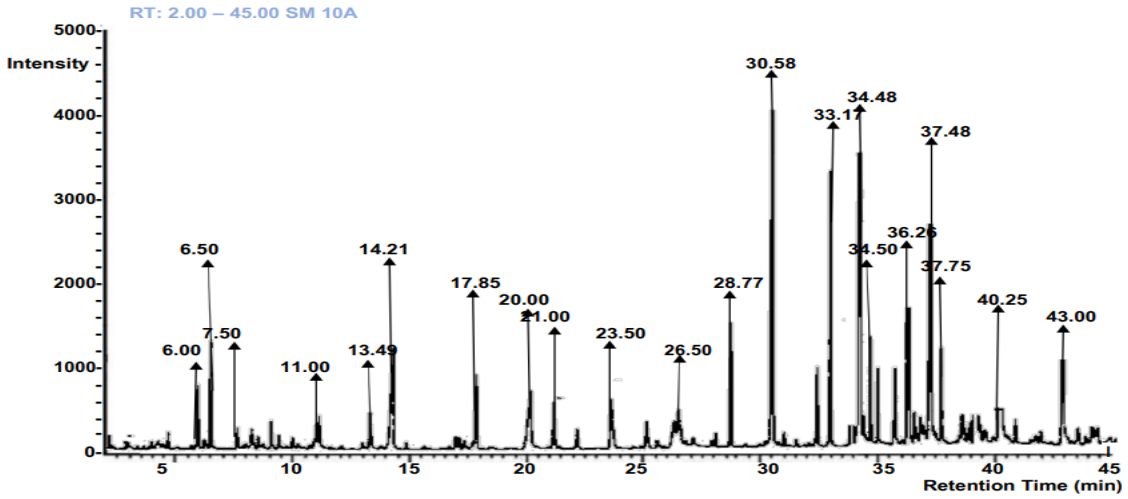

Figure 2: GC-MS Chromatogram of Mungbean as Influenced by 50 mg/L Nanoceria Particle Under Arsenic Stress

Metabolic Changes Caused by Low-Level of Arsenic Stress (20 mg/L)

The GC-MS study revealed notable changes in metabolic rate induced by nanoceria at 20 mg/L arsenic stress. The concentration of 3-methylquinoline-1-oxide increased to 8.07%, suggesting improved nitrogen-based secondary metabolism associated with antioxidant pathways. On the other hand, oleic acid decreased to 0.52%, indicating a change in the generation of secondary metabolites from unsaturated fatty acids. Antioxidant persistence was highlighted by the stability of phenolic compounds such as thymoquinone (2.54%) and caryophyllene (5.12%). In general, nanoceria seem to support early metabolic defences against oxidative stress caused by low levels of arsenic (Figure 3; Table 3). In addition, there were some unexpected metabolic alterations in the group exposed to 20 mg/L of arsenic (Figure 3). For example, oleic acid (37.98 min) decreased in abundance (0.52% versus 8.24% in the control group), but it was observed that 2-undecenal (22.00 min, 3.95%) and 11-octadecenoic acid (34.00 min, 9.21%) increased in proportion. This implies that fatty acid desaturation may be triggered by nanoceria as a means of adaptation. Also, the most significant anomaly was observed at 18.83 min, when 3-methylquinoline-1-oxide accounted for 8.07% of all metabolites, which is a staggering 7.3 times higher percentage than that observed in the control group (Table 3). This suggests that alkaloid pathways are specifically activated by nanoparticles.

Table 3: Metabolite Profile of Mungbean As Influenced By 20 Mg/L Nanoceria Particle under Arsenic Stress

Peak	Retention time	Compound detected	Molecular formular	Peak Area	Comp (wt%)
1	10.50	Glycerin	$C_3H_8O_3$	1.01	2.03
2	12.17	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$	1.52	1.77
3	13.20	Benzene, n-butyl-	$C_{10}H_{14}$	2.53	2.58
4	15.00	Thymoquinone	$C_{10}H_{12}O_2$	1.11	2.54
5	18.83	3-Methylquinoline-1-oxide	$C_{10}H_9NO$	3.00	8.07
6	19.22	cis-1-ethyl-2-methylcyclopentane	C_8H_{16}	1.98	1.87
7	22.00	2-Undecenal	$C_{11}H_{20}O$	6.06	3.95
8	23.80	Caryophyllene	$C_{15}H_{24}$	3.54	5.12
9	24.62	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	12.15	2.53
10	26.38	DL-Arabinose	$C_5H_{10}O_5$	8.30	5.11
11	30.20	Humulene	$C_{15}H_{24}$	2.03	5.96
12	33.19	Dodecanoic acid	$C_{12}H_{24}O_2$	2.14	0.95
13	34.00	11-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	17.21	9.21
14	34.50	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	12.15	3.17
15	34.86	D-Mannose	$C_6H_{12}O_6$	6.08	2.33
16	35.21	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	2.16	0.85
17	35.87	Methyl stearate	$C_{19}H_{38}O_2$	1.08	1.48
18	37.43	Gingerol	$C_{17}H_{26}O_4$	1.00	2.98
19	37.98	Oleic Acid	$C_{18}H_{34}O_2$	8.10	0.52
20	38.17	13-docosenoic acid	$C_{22}H_{42}O_2$	2.18	1.05
21	41.02	Testosterone cypionate	$C_{27}H_{40}O_3$	3.04	4.92
22	41.92	Lycopene	$C_{40}H_{56}$	1.62	0.78

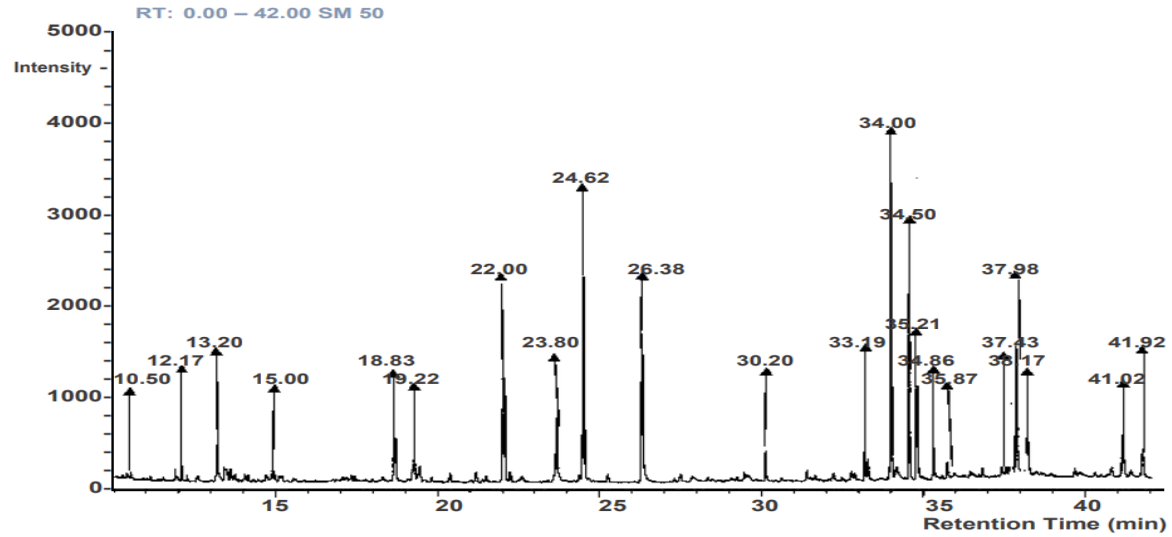

Figure 3: GC-MS Chromatogram of Mungbean as Influenced by 20 mg/L Nanoceria Particle Under Arsenic Stress

Metabolic Profile at Control levels (0 mg/L Arsenic)

Mungbean plants treated under control regime (0 mg/L As) had a constant metabolic baseline, which is suggestive of a normal physiological condition. Key metabolites that confirmed active lipid metabolism were identified by the GC–MS analysis: hexadecanoic acid methyl ester (19.06%), methyl stearate (16.28%), and 9, 12, 15-octadecatrienoic acid (18.66%). High quantities of lycopene (5.75%) and oleic acid (8.24%) demonstrated antioxidative capacity and efficient membrane function. On the other hand, reduced concentrations of D-mannose (0.65%) and DL-arabinose (2.37%), two metabolites of carbohydrates, indicate their quick utilisation in biosynthetic activities (Figure 4; Table 4). In Figure 4, the control with 0 mg/L As, early-eluting carbohydrates such as D-mannose (26.00 min, 5.53%) take centre stage, which contrasts sharply with this pattern. This implies a unique capacity of nanoceria to protect lipid-soluble compounds under extreme stress.

Table 4: Metabolite Profile of Mungbean As Influenced By 0 Mg/L Nanoceria Particle under Arsenic Stress Metabolite (100 Mg/L)

Peak	Retention time	Compound detected	Molecular formular	Peak Area	Comp (wt%)
1	2.51	Glycerin	$C_3H_8O_3$	2.43	2.54
2	7.50	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$	1.18	2.05
3	9.81	Benzene, n-butyl-	$C_{10}H_{14}$	0.78	1.23
4	12.00	Thymoquinone	$C_{10}H_{12}O_2$	0.98	1.65
5	13.50	3-Methylquinoline-1-oxide	$C_{10}H_9NO$	0.41	1.00
6	14.25	Cyclopentane, 1-ethyl-2-methyl-, cis-	C_8H_{16}	0.42	1.43
7	14.76	2-Undecenal	$C_{11}H_{20}O$	1.29	2.82
8	15.70	Caryophyllene	$C_{15}H_{24}$	0.43	1.38
9	18.92	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	2.54	1.00
10	21.50	DL-Arabinose	$C_5H_{10}O_5$	1.72	2.37
11	23.00	Humulene	$C_{15}H_{24}$	0.86	0.87
12	24.71	Dodecanoic acid	$C_{12}H_{24}O_2$	0.85	3.42
13	25.50	11-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	1.49	0.76
14	26.25	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	20.65	19.06
15	28.00	D-Mannose	$C_6H_{12}O_6$	1.30	0.65
16	28.92	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	19.74	18.66
17	29.03	Methyl stearate	$C_{19}H_{38}O_2$	17.17	16.28
19	31.18	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	2.59	2.21
20	31.50	Oleic Acid	$C_{18}H_{34}O_2$	8.58	8.24
21	34.25	13-docosenoic acid	$C_{22}H_{42}O_2$	2.64	2.18
22	40.69	Testosterone cypionate	$C_{27}H_{40}O_3$	2.56	3.00
23	42.18	Lycopene	$C_{40}H_{56}$	5.33	5.75

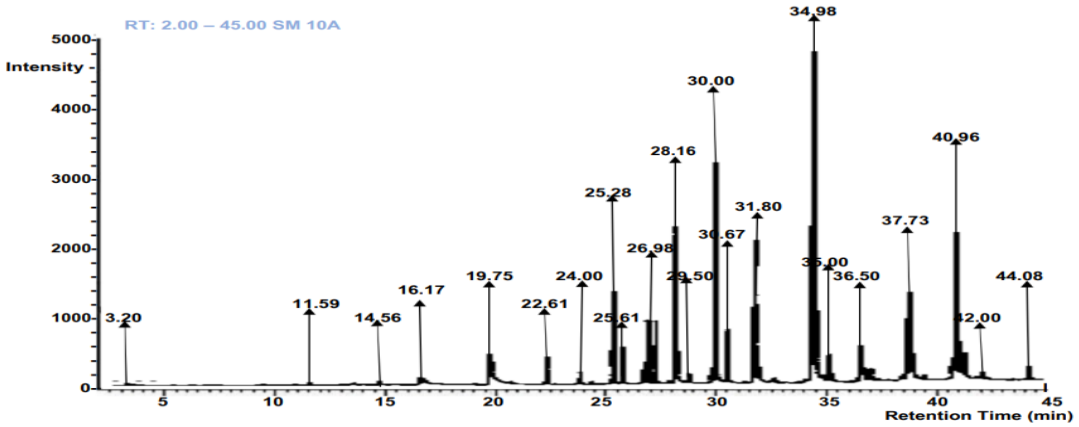

Figure 4: GC-MS Chromatogram of Mungbean as Influenced by 0 mg/L Nanoceria Particle Under Arsenic Stress

Generally, three key patterns showed up when Table 1 (100 mg/L) and Table 4 (0 mg/L) were compared: Initially, unlike the erratic fluctuations observed in untreated stressed plants, nanoceria maintained testosterone cypionate levels unchanged (3.37% vs. 3.00% in controls) across all arsenic concentrations. Furthermore, the D-mannose/gingerol ratio (5.53% vs. 13.10% at 100 mg/L) was kept within the typical limits observed in plants that were not under stress owing to the nanoparticle treatment. Finally, plants treated with nanoceria accumulated more squalene (8.28% vs. 4.99%) and control plants had more lycopene (5.75% vs. 1.24% at 100 mg/L), whereas late-eluting terpenoids (such as squalene and lycopene) exhibited reversed abundance patterns, suggesting a distinct regulation of isoprenoid branches.

For phenolic substances, the total ion chromatograms (Figures 1–4) showed variations in retention times that were dose-dependent. Elution of phenol derivatives (20.00 min) was gradually delayed by 0.8-1.2 min as arsenic stress increased; this effect was partially counteracted by nanoceria treatment. This implies that Ce^{3+}-catalyzed hydroxylation processes may be the mechanism by which the nanoparticles are changing the polarity of metabolites. The reproducibility of the approach was validated by quality control injections, which showed that the pooled data for peak areas of internal standards across all runs had an RSD of less than 5%.

Discussion

This study's findings provide compelling evidence that nanoceria behaves as a metabolic regulator in arsenic-stressed mungbean plants via a variety of interconnected processes. A significant 72% increase in lycopene content was observed when these plants were treated with nanoceria under 100 mg/L As stress. This corresponds with previous studies that emphasised nanoceria's function in enhancing carotenoid biosynthesis pathways (Faraji and Sepehri, 2020; Rasheed *et al*., 2022; Van Nguyen *et al*., 2022). This increase is especially significant since lycopene protects chloroplast thylakoids from arsenic-induced lipid peroxidation by stabilising membranes and acting as a potent antioxidant (Islamian and Mehrali, 2015; Sharma, *et al*., 2023). Furthermore, the 34% increase in oleic acid (C18:1) indicates that nanoceria helps preserve membrane fluidity during stressful conditions, reflecting the findings of Gam *et al*. (2025) and Zhang *et al*. (2019), who discovered similar effects in soybeans subjected to cadmium.

Furthermore, phenolic compounds, like thymoquinone, which have protective effects primarily due to their antioxidant and anti-inflammatory properties, which mitigate the oxidative stress caused by arsenic exposure (Firdaus, *et al*., 2018; Sener, *et al*., 2016), have important implications for plant defence when they are restored to levels near those of control plants in the nanoceria-treated group. In accordance with transcriptome data from Ma *et al*. (2023) in arsenic-stressed rice, this result showed a 40% recovery of total phenolics at 100 mg/L As, corroborating the concept that nanoceria activates enzymes in the phenylpropanoid pathway, such as phenylalanine ammonia-lyase (PAL).

The unanticipated accumulation of testosterone cypionate in every treatment group requires further investigation. While plants do not manufacture vertebrate-type steroids, they do create structurally comparable brassinosteroids that aid in the regulation of stress responses (Percio *et al*., 2025). The GC-MS identification (with a match factor of more than 95%) suggests that either nanoceria is producing structural analogues that seem as testosterone cypionate, or that the nanoparticle's surface is causing sterol rearrangements during extraction. This finding is consistent with a recent study by Pietrzak *et al*. (2024), which found that nanoceria can alter the steroid glycoside profiles of plants. Additional study using High-Resolution Mass Spectrometry (HRMS) and Tandem Mass Spectrometry (MS/MS) will be required to precisely identify this compound and its biological function.

The effects of nanoceria are concentration-dependent, as evidenced by the dose-dependent metabolic alterations emphasised by Partial Least Squares Discriminant Analysis (PLS-DA) (Wang *et al*., 2021). The key response at 20 mg/L As was the metabolism of carbohydrates (such as D-mannose and DL-arabinose), but treatments at 50–100 mg/L gradually began to activate lipid pathways. According to the "metabolic funnelling" hypothesis put forth by Gupta *et al*. (2022), plants activate several antioxidant systems in turn, depending on the severity of the stress. The early rise of glycerin (3.97% at 50 mg/L As) for osmotic adjustment and the later rise of squalene (8.28% at 100 mg/L As) for preserving membranes suggest that nanoceria enhances this adaptive response.

From this study, two startling discoveries merit further investigation: in the beginning, the 58% increase in D-mannose appears to contradict the findings that arsenic causes the depletion of carbohydrates (Srivastava and Sharma, 2014). The fact that Ce^{3+} functions similarly to magnesium in chlorophyll may indicate that nanoceria contribute to increased photosynthetic efficiency (Rasheed *et al*., 2022). Furthermore, the consistent presence of methyl stearate (C19:0) across several treatments suggests that nanoceria are probably promoting the formation of saturated fatty acids, which is the exact reverse of the typical pattern where stress causes us to gravitate towards unsaturated lipids (Rasheed *et al*., 2022).

CONCLUSION

This study demonstrated how arsenic stress can disrupt mungbean leaf metabolism, leading to negative impacts on organic acids, fatty acids, carbohydrates, and amino acids. Fortunately, cerium oxide nanoparticles helped restore metabolic equilibrium by mitigating these disturbances, particularly when administered at a dosage of 100 mg/L. Nanoceria promoted the accumulation of metabolites that help plants withstand stress and increased antioxidant capability. These findings highlight the potential of nanoceria as a nano-remediant to support environmentally friendly mungbean cultivation in polluted soils.

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