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Phytomediated Selenium Nanoparticles Improved Physio-morphological, Antioxidant, and Oil Bioactive Compounds of Sesame under Induced Biotic Stress

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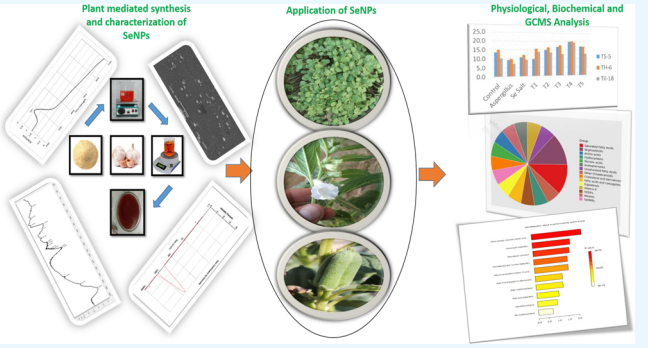
ABSTRACT: Vegetable oil consumption is expected to reach almost 200 billion kilograms by 2030 in the world and almost 2.97 million tons in Pakistan. A large quantity of edible oil is imported annually from other countries to fill the gap between local production and consumption. Compared to other edible oil crops such as soybean, rapeseed, peanut and olive, sesame has innately higher (55%) oil content, which makes it an excellent candidate to be considered to meet local edible oil production. Oil seed crops, especially sesame, are affected by various pathogens, which results in decreased oil production with low quality oil. Selenium nanoparticles (SeNPs) work synergistically, as it has antifungal activity along with improving plant growth. Different concentrations of SeNPs were used, on three different varieties of sesame (TS-5, TH-6, and Till-18). Plant growth and development were accelerated by SeNPs, which ultimately led to an increase in crop yield. Morphological parameters revealed that SeNPs resulted in a growth increase of 55.7% in root length, 48% increase in leaf number/plant, and 38% in stem diameter. Out of three sesame varieties, TS-5 seedlings treated with 40 mg/L SeNPs showed 96.7% germination and 53% SVI at 40 mg/L. Sesame varieties dramatically increased antioxidant capability using SeNPs, resulting in 147% increase in SOD and 140% increase in POD enzyme units in TH-6 and 76% elevation in CAT enzymes in TS-5 (mean ± S.E). GCMS analysis revealed that bioactive compound I, sesamin, sesamol, and tocopherol contents were increased along with enhanced production of different unsaturated fatty acids. Kegg pathway analysis and MSEA revealed that these compounds were mainly involved in biosynthesis of unsaturated fatty acids, suggesting that SeNPs have elicited the biosynthesis of unsaturated fatty acids such as oleic acid, linoleic acid, and α-linoleic acid. This study concluded that SeNPs (40 mg/L) have an excellent capability to be used for crop improvement along with better oil quality.



1. INTRODUCTION

Growing needs of an increasing population are pushing the country toward food insecurity, which calls for increasing agriculture products on war-footing, especially oil-seed crops. Pakistan is managing to produce only 11% of edible oil of its requirements, while the rest of the 89% is imported by spending revenue of about US$ 3.419 billion. This higher dependency on oil seed imports not only puts pressure on revenue but also is the major reason of hike of edible oil prices in Pakistan.[1]

Sesamum indicum which is commonly called sesame or "Till" or benne belongs to the group of flowering plant family Pedaliaceae. Sesame is considered as one of the oldest oil seed crops whose cultivation dates back to Babylonian civilization.[2] Sesame has a very good phytochemical profile by having about 50−60% oil, 17−32% proteins, and 14−25% of carbohydrates.[3] Sesame oil is rich in diunsaturated and triunsaturated glycerides, which account for 58 and 36%, respectively, of the total fatty acid content. The lignans (sesamin, sesamol, etc.) add pharmaceutical properties to its oil.[3] The γ-tocopherol is the most abundant tocopherol in its oil.[4] Furthermore, sesame oil has antioxidant properties which prevent oxidative decay and improve oil storage quality.[5]

Plants are always subjected to a variety of biotic and abiotic stresses. Losses from biotic stress likely exceed those from all other factors combined. As like all other crops, sesame is also greatly affected by various pathogens, which not only limits the yield but also changes the oil quality of sesame. One of the major pathogens of the sesame is *Aspergillus* sp. which by

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Table 1. Layout of the Present Study: There Were Three Different Application Methods, Namely, Seed Pretreatment Only, Foliar Spray Only, and Seed Pretreatment + Foliar Spray[a]

Treatments			TS-5			TH-6			Till-18		
			R1	R2	R3	R1	R2	R3	R1	R2	R3
To	Control	Seed Treatment									
T+	Only Aspergillus										
T-	Aspergillus+Se Salt										
T1	Aspergillus+ 10ppm SeNPs										
T2	Aspergillus+ 20ppm SeNPs										
T3	Aspergillus+ 30ppm SeNPs										
T4	Aspergillus+ 40ppm SeNPs										
T5	Aspergillus+ 50ppm SeNPs										
T-	Aspergillus+Se Salt	Foliar									
T1	Aspergillus+ 10ppm SeNPs										
T2	Aspergillus+ 20ppm SeNPs										
T3	Aspergillus+ 30ppm SeNPs										
T4	Aspergillus+ 40ppm SeNPs										
T5	Aspergillus+ 50ppm SeNPs										
T-	Aspergillus+Se Salt	Seed+ Foliar									
T1	Aspergillus+ 10ppm SeNPs										
T2	Aspergillus+ 20ppm SeNPs										
T3	Aspergillus+ 30ppm SeNPs										
T4	Aspergillus+ 40ppm SeNPs										
T5	Aspergillus+ 50ppm SeNPs										

[a]Foliar spray was carried out at three different stages of the plant life cycle, that is, at five-leaf stage, flowering stage, and Fruit setting stage. All the treatments were performed in triplicates.

affecting sesame plants reduces crop yield and oil quality along with producing carcinogens such as afaltoxins.[6,7] There are many chemically prepared commercial fungicides and pesticides available in the market, but these chemicals are not safe to crops, and they have well-documented toxicity to humans. We can now benefit from advanced technologies, such as nanotechnology, which deals with particles smaller than 100 nanometers.[8] Nanomaterials have many potential applications in agriculture to cope with biotic stress, enhance crop productivity, and improve its physio-biochemical and oil qualities. This essential micronutrient is the only one metalloid which is incorporated in certain proteins which are called seleno-proteins, and also, it makes the 21st amino acid called "selenocysteine".[9] As the use of selenium itself poses various problems due to its bulk size and solubility in water, selenium nanoparticles (SeNPs) come into action, which can resolve both these problems. As these SeNPs are less than 100 nm in size, they are more potent and can penetrate the cellular and sub-cellular membrane with better interaction with seleno-proteins. Due to all these advantages, SeNPs are now being used in a wide variety of agricultural applications, that is, to cope with both biotic and abiotic stresses and to make biofortified crops.[10−12] As SeNPs have anti-fungal activity along with their potential to enhance the crop yield and their phytochemical constituent, this study was conducted to check the effect of plant-based SeNPs on sesame growth and yield under biotic stress. To the best of our knowledge, this is the first ever report on elucidating the effect of plant-mediated nanoparticles on sesame with extensive morphological, physiological, antioxidant, and metabolic profiles of *S. indicum*. As SeNPs have both antifungal and growth improvement attributes, it was hypothesized that plant-mediated SeNPs may have the ability to improve growth biomarkers and oil bioactive compounds with improved oil yield in sesame under induced biotic stress.

2. MATERIALS AND METHODS

2.1. Phytofabrication of SeNPs. About 25 g of garlic (*Allium sativum*) powder was added to 250 mL of water and heated at 220 °C for 25 min. The extract was filtered thrice and stored at 4 °C for the synthesis of SeNPs. About 0.43 g of selenium salt (sodium selenite) was dissolved in 500 mL of double-distilled water to make a 1 mM salt solution. The salt solution was placed on a magnetic stirrer for continuous stirring at 150 °C for 5 h. The solution was further kept in the dark for 4 days, and the color change from colorless to brick red confirmed the synthesis of SeNPs. The resulting solution was centrifuged at 13,000 rpm for 20 min, the supernatant was removed, and the pellets were washed with 10 mL of methanol. SeNPs were placed in Petri plates and dried in an oven at 40 °C for 48 h. These plant-mediated SeNPs were used for further study.[13]

2.2. Characterization of *A. sativum*-Mediated SeNPs. Characterization of phytomediated SeNPs was carried out using different instrumentation techniques. The color change from colorless to brick red confirmed the synthesis of SeNPs. Furthermore, it was confirmed by UV−visible spectroscopy (Uv-752pc spectrophotometer), by recording the spectrum and absorption at 200−800 nm[14] (37 in nanomaterial). Scanning electron microscopy (SEM) was carried out using model JSM5910, JEOl, JAPAN with an energy of 30 KV with a magnification of 2.3 nm to 30,000× as the minimum and maximum, respectively. A small amount of sample was placed on a carbon-coated copper grid, and the obtained films were then air-dried. Micrographs at different magnifications were obtained[15] Fourier transform infrared (FTIR) is an important instrumentation technique which reveals which of the functional groups from the plant extract are involved in phytomediated synthesis of SeNPs. Dried SeNP powder was pelleted with potassium bromide, and the FTIR spectrum (Perkin-Elmer FTIR-Spectrum, Akron, OH, USA) was obtained as a wavenumber in the range of 500−4000 cm^{-1}. Dynamic light scattering (DLS) was employed for zeta potential and particle size analysis for phytomediated SeNPs using the Malvern Zetasizer nanosizer (size range 0.1−10,000 nm).

2.3. Seed Collection and Experimental Layout. Seeds of three sesame varieties, that is, TS-5, TH-6, and Till-18, were

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ACS Omega 2023, 8, 3354−3366

collected from National Agriculture Research Center Islamabad (NARC). Seeds were surface-sterilized with 0.5% sodium hypochlorite for 20 min and washed repeatedly with sterile distilled water. An inoculum of *Aspergillus flavus* was obtained from Mycology lab of PMAS-Arid Agriculture University, Rawalpindi, and was subcultured on potato dextrose agar (PDA) at 28 °C ± 2 for 5 days. Spore solution of *A. flavus* was made with a spore count of 2×10^6, and the seeds were soaked in spore solution for 2 h. Different nanoformulations (10, 20, 30, 40, and 50 ppm) of plant-based SeNPs were made, and three different treatment methods, namely, seed pretreatment only, foliar spray, and seed pretreatment + foliar spray, were used. Apart from these nanoparticle treatments, three other groups were also made in which one group got only *Aspergillus* pretreatment, second group received *Aspergillus* + selenium salt, and third was placed as the control. Pot experiment was performed, and all the treatments were performed in triplicates. Plastic pots of 12 × 15 inches (length × width) were used which were filled with well-drained, sandy loam soil with a pH of 7.6. The first group of seed pretreatment was treated with different concentrations of SeNPs for 2 h. The second and third groups (foliar only and seed + foliar) were sprayed with SeNPs on three different growth stages, that is, at five-leaf stage, flowering stage, and fruit setting stage. Each pot was sprayed with about 200 mL of SeNP solution with spray bottles.[16] Crops were sown on July 18, 2021 and were harvested between 94 and 125 days of sowing depending upon the ripening of the pods. Watering was carried out at alternate days, while no fertilizer was applied during the study (Table 1).

2.4. Germination Parameters. Germination experiment was performed under controlled conditions. Petri plates were sterilized in an autoclave at 121 °C for 15 min. About 10 surface sterilized seeds were taken and kept in Petri plates containing three layers of filter paper wet with Milli-Q water. Petri plates were placed under dark conditions at 4 °C for two (02) days and then transferred to the growth chamber where illuminating light was adjusted to 200 μmol m^{-2} s^{-1}, 16 h light period per day, and the temperature was set at 22 °C. After 4 days of germination, seedlings were treated with foliar spray of SeNPs (only foliar and seed + foliar treatments) with a concentration of 10−50 ppm.

2.5. Estimation of Growth Biomarkers. After 90 days of sowing, growth parameters of SeNP-treated plants were recorded. The length of plant organs, that is, root and shoot length, was measured using the meter scale. Plant fresh weight was recorded at the time of harvesting, while dry weight was calculated after placing the plant in a hot air oven at 65 °C for 8 h. Also, the number of leaves was calculated at the time of maturity.

2.6. Estimation of Leaf Area and Chlorophyll Content. Leaf area was determined with the help of a leaf area meter. Three plants were randomly selected from each treatment, and leaf area was noted in cm^2. Chlorophyll content was measured using the method mentioned in ref 17 with slight modification. Leaf tissue was carefully weighed and homogenized in 80% chilled acetone. The resulting solution was filtered twice, and absorbance was noted at three different wavelengths, that is, 633, 645, and 663 nm, on the spectrophotometer. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll were calculated with the help of the following formula

$$\text{Chlorophyll a} = 12.7 \times \text{A663} - 2.7 \times \text{A645}$$

$$\text{Chlorophyll b} = 22.9 \times \text{A645} - 4.7 \times \text{A633}$$

$$\text{Total Chlorophyll} = 22.9 \times \text{A645} - 4.7 \times \text{A633}$$

2.7. Total Proline Content. Proline content was determined by the method described by ref 18. About 0.2 g of leaf tissue was crumbled in 10 mL of sulfosalicylic acid, filtered through Whatman filter paper no.1, and centrifuged at 10,000 rpm for 10 min. In a test tube, 2 mL of the filtrate was mixed with 2 mL of glacial acetic acid along with 2 mL of ninhydrin and incubated at 100 °C on the water bath. After 1 h, the test tubes were placed in a desiccator, and 4 mL of toluene was added to each test tube, mixed well by placing on the vortex for 5 min. From the two layers of solution, the upper translucent layer was separated, and absorbance at 520 nm was determined. The following formula was used to calculate proline content.

$$\text{Proline content } (\mu\text{g/mL}) = \frac{\text{Absorbance reading} \times K \text{ value} \times \text{Dilution factor}}{\text{Weight of fresh tissue}}$$

2.8. Estimation of Antioxidant Enzyme Activity. Fresh leaf tissue was ground with liquid nitrogen and homogenized with the prechilled pestle mortar, and the powered material was transferred to a prechilled tube and kept at −80 °C for further use.

2.8.1. Catalase Activity (CAT). For determination of CAT activity, the method mentioned in ref 19 was used. The activity was measured by disappearance of H_2O_2. The reaction mixture contained 20 μL of the enzyme extract, 50 mM potassium phosphate buffer (pH 6.8), 1 mM EDTA, and 15 mM H_2O_2. The activity of CAT was measured using the decrease in H_2O_2 in 1 min at an absorbance of 240 nm. CAT activity was measured as per its extinction coefficient of 6.93 mM^{-1} cm^{-1}.

2.8.2. Peroxidase (POD). POD activity was determined using methods mentioned in ref 20 with little modification. The 1 mL reaction mixture contained 5 mM H_2O_2, 15 mM guaiacol, and 40 mM phosphate buffer (pH 6.8). The reaction mixture was kept for some time, and then, the reaction was started by adding H_2O_2, and the increase in absorbance at 470 nm was noted for 1 min. Measurement of POD activity was carried out as per its extinction coefficient of 25 mM^{-1} cm^{-1}.

2.8.3. Superoxide Dismutase (SOD). Superoxide dismutase was determined by the method mentioned in ref 20. Ten milliliters of prechilled sodium phosphate buffer was used to grind 0.5 g of the leaf material followed by centrifugation for 15 min at 15,000 rpm and 40 °C. On settling down of solution, a separate set of test tubes containing 0.1 mL of the extract was supplemented with 0.1 mL of riboflavin and 3 mL of SOD buffer. This reaction mixture was placed under the fluorescent lamp for 8 min to start the reaction. The same reaction mixture was prepared for dark reaction in another set of test tubes. The absorbance of both sets was recorded at 560 nm wavelength.

2.9. Oil Extraction and GCMS Analysis. About 50 gm of each seed sample was put into the porous thimble and placed in a Soxhlet extractor. About 170 mL of *n*-hexane was used as the solvent, with a boiling point of 50−60 °C. The oil extraction process was repeated continuously for 8 h which yielded the required amount of oil. The obtained oil sample was then evaporated using the rotary evaporator at 70 °C to remove the excess amount of solvent in the oil sample. The resulting oil sample was then stored in a refrigerator for GCMS analysis.[21]

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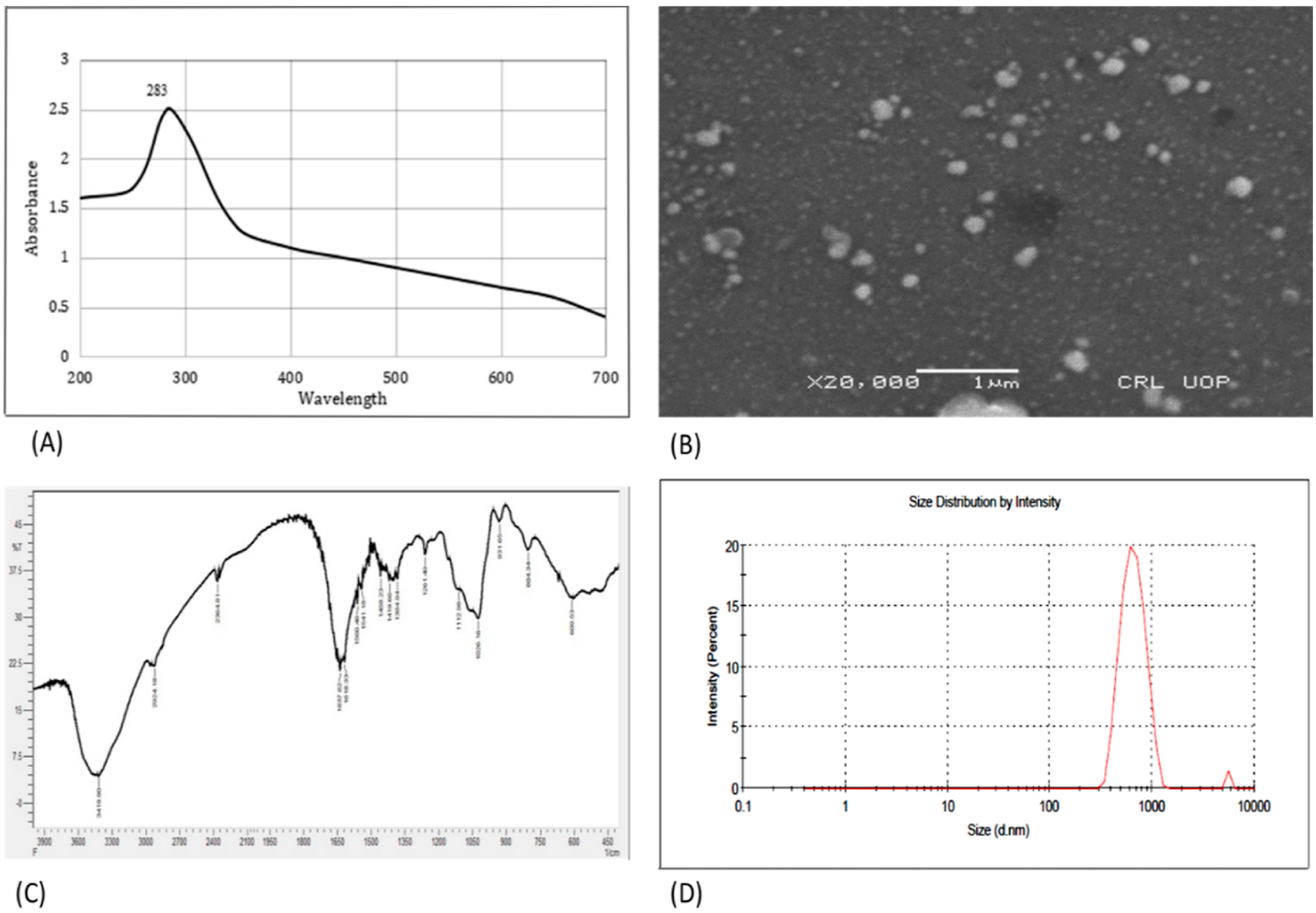

Figure 1. Characterization of plant-based SeNPs, (A) UV−visible spectrum of phytomediated SeNPs, (B) SEM spectrum of plant-based SeNPs, (C) FTIR spectrum of plant-based synthesized SeNPs, and (D) size distribution of SeNPs.

GCMS analysis was carried out at Centralized Resource Laboratory, University of Peshawar, Pakistan. Clarus 500 model, PerkinElmer, was used for analysis which was equipped with FID, TCD, FPD, and ECD. Helium gas was used as carrier gas, while the temperature was set at 70 °C. The peaks obtained were subjected to ChemStation and compound database NIST11.L to identify the compounds in the oil sample. Further confirmation of compounds was carried out by comparing the retention time and molecular weight of the compounds with already present data in the literature.

2.10. Identification and Enrichment Analysis of Compounds. All the identified compounds were subjected to Kegg compound database to retrieve their Kegg IDs (http://www.kegg.jp/kegg/compound/). These Kegg IDs were subjected to MetaboAnalyst 5.0 for metabolite set enrichment and pathway enrichment (https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml).

2.11. Statistical Analysis. The experiment of the germination process was performed in triplicates. However, in order to avoid confusion in labeling and for better understanding of the results, average values were taken. Data were subjected to different statistical software (SPSS; 26, XLSTAT). Different online compound identification and metabolomics tools such as KEGG compound database and MetaboAnalyst were used for metabolomics data analysis.

3. RESULTS

3.1. Characterization of Nanoparticles. *3.1.1. UV−Visible Spectrometry.* The SeNP synthesis was confirmed using the UV−visible spectrum within the range of 200−800 nm. The results revealed that the characterization peak for the plant-mediated SeNPs was in the range of 200−400 nm. However, the characterization absorption peak was acquired at 283 nm. Our results are similar to the previous results of ref 22, in which the UV absorption peak for phytomediated SeNPs was acquired at 257 nm.

3.1.2. FTIR Analysis. The FTIR spectrum of SeNPs extracted from garlic clove powder was applied to determine the functional group responsible for the production and stability of green synthesized SeNPs. According to FTIR spectra at a specific absorbance, the wavenumber ranges from 400 (far IR spectrum) to 4000 (mid IR spectrum) for green synthesized SeNPs. The FTIR spectra of SeNPs synthesized from garlic cloves generated 16 absorption peaks at 609.5, 804.34, 931.65, 1026.16, 1112.98, 1261.49, 1384.94, 1419.66, 1458.23, 1541.18, 1560.46, 1618.33, 1637.62, 2364.81, 2924.18, and 3419.90, as shown in Figure 1C. Each peak represents the presence of particular functional groups that serve as capping agents to stabilize SeNPs. The trajection of the peak at 3200−3420 cm^{-1} wavenumber may be due to the presence of the hydroxyl (0 H) group and amino (N−H) group. The strongest absorption peak was observed at 3419.90 cm^{-1}, determining the presence of the amino group, whereas

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ACS Omega 2023, 8, 3354−3366

the weakest absorption peaks were obtained at 609.5 and 804.34 for functional group (C−H) cis and trans alkenes, respectively. The FTIR spectra of SeNPs at 1026.16 and 1112.98 indicated the presence of aromatic hydrocarbon and cyclic ether, respectively. The presence of the particular functional group lies in the specific wavenumber such as methyl ($-CH_3$) at 1384.94 and the aromatic aryl group having the absorption peak at 1458.23. Similarly, the wