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Original Research Article

Selenium nanoparticle-mediated enhancement of plant biochemistry and metabolite enrichment: GC-MS profiling of selenium-enriched sesame seed oil

Zohaib Younas[1]✉*, Ilyas Ahmad[1,3], Tayyaba Yousaf[1], Naveed Iqbal Raja[1], And Zia-ur-Rehman Mashwani[1,2]✉*

[1]Department of Botany, PMAS Arid Agriculture University Rawalpindi, 46300 Pakistan
[2]Pakistan Academy of Sciences, Islamabad, 44010 Pakistan
[3]Department of Food Science and Nutrition, College of Food Agriculture and Natural Resources, University of Minnesota, Twin Cities, St Paul, MN 55108, USA

ABSTRACT

This study explored the impact of selenium nanoparticles (SeNPs) on the biochemistry and seed oil profile of sesame (*Sesamum indicum* L.). Sesame plants were treated foliarly with varying dosages of SeNPs (10, 20, 30, 40, and 50 mg/L) and 5 mg/L of selenium salt. UV-Visible spectrum indicated a peak absorption at 279 nm for SeNPs, while FTIR analysis confirmed the reduction of sodium selenite to SeNPs using *Allium sativum* extract. The highest total flavonoid content (TFC) and total phenolic content (TPC) were recorded at 14.72 and 14.24 mg/g, respectively, for the 40 mg/L treatment. Additionally, GC-MS analysis identified thirty-five chemical compounds in sesame seed oil. The Kyoto encyclopaedia of gene and genomics (KEGG) pathway analysis revealed significant metabolite enrichment across eight pathways, particularly in pyruvate metabolism. Overall, SeNPs enhanced the biochemical profile and metabolite detection in sesame seed oil, potentially improving crop yield and stress resilience.

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1. Introduction

Sesamum indicum L., commonly known as sesame or benne, belongs to the Pedaliaceae family and is considered one of the earliest domesticated crops (Ashri, 2007). *Sesamum indicum* L. (sesame) has a long history, stretching back to the bronze age indus valley culture and ancient Anatolia, later domesticated and cultivated on the Indian subcontinent (Howlader et al., 2018; Pathak et al., 2019). Currently, China and India lead the world in sesame production, subsequently followed by Myanmar, Sudan, Uganda, Nigeria and Pakistan (Pham et al., 2010). Fluctuations in worldwide output are due to local economic factors, challenges in agricultural production, and variable weather conditions (Cagirgan, 2006). Sesame thrives on well-drained, medium-textured fertile soil (sandy loam) with a pH over the range 5-8. Furthermore, it is a temperature-sensitive and robust crop that demands extreme temperatures (25-37°C) for the best development and output (Yemata and Bekele, 2024). According to Terefe et al. (2012), a plant can reach a height of 1.5-2.0 m under ideal growth conditions. Plant varieties exhibit considerable variability in terms of flower color, form, seed size, seed color, and composition. Sesame is primarily an annual plant, although it can be somewhat perennial. It has a dense root system that helps keep the plant upright as it grows (Terefe et al., 2012). There is considerable variation in leaf shape, including lobed, entire, and toothed forms. Sesame leaves can be light or dark green, with surfaces that are typically glabrous, though they may be pubescent in rare cases. The arrangement of leaves can be opposite, alternate, or mixed, varying greatly among different varieties. The plant features a weak, herbaceous, and rectangular-branched stem, which

✉ Corresponding authors: Zohaib Younas and Zia-ur-Rehman Mashwani
Tel.: ++92-333-9022077, Fax: ++92-333-9022077
E-mail addresses: zohaibyounas97@gmail.com, mashwani@uaar.edu.pk, **https://doi.org/10.71596/tpr.2024.936631**

is multiflowered and produces capsules containing numerous small, oil-rich seeds. Flowers emerge in the axils of the leaves, with multiple blooms on the upper stem or branches. The calyx consists of five short, velvety lobes, while the corolla (petals) is bilabiate and tubular, with five lobes. There are five stamens fused to the corolla, four of which are functional, while the fifth is sterile. The plant has a bicarpellary female organ, with a superior ovary that is two-celled, a terminal and filiform style, and a bi-lobed stigma that is hairy in texture (Nurdjannah and Bermawie, 2012).

For thousands of years, India, Egypt, and the Persian region have utilized sesame in religious rituals. In Nigeria, the Bura tribes throw benne (sesame) seeds over their shoulders to ward off evil or place them in a habtu (a pot used for ceremonial purposes) to bring good luck. In Sierra Leone, benne seeds are employed in Poro-Sande rituals as a form of punishment. Offenders, particularly those guilty of serious crimes like theft, must pick up a quart of benne seeds that an elder has poured on the ground (Bedigian, 2013). Sesame seeds can be used to decorate bread, create paste for certain dishes, or make sweetened tahini for desserts. The high quality of sesame oil has earned it the title of "queen of oilseeds" (Shimoyoshi et al., 2020; Zhang et al., 2021). Its high oil content, particularly in poly unsaturated fatty acids (PUFA), makes it very appealing to customers. Sesame oil is commonly used in cooking and as a food ingredient, and it may have health advantages. An estimated 65.0% of the sesame production is consumed as edible oil and 35.0% as a food ingredient (Wan et al., 2015). Fats are highly stable and do not develop rancidity in their oil compared to other oils, which helps reduce oxidative damage (Ahmad et al., 2024). Furthermore, sesame contains bioactive substances such as sesamin, sesamolin, and α-tocopherol, which have been linked to health benefits such as reduced blood pressure, lower cholesterol levels, and a lower risk of myocardial infarctions (Jeng and Hou 2005; Phatak et al., 2019). In addition to their anti-carcinogenic and anti-inflammatory properties, these lignans also possess anti-spasmodic effects (Pathak et al., 2019).

Selenium (Se) can be found in both inorganic forms, *e.g.*, selenide, selenite and selenate and organic forms viz. selenomethionine and selenocysteine). Selenium enhances plant growth and provides health benefits in human nutrition, thereby improving agriculture and food technologies (Vinkovic Vrcek, 2018). Selenium nanoparticles (SeNPs) serve as bio-stimulants, nutraceuticals, stress alleviators, antioxidants, and nano-fertilizers in plants.

The objective of this study was to assess the potential influence of SeNPs on both plant biochemistry and the enrichment of metabolites in sesame seed oil.

2. Experimental

The seeds of sesame variety (TH-6) were collected from gene bank, Plant Genetic Resources Prog (PGRP) of National Agriculture Research Centre (NARC), Islamabad, Pakistan. Seeds were pretreated with sodium hypochlorite (0.5%) to remove any pathogenic spores. Seeds were germinated on wet filter paper and were then transferred to pots. Sandy loam type of soil with pH of 6.8 was filled in pots and about 15 seeds were placed initially in each pot. Watering and fertilizer requirements were fulfilled according to the crop requirements. Seeds were pre-treated with different concentration of SeNPs such as (T1; 10, T2; 20, T3; 30, T4; 40, T5; 50 and 5 mg/L (sodium selenite salt). The SeNPs applied at time of three different growth stages five leaf stage, flowering and fruiting stages. This study was conducted in 2022-2023 at PMAS, Arid Agriculture University, Rawalpindi, Pakistan.

2.1. Plant-mediated synthesis and characterization of SeNPs

Thirty grams of fresh leaf paste of *Allium sativum* was added to 300 mL of double-distilled water, and the mixture was heated at 180 °C for 45 minutes. The mixture was then filtered twice using Whatman filter paper to obtain a pure plant extract, which was stored at 4 °C.

To prepare a 1 mM precursor salt solution, 0.22 g of sodium selenite (Na_2SeO_3) was dissolved in 250 mL of double-distilled water. The steps involved in the plant-mediated synthesis of SeNPs are depicted in Fig. 1. The plant extract was added dropwise in salt solution, while maintaining the hotplate temperature at 170 °C. The colour of the precursor salt solution changed from colourless to pale yellow, eventually turning dark red brick after a certain interval. This colour change indicates the synthesis of phyto-fabricated SeNPs from the plant extract.

Centrifugation was performed at 10,000 rpm for 10 minutes, and the supernatant was removed to process the pellet formed at the bottom of the Falcon tubes. The pellet was then incubated at 40 °C while being shaken with 5 mL of a volatile organic solvent (methanol). The solution was placed in a petri dish and left to dry in an oven at 65 °C for twenty-four hours (Anu et al., 2020). Three different characterization techniques were used for the SeNPs, including scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and UV-Visible spectroscopy.

2.2. Quantification of phytochemical parameters

2.2.1. Determination of total flavonoid contents (TFC)

To determine the total flavonoid content in the plant leaves, the protocol outlined by Chang et al. (2002) was followed with minor modifications. First, 0.5 g of fresh sesame leaves were ground in 5 mL of phosphate buffer solution to prepare the sample for quantification. Then, 150 µL of CH_3CO_2K (1.0 M), 500 µL of double-distilled water, and 200 µL of $AlCl_3$ (10%) were mixed to create the solution mixture. For each tested sample, 300 µL of the plant extract was added to the solution mixture, and the absorbance was measured at 415 nm after incubating in the dark for one hour at ambient

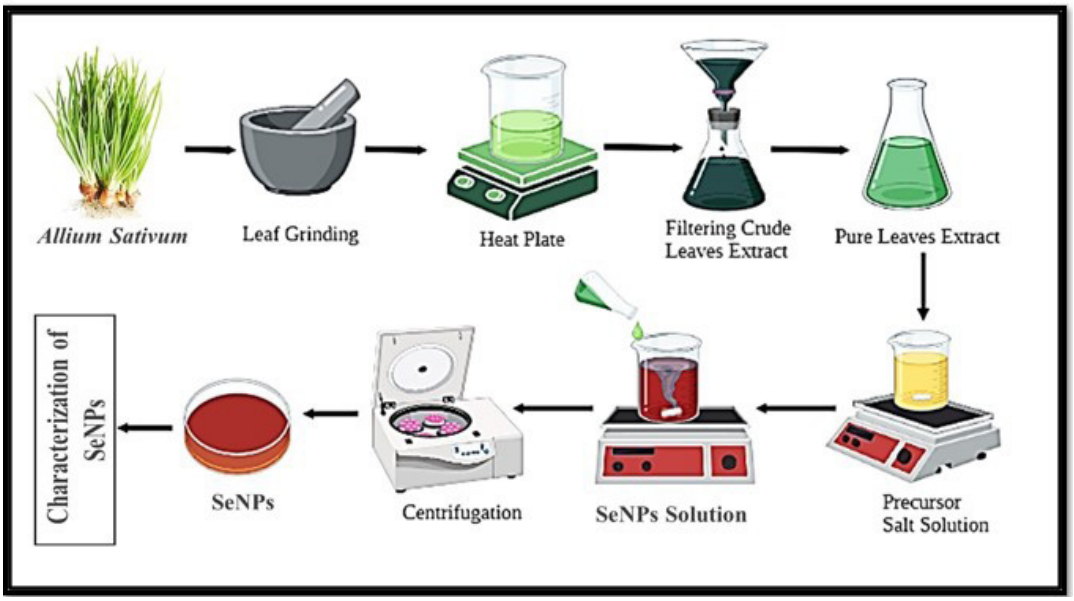

Fig. 1. Steps involved in the synthesis of plant-mediated selenium nanoparticles (SeNPs) using *Allium sativum* extract.

temperature.

2.2.2. Determination of total phenolic contents (TPC)

The evaluation of total phenolic content was conducted according to the standard method developed by Veilaogu et al. (1998). In this regard, 0.5 mL of Folin-Ciocalteu reagent was mixed with 100 µL of the plant extract. Subsequently, a resultant mixture was obtained by adding 750 µL of sodium carbonate solution (7.5%) and 100 µL of methanol. The mixture was incubated in the dark for ten minutes at 21°C. Absorbance readings were taken at 725 nm for all plant samples tested.

2.2.3. Determination of soluble protein content (SPC)

To determine the SPC in fresh sesame leaf extract, the methodology suggested by Parida et al. (2002) was employed. The plant extract was obtained by grinding 0.5 g of fresh leaves in phosphate buffer. According to this method, each test tube contained 500 µL of plant extract, 500 µL of distilled water, and 2 mL of Bio Red dye. The absorbance of all samples was measured at 650 nm using a Cecil 2021 spectrophotometer.

2.2.4. Determination of total soluble sugar content (TSSC)

The total soluble sugar content (TSSC) in the leaf extract of *Sesamum indicum* was quantified using the standard procedure of Dubois et al. (1956) with minor adjustments. Fresh leaves (2.0 g) were ground in 5 mL of ethanol (80%). The reaction mixture was then placed in a water bath at 80°C for 1 hour. Immediately afterward, 1 mL of phenolic solution was added to the reaction mixture, followed by the addition of 1.5 mL of sulfuric acid. The absorbance of the tested samples was finally measured at 490 nm using a spectrophotometer.

2.3. Soxhlet extraction

After sampling, seed pods were collected from the plant material and shade-dried to remove moisture. The seeds were then ground into a fine powder and homogenized using an electric grinder. The oil was extracted using the Soxhlet apparatus using *n*-hexane as the solvent. For the Soxhlet extraction, 240 mL of *n*-hexane was poured through a thimble containing 20 g of the fine sesame powder (Fig. 2 A&B). Extraction cycle was repeated for 8 hours at 120°C and the resulting mixture was subsequently evaporated in a water bath at 103 °C, followed by drying in an oven at 40°C for 24 hours. Resulting oil was stored at room temperature until GC-MS analysis was performed (Waheed et al., 2024).

2.3.1. GC-MS analysis

The phytoconstituents present in the *n*-hexane extract of sesame seed oil were identified using gas chromatography-mass spectrometry (GC-MS) analysis. The analysis was conducted with an Agilent Technologies 7000 GC-MS triple quadrupole (TQQQ) system at Fatima Jinnah Women University, Rawalpindi, Pakistan. The software utilized was Hunter Workstation, version B.04.00. The electron ionization potential during the analysis was set to 70 eV. For compound separation,

Fig. 2. A): Powder of sesame seed, **B)**: Sesame seed oil for GC-MS analysis.

an OPTIMA-5 column was used, with a temperature set at 360 °C and dimensions of 250 µm × 0.25 µm. Helium served as the carrier gas at a flow rate of 1.0 mL/min. A sample volume of 2.5 µL was injected using an automated liquid sampler. Various compounds were identified by comparing their mass spectra with literature data from the NIST (National Institute of Standards and Technology) database.

2.4. Statistical analysis

The randomized complete block design was used to conduct the experiment, and observations were made in triplicate. The Tukey's test was performed for the phytochemical analysis to evaluate significant differences between groups using Minitab 18.1 software. Additionally, TurboMass version 5.2 was used to interpret the mass spectra and chromatograms (Ahangari and Sargolzaei, 2012). The KEGG compound database was utilized to retrieve KEGG IDs, which were then subjected to MetaboAnalyst for metabolite set enrichment and pathway enrichment analysis.

3. Results and Discussion

3.1. Characterization of phyto-synthesized SeNPs

The UV-Visible spectrum of the synthesized SeNPs ranged between 200-300 nm, confirming their synthesis. The peak absorbance in the current study was observed at 279 nm, as shown in Fig. 3A. The size and shape of the SeNPs were determined using scanning electron microscopy (SEM), revealing irregular nanoparticles with an average size of 11.98 nm (Fig. 3B).

FTIR analysis demonstrated that functional groups present in the plant extract that are involved in the stabilization of the plant-mediated selenium nanoparticles, acting as capping and stabilizing agents. The wavelength and position of the specific functional groups ranged between 400-4000 nm. In this study, the amide and hydroxyl groups were found to be the most potent functional groups participating in the stabilization of the SeNPs, while the hydrocarbon group was considered the weakest among all (Fig. 3C).

FTIR analysis also provided information about the absorption peaks, peak shapes, appearances, and compound classes. The methyl and ether groups exhibited weak and broad peaks at 609.63 and 1028.16 cm^{-1}, respectively. In contrast, the amide, hydroxyl, and carboxylic groups showed strong and broad peak spectra at 1637.62 and 3419.90 cm^{-1}. These functional groups are present in the plant extract, which is abundant in metabolites that reduce the metallic salt into a stable zero-valent form.

Comparable results were reported by Anu et al. (2017) and Satgurunathan et al. (2017), who detected maximum absorbance in the range of 250-300 nm. Ahmad et al. (2023) revealed that SeNPs synthesized from garlic extract were spherical in shape, which differs from the irregular shape observed in the present study. Factors such as time, extract concentration, and pH can influence the size and shape of the synthesized SeNPs.

Husen and Siddiqi (2014) successfully synthesized selenium nanoballs from *Vitis vinifera* leaf extract, and their FTIR results showed sharp peaks at 3420 and 1620 cm^{-1} for the hydroxyl and alkyl groups, respectively. In contrast, the SeNPs synthesized from garlic powder extract in the current study exhibited broader and stronger peaks at 3419.93 and 1618.23 cm^{-1} for the hydroxyl and carboxylic groups. The differences in the results can be attributed to the use of different extracts for reducing the selenium salts.

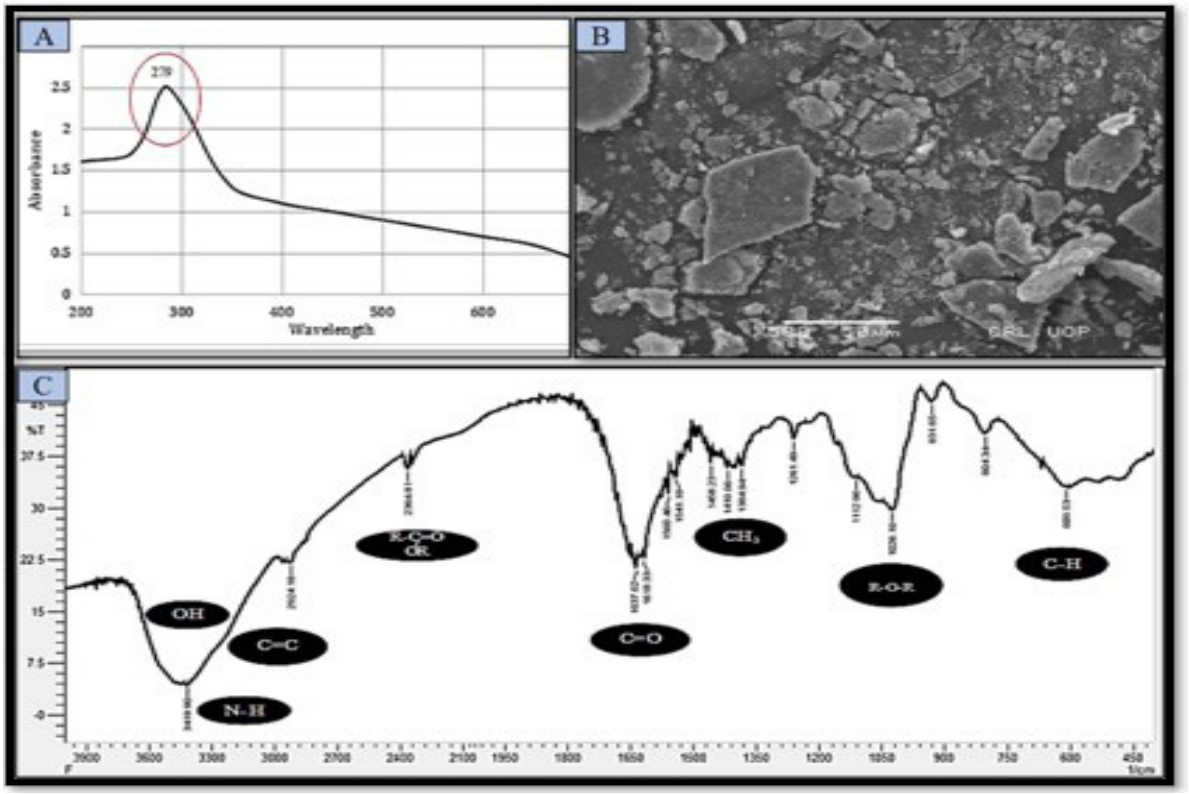

Fig. 3. The method employed for characterization of nanoparticles. **A:** UV-Visible absorption spectrum range, **B:** Scanning electron microscopy (SEM) determines structure morphology and size of nanoparticles, **C:** Fourier transform infrared (FTIR) applied for determination of numerous functional groups in nanoparticle sample synthesized from *Allium sativum*.

3.2. Quantitative phytochemical analysis

Plant biochemistry exhibited remarkable changes when treated with SeNPs. Flavonoids, which are structurally diverse secondary metabolites found in plants (Mohammadhosseini et al., 2019; Bailly, 2021; Mohammadhosseini et al., 2021; Moncayo et al., 2021), serve a variety of functions, including regulating plant growth, pigmentation, UV protection, facilitating defense and signaling activities between plants and microbes. The highest total flavonoid content (TFC) of 14.6 mg/g was recorded at a concentration of 40 mg/L of SeNPs, while the lowest TFC of 5.8 mg/g was observed at 5 mg/L of selenium salt. Notably, there was a 185% increase in TFC at 40 mg/L and an 18.0% decrease compared to the untreated control (Fig. 4A). Additionally, the plant exhibited a maximum increase in phenolic content of 14.2 mg/g when treated with 40 mg/L of SeNPs compared to the control (Fig. 4B), resulting in a percentage increase of 132%. Previous findings by Wang et al. (2023) revealed that applying various concentrations of ZnO nanoparticles (25-100 mg/L) induced early callus formation in *Ginkgo biloba*, with lower total flavonoid contents observed at 25 mg/L of ZnO nanoparticles. The current study shows similar findings to earlier research, despite both studies using different nanoparticles. Hernandez et al. (2019) demonstrated that flavonoid and phenolic concentrations increases when plants are exposed to stress conditions. These compounds act as vital antioxidants, helping plants cope with free radicals generated by the application of a nano-composite of SeNPs and copper nanoparticles (CuNPs). The amount of sugar in plants reflects the rate of photosynthesis, with excessive sugar indicating a high rate of photosynthesis. A pronounced increase of 86% in soluble sugar content was observed at 5 mg/L of selenium salt. Increasing the dosage of SeNPs resulted in an increase in TSSC), with maximum and minimum TSSC values of 82.4 and 57.6 mg/g at 50 mg/L and 30 mg/L, respectively. However, a 10.0% decrease in TSSC was observed at 40 mg/L, while an increase in TSSC levels was recorded at 50 mg/L (Fig. 4C). At 40 and 30 mg/L of SeNPs treatment, total protein contents of 6.2 and 6.1 mg/g were observed, respectively. A significant increase of 52% in total protein content (TPrC) was recorded at 30 and 40 mg/L of SeNPs. Higher doses may have negatively influenced the soluble protein content, as 5 mg/L of selenium salt exhibited the lowest protein content at 4.3 mg/g compared to the untreated control (Fig. 4D). Similar results were noted by Zeid et al. (2019), who reported significant increases in total soluble sugar and protein contents in cowpea plants.

3.3. GC-MS analysis of *Sesamum indicum* L. (sesame) seed oil

The GC-MS analysis of selenium-treated sesame seed oil revealed the presence of forty-seven

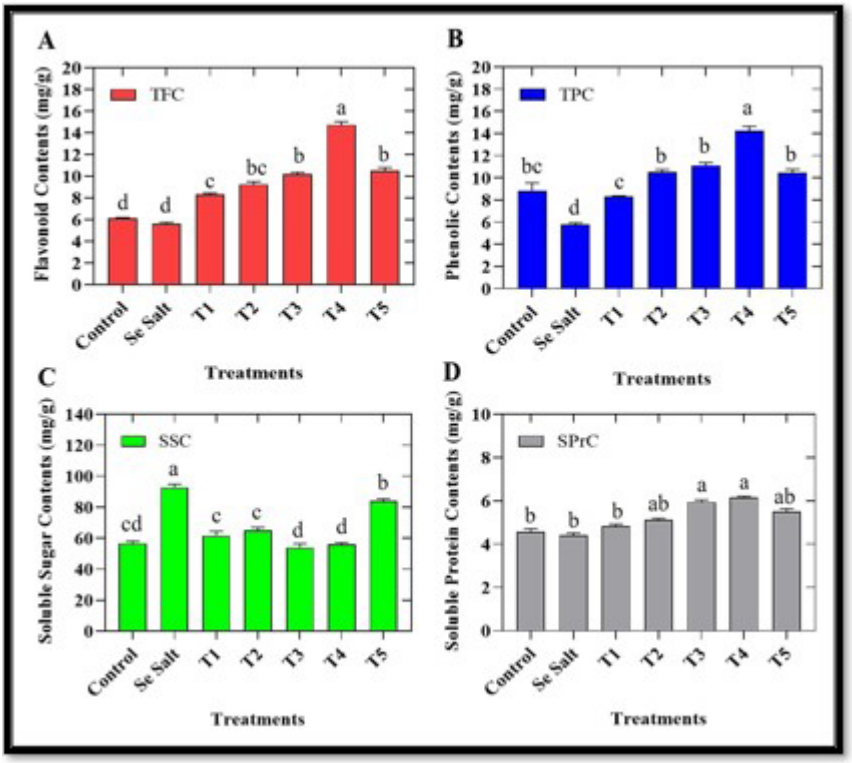

Fig. 4. Biochemical composition in sesame utilizing seed priming combine with foliar spray of SeNPs, **A:** Total flavonoid contents, **B:** Total phenolic contents, **C:** Soluble sugar contents, and **D:** Total protein contents.

different bioactive compounds, accounting for a cumulative percentage area of 89.2%. Among these compounds, oleic acid comprised 42.8%, followed by benzohydrazide (11.6%), 1,3-benzodioxole (10.0%), hexadecanoic acid (7.4%), octadecanoic acid (5.6%), γ-sitosterol (4.5%), and tetracosane (0.2%) as the most abundant constituents. Emerging research indicates that these phytoconstituents have diverse applications across various fields, exhibiting significant antibacterial, antifungal, antidiabetic, analgesic, anticancer, neuroprotective, anti-inflammatory, and anti-aging activities. These bioactive compounds are widely reported to be used in the cosmetics industry, as food additives, in drug discovery, as emulsifiers, and as fragrances in soaps and perfumes.

Fig. 5 demonstrates the presence of sixteen bioactive compounds that were exactly matched using the National Institute of Standards and Technology (NIST) MS Search 2.23 and AMDIS chromatograms. GC-MS chromatogram shows retention time for particular compound identified in sesame oil as depicted in Fig. 6. The present study reveals that these tentative compounds are characterized by high levels of fatty acids, with decreasing amounts of ketones, aldehydes, esters, and alcohols. Table 1 highlights the phytoconstituents identified through GC-MS analysis based on hydrocarbons, which are useful for calculating Kovats indices and retention index properties, along with the top five matching peaks using NIST MS Search 2.23.

The interpretation of the mass spectrum from the GC-MS analysis was conducted using the NIST database. In addition, the molecular masses of the compounds are provided in Table 1, and the detected compounds at specific retention times from the GC-MS chromatogram are illustrated in Fig. 7. The peaks represent possible fragment ion clusters interpreted through the MS interpreter, which dissociate from functional molecular ions when subjected to 70 eV. The fragment ions are stable isotopes with specific natural abundances, with the isotope of lowest mass being the most abundant for all elements reported in the literature.

Furthermore, the results indicate that 5-cholestene-3-ol, 24-methyl has the highest hydrocarbon (HC) number (28) with a Kovats retention index (KRI) of 2800, while cyclopentanol has the lowest HC number of five with a KRI of 500. Interestingly, no change was found in the retention index, and the estimated retention index for oleic acid was 2085 IU. The list of identified phytoconstituents in the sesame seed oil, along with their molecular masses, percentage areas, number of hydrocarbons, Kovats retention indices, and reported activities from the literature, is presented in Table 2.

The spectrum of unknown components was compared with the spectra of known components stored in the NIST library. Mass Hunter demonstrates that the peak spectra associated with these phytoconstituents differ significantly from those found in the NIST MS database

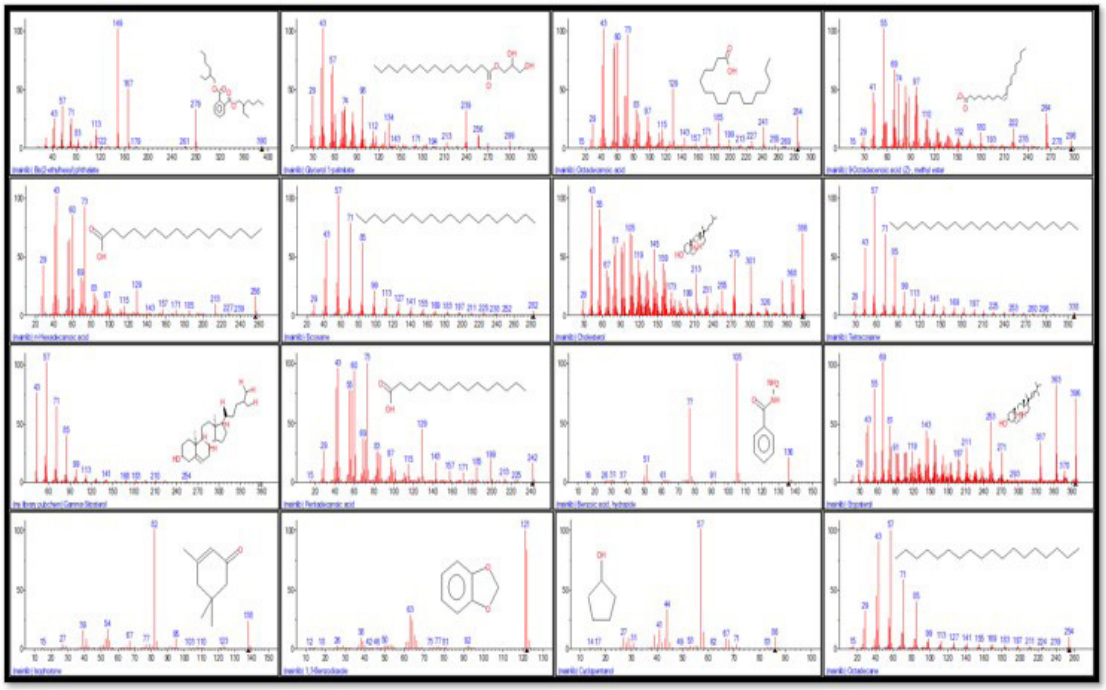

Fig. 5. Mass spectral patterns of the main constituents of of *Sesamum indicum* seed oil.

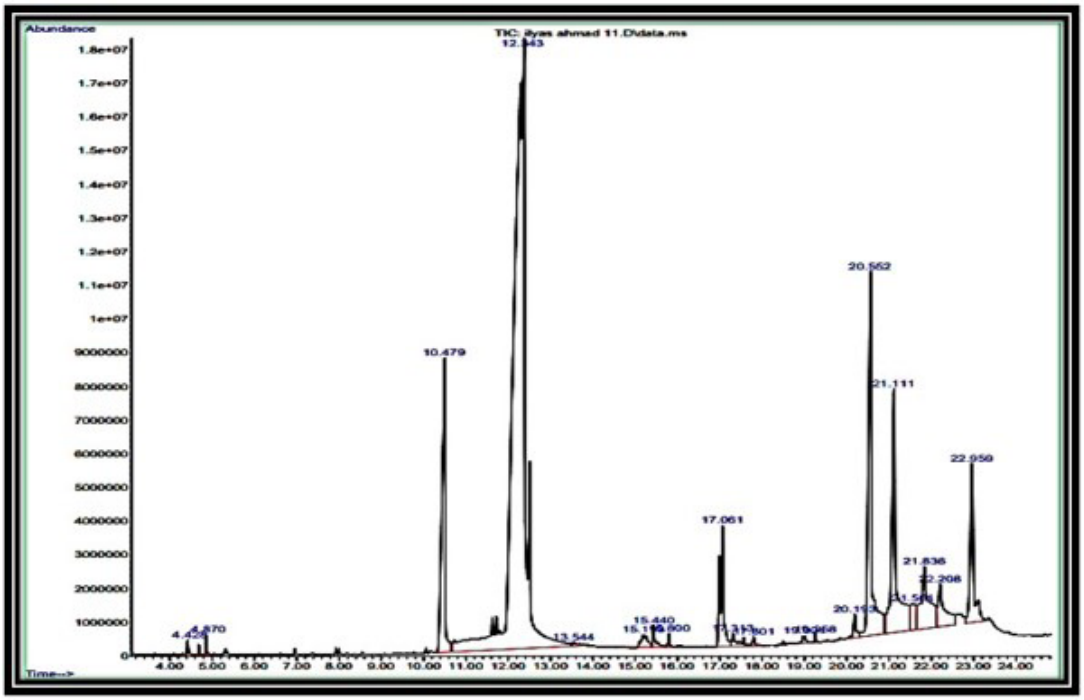

Fig. 6. GC-MS chromatogram of *Sesamum indicum* seed oil.

Table 1
Compounds with Kovats retention indices, percentage area, estimated (RI) using NIST database.

Compounds	MW	Exact mass	Area (%)	Retention index (iu)	Estimated RI	5 Largest fragments
Hexadecane	226	226.36	0.17	268.29	272.09	29, 43, 57, 71, 85
Octadecane	254	254.29	0.16	296.84	301.71	29, 43, 57, 71, 85
Eicosane	282	282.32	0.08	331.9	327.63	29, 57, 85, 113, 169
Tetracosane	338	338.39	0.16	366	382.35	29, 57, 71, 85, 155
Isoacetophorone	138	138.1	0.24	1111	1124.2	39, 54, 82, 95, 138
Benzohydrazide	136	136.06	11.59	1205	1205	16, 51, 77, 105, 136
Cyclopentanol	86	86.07	0.17	1323	1339	27, 29, 31, 41, 83
1,3-Benzodioxole	122	122.03	10	1530	1531	38, 65, 103, 121, 122
Pentadecanoic acid	242	242.22	0.12	1877	1890	29, 60, 73, 129, 200
Hexadecanoic acid	256	256.24	7.42	1975	1968	27, 43, 73, 129, 256
Oleic acid	296	296.27	42.75	2085	2102	55, 83, 97, 111, 264
Octadecanoic acid	284	284.27	5.62	2177	2177	43, 73, 129, 185, 284
γ-Sitosterol	358	358.32	4.53	2462	2462	43, 57, 71, 85, 154
Glycerol 1-palmitate	330	330.27	0.43	2482	2482	43, 74, 98, 134, 239
Phthalic acid	390	390.27	0.24	2539	2544	43, 57, 149, 167, 279
5-Cholestene-3-ol	386	386.35	2.76	3098	3098	43, 81, 145, 213, 255
5-Cholestene-3-ol, 24-methyl	396	396.33	2.76	3254.2	3254.2	43, 81, 145, 253, 337

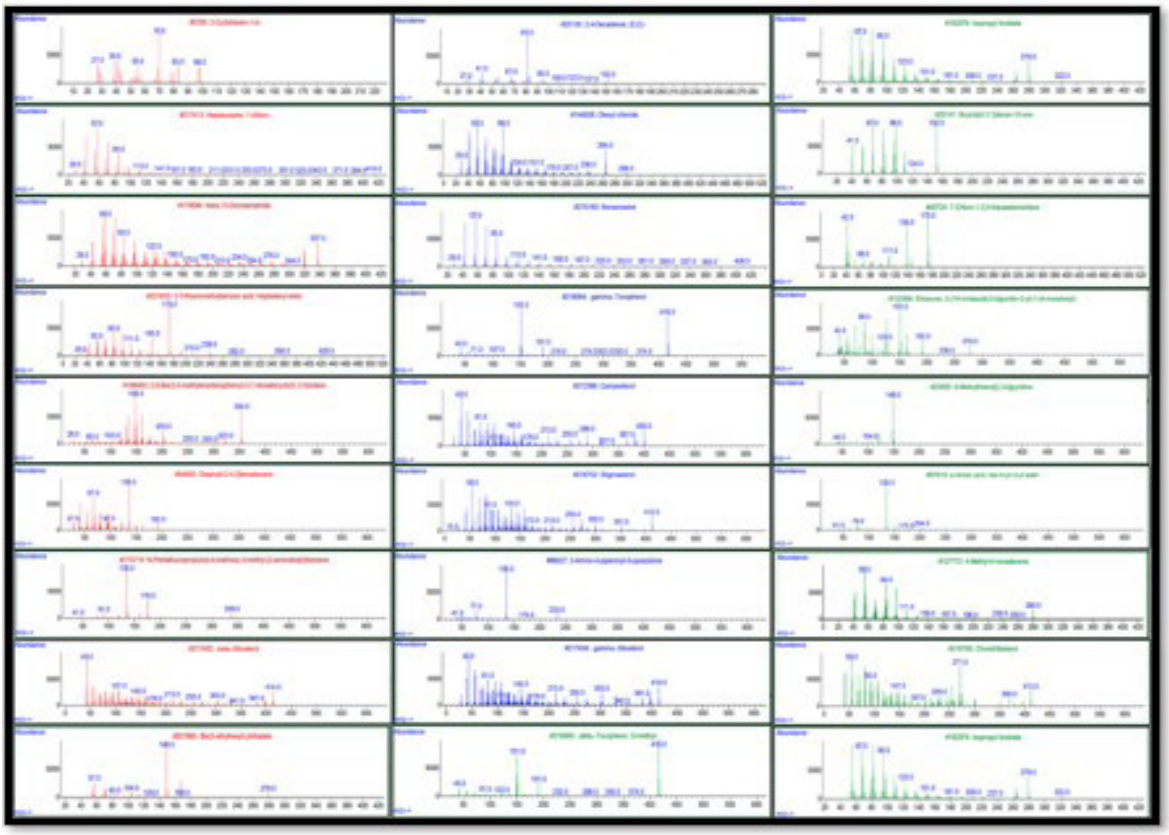

Fig. 7. Identification of chemical compounds characterized through Mass Hunter, showing the best hits for sesame seed oil.

Table 2
GC-MS analysis of *n*-hexane extract of sesame seed oil.

Compounds	Area (%)	Reported activities/Applications	(HCs)	Kovats retention indices (KRI)
2-Cyclohexen-1-ol	0.12	Used in synthetic fragrance, drug and personal good cares, flavoring agent	6	600
7-Chloro-1,3,5,triazaadamantane	0.3	To measure second harmonic generation (SHG) properties and piezoelectric effect	7	700
6-Methylthieno [2,3-b] pyridine	10	PI3K activity and antiviral activity	8	800
p-Anisic acid	9.23	Antiseptic properties	8	800
3-(Trifluoromethyl)benzoic acid	0.3	Highly potent Bcr-Abl kinase inhibitor	8	800
2,4-Decadienal	0.28	Agrochemical, drug discovery, natural food and flavor ingredients	10	1000
Ethanone, 2-(1H-imidazo(4,5-b)pyridin-2-yl)-1-(4-morpholyl)-	0.92	HSP inhibitor	12	1200
Dispiro [4.2.4.2] tetradecane	1.15	Used as a catalyst	14	1400
Palmitoyl chloride	0.43	Used in cosmetic industry	16	1600
Oleoyl chloride	0.3	Used in coating, polymer and adhesive purposes	18	1800
7,11-Hexadecadien-1-ol, acetate, (Z,E)-	3.87	To control pest attach (disrupt mating of cotton bollworm)	18	1800
Sesamolin	0.12	Antioxidant, neuroprotective and anticancer activities	20	2000
Bicyclo (4.3.1)decan-10-one	3.17	Extensively used in polymer additives and pesticides	20	2000
2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3,3,0)octane	10	Inhibit the metabolism of cholesterol	20	2000
4-Methyl-4-nonadecene	0.42	Antidiabetic and anticancer activities	20	2000
Isopropyl linoleate	0.61	Antioxidant scavenging activity	21	2100
trans-13-decosenamide	0.17	Antimicrobial, antinociceptive and anti-inflammatory activities	22	2200
Butyl linoleate	0.61	Reduce cholesterol and prevent heart diseases	22	2200
Erucamide	1.15	Used in plastic manufacturing industry	22	2200
Bis(2-ethylhexyl) phthalate	0.21	Uses in PVC preparation	24	2400
Heptacosane	0.16	Drug discovery	27	2700
δ-Tocopherol	0.52	Antidiabetic, anticancer and used to treat CVDS	27	2700
γ-Tocopherol	0.52	Anti-inflammatory and antiaging activities	28	2800
Campesterol	3.92	Used as emulsifier and stabilizer in food industry	28	2800
Nonacosane	0.11	Antioxidant and antibacterial activities	29	2900
Stigmasterol	2.29	Role in reduction of LDL cholesterol	29	2900
γ-Sitosterol monohydrate	5.17	Antidiabetic, and hypolipidemic agents	29	2900
Chondrillasterol	2.29	Effectiveness against leukemia and breast cancer	29	2900
β-Sitosterol acetate	4.53	Lowering cholesterol level, CVDs, Male pattern baldness	31	3100
1,3-Dioleoyl-2-lauroyl glycerol	0.16	Human milk fat substitute	51	5100

(2.23). The results indicate the presence of six amide compounds, including 6-methylthieno [2,3-b] pyridine, erucamide, bicyclo (4.3.1) decan-10-one, *trans*-13-decosenamide, 7-chloro-1,3,5-triazaadamantane, and ethanone. The bioactive compounds containing halo-groups (Cl, F, Br) include palmitoyl chloride, 7-chloro-1,3,5-triazaadamantane, oleoyl chloride, and 3-(trifluoromethyl)benzoic acid.

Kumari and Meenatchi (2017) reported the presence of twenty-one bioactive compounds through GC-MS analysis of *n*-hexane leaf extract from *Lantana camara*. When comparing the two findings, we discovered that the leaves contained fewer beneficial compounds than the seed oil. Recently, Tanta et al. (2024) performed an analysis of *Ficus articulata* and detected twenty-three phytoconstituents, with hexadecanoate being the most abundant component. Sesame seed oil predominantly comprises fatty acids, especially oleic acid, which accounts for 42.75% of the area. Yunus et al. (2021) identified the presence of amides, fatty acids, flavonoids, steroids, and phenolic derivatives in *Ficus articulata* using ^{1}H-NMR metabolomics approach and UHPLC-MS/MS analysis. Previous studies have established that phytosterols, such as sitosterol, are used as sunscreen emulsions and anti-aging additives in cosmetic products. The presence of unsaturated fatty acids lowers the risk of cardiovascular diseases and exhibits significant antidiabetic activity, according to Farhana et al. (2022). The presence of flavonoids also indicates antioxidant activity. The current investigation reveals the presence of phytosterols, flavonoids, and unsaturated fatty acids.

Waheed et al. (2019) performed GC-MS analysis on the seed oil of *Raphanus sativus* L., isolating thirteen different compounds. Comparing the outcomes for both Brassicaceae members indicates that sesame seed oil is richer in metabolites than radish. Similar studies by Hariram and Vasanthaseelan (2016) revealed that the GC-MS spectrum of *Brassica napus* L. (Canola) seed biodiesel isolated nine different compounds, which is fewer in number than those found in the present study. It is concluded that SeNPs may play a significant role in enriching the metabolites in sesame seed oil in the current study. In sesame seed oil, bis(2-ethylhexyl) phthalate was unexpectedly identified by GC-MS analysis. Bianco et al. (2014) found phthalates esters in plant materials sourced from plastic containers containing organic solvents used in the extraction process. Furthermore, Venditti (2018) presented a crescent number of studies emphasizing the identification of phthalate monomer as a naturally occurring component of plant extracts. According to Mouminah (2024), the amount of phthalates in food is affected by factors such as contact surface, temperature, storage time, and packing material content. The results of Abd-Allah et al. (2016) and Kirillov et al. (2015) are comparable to the current findings in context of the presence of phthalates in plant extracts. According to Mouminah (2024), the amount of phthalates in food is affected by factors such as contact surface, temperature, storage time, and packing material content.

3.4. Kyoto encyclopaedia of gene and genomics (KEGG) pathway analysis

The compounds isolated from the GC-MS analysis spectrum were analyzed for enrichment pathways using MetaboAnalyst (Version 6.0). The HMDB IDs were retrieved and further processed through KEGG pathway analysis, resulting in enrichments of metabolite sets, as depicted in Fig. 7. The enrichment ratio was calculated using the following formula.

Hits/expected ratio = Observed hits/Expected hits (Eqn. 1)

The results indicated that the metabolites play a major role in eight different enriched metabolic pathways, including pyruvate metabolism, glycolysis/gluconeogenesis, glycerophospholipid metabolism, biosynthesis of unsaturated fatty acids (USFA), fatty acid elongation/degradation, steroid biosynthesis, and fatty acid biosynthesis, as depicted in Fig. 8A. Based on the metabolites, the pathway impact analysis shows that larger bubble structures correspond to more significant results (Fig. 8B). Acetaldehyde was identified in both glycolysis and pyruvate metabolism, with a *p*-value of less than 0.05. The false discovery rate (FDR) recorded was 0.889, with a *p*-value of 0.044 for pyruvate metabolism. Moreover, a lower *p*-value indicates greater confidence in the results. Glycolysis also showed significant results, with an FDR value of 0.889 and a *p*-value of less than 0.05 (Fig. 8C).

The rounded bubble for pyruvate metabolism, located at 1.4 -log10 (*p*-value), has the highest enrichment factor of 1, followed by another bubble for glycolysis/gluconeogenesis at 1.3 -log10 (*p*-value) with the second highest enrichment factor of 2. Fig. 8D illustrates the evidence of interactions, strength, modes of action, and binding affinities among the metabolites present in the network pathway. The protein-chemical compound edges exist between palmitate, elaidic acid, and pentadecanoic acid, exhibiting the highest binding affinity. A total of six nodes were created using *Arabidopsis thaliana* as a model. The highest binding affinity is found at the protein-chemical edge between palmitate, elaidic acid, and pentadecanoic acid. Based on KEGG pathway enrichment analysis. Peng et al. (2021) identified nine distinct pathways for metabolite enrichment in soybean varieties. Both studies found that soybean and sesame had opposing metabolite enrichment sets, although both oilseed crops exhibited some similarities.

Pyruvate is an essential step in carbohydrate metabolism in plants. It serves as a crucial intermediate metabolite during seed germination in oil plants, playing a role in converting lipids to sugars via β-oxidation and gluconeogenesis. Enrichment in this pathway suggests that these metabolites are not only important for plants during seed germination but also indicate that these germinated seeds can be utilized for various human health applications where pyruvate or its intermediates are required (Sattler et al., 2004). It is important to note that SeNPs resulted in an enrichment of metabolites related to pyruvate metabolism, suggesting that the

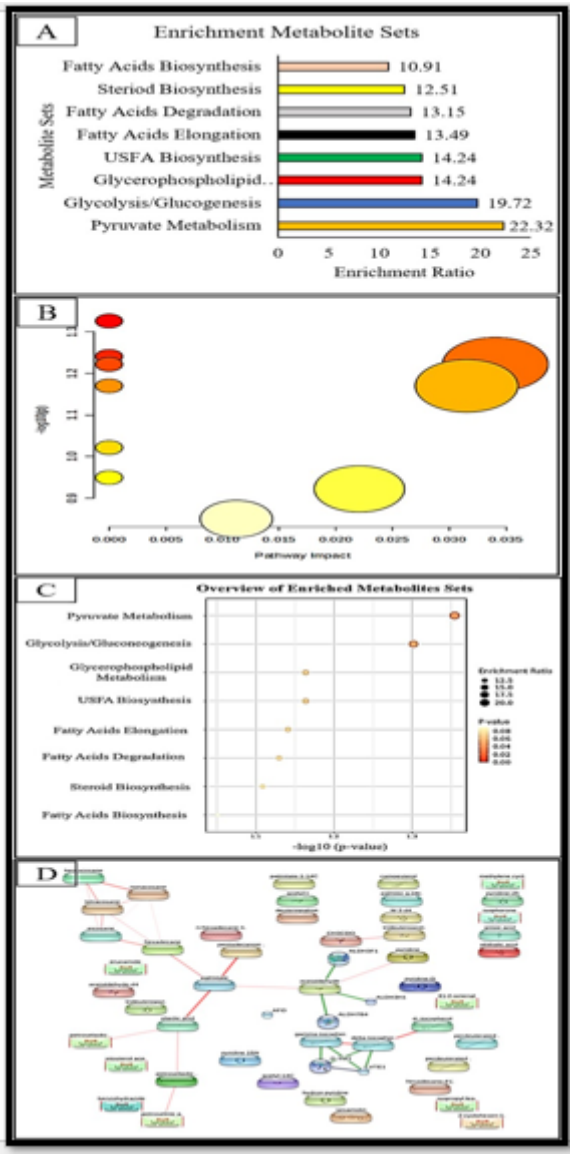

Fig. 8. Metabolites enrichment analysis of *Sesamum indicum* L. seed oil, **7A:** Enrichment metabolites set of sesame seed oil, **7B:** Overview of pathway analysis, **7C:** Enrichment ratio on basis of *p*-value and size, **7D:** kegg metabolomic pathway showing the mapped 35 metabolites obtained from sesame seed oil through metabolomic approaches.

energy conversion pathway is enhanced in plants treated with SeNPs, leading to better distribution, storage, and conversion of photosynthetic products into essential secondary metabolites.

Furthermore, since sesame contains a high amount of oil, the pathways for fatty acid synthesis and elongation were also upregulated. Sesame seeds are primarily enriched with oleic and linoleic acids, and these simple compounds play significant roles in plants while yielding high-quality, health-promoting edible oil for human consumption (He et al., 2020). It is also noteworthy that regulating fatty acid and lipid metabolism at the genetic level is crucial for plants. The enrichment of metabolites in these pathways suggests and predicts a high oil content in plants, which will ultimately be beneficial for human use. While increasing oilseed production through breeding and other methods is useful, it is also time-consuming. Moreover, it is essential to have a foundational understanding of the genes responsible for the synthesis and regulation of fatty acid biosynthesis (Bu et al., 2023).

Enrichment in unsaturated fatty acid biosynthesis is another important consideration, as these carboxylic acids with one or more double bonds are fundamental to plants. These compounds are closely linked to both biotic and abiotic stressors and perform a variety of vital functions. In addition to serving as carbon and energy reserves in triacylglycerols (TAGs) and as membrane components and modulators in glycerolipids, C18 unsaturated fatty acids (UFAs) also function as intrinsic antioxidants, precursors to various bioactive molecules (often including the stress hormone jasmonic acid (JA), and components of the extracellular barrier, such as cutin and suberin (Ohlrogge and Browse, 1995; Harwood, 1996; He et al., 2018). These unsaturated fatty acids are also essential for humans, as they cannot biosynthesize the PUFAs 18:2 and 18:3, making them dietary necessities. Furthermore, these organic compounds serve as raw materials for various products, including biofuels, cosmetics, detergents, and pharmaceuticals.

In Fig. 8D, the red lines represent interactions between chemical compounds, blue lines indicate protein-protein interactions, and green lines denote protein-chemical interactions. The proteins associated with these metabolites include ALDH3H1 (aldehyde dehydrogenase 3H1), ALDH7B4 (aldehyde dehydrogenase 7B4), and ALDH3F1 (aldehyde dehydrogenase 3F1). γ-Tocopherol and δ-tocopherol show interactions with G-TMT (tocopherol *O*-methyltransferase) and VTE1 (tocopherol cyclase).

4. Concluding remarks

Sesame seeds are cultivated worldwide due to their high nutritional content and numerous health benefits. The nutritional and flavor profiles of the seeds were enhanced using advanced nanotechnology methods. This study aimed to explore the impact of SeNPs on the biochemistry and seed oil profiling of sesame (*Sesamum indicum* L.). The sesame plants were treated foliarly with

various dosages, including T1 (10 mg/L), T2 (20 mg/L), T3 (30 mg/L), T4 (40 mg/L), T5 (50 mg/L of SeNPs), and 5 mg/L of selenium salt. The UV-Visible spectrum revealed a peak absorption spectrum at 279 nm for SeNPs. The FTIR analysis demonstrated the reduction of sodium selenite salt to SeNPs using *Allium sativum* extract through a biological approach. The functional groups identified included hydroxyl, carboxylic, amide, methyl, and ether groups. Biochemical parameters such as TFC, TPC, TSSC, and total protein content (TPrC) were also measured. The treatment with 40 mg/L resulted in the highest TFC and TPC at 14.72 and 14.24 mg/g, respectively, compared to the control. The lowest phenolic content of 5.8 mg/g was recorded for selenium salt at 5 mg/L, representing increases of 185% and 132%, respectively. A rise in soluble sugar content and TPrC of 92.78 and 6.15 mg/g was noted at 5 and 40 mg/L of sodium selenite and SeNPs application. The results indicated a 10% reduction in TSSC at 40 mg/L, while a substantial 52% increase in TPrC was observed at 30 and 40 mg/L of SeNPs, respectively. GC-MS analysis of sesame seed oil identified a total of thirty-five different chemical compounds at specific retention times (RT). The analysis indicated that SeNPs were beneficial in enhancing metabolite enrichment. KEGG pathway analysis revealed metabolite enrichment in eight different pathways, with pyruvate metabolism showing the lowest *p*-value of 0.044 and an FDR of 0.889.

Overall, the findings suggest that SeNPs significantly enhanced the sesame seed oil profile, leading to a greater number of metabolite detections. SeNPs positively affect plant biochemistry, ultimately increasing crop yield. The increase in flavonoid and phenolic content may help the plant cope with stress conditions.

Author contribution statement

Conceptualization and literature search was performed by Zia-ur-Rehman Mashwani and Zohaib Younas. The first draft of manuscript, data curation, review and editing were handled by Zohaib Younas and Ilyas Ahmad. Methodology was developed by Ilyas Ahmad. Formal analysis was carried by Tayyaba Yousaf and Naveed Iqbal Raja. The investigation was performed by Tayyaba Yousaf, Naveed Iqbal Raja and Ilyas Ahmad. Supervision was provided by Zia-ur-Rehman Mashwani and Naveed Iqbal Raja. All authors read and approved the final manuscript.

Abbreviations

ALDH3F1: Aldehyde Dehydrogenase 3F1; **ALDH3H1:** Aldehyde Dehydrogenase 3H1; **ALDH7B4:** Aldehyde Dehydrogenase 7B4; **CuNPs:** copper nanoparticles; **DEGs:** Differentially Expressed Genes; **FDR:** False Discovery Rate; **FTIR:** Fourier-Transform Infrared; **GC-MS:** Gas Chromatography-Mass Spectrometry; **G-TMT:** Tocopherol *O*-Methyltransferase; **HC:** Highest Hydrocarbon; **KRI:** Kovats Retention Index; **NARC:** National Agriculture Research Centre; **NIST:** National Institute of Standards and Technology; **PGRP:** Plant Genetic Resources Prog; **RT:** Retention Times; **SEM:** Scanning Electron Microscopy; **SeNPs:** Selenium Nanoparticles; **TAGs:** Triacylglycerols; **TFC:** Total Flavonoid Content; **TPC:** Total Phenolic Content; **TPrC:** Total Protein Content; **TQQQ:** Triple Quadrupole; **TSSC:** Total Soluble Sugar Content; **USFA:** Unsaturated Fatty Acids; **VTE1:** Tocopherol Cyclase.

Conflict of interest

The authors declare that there is no conflict of interest.

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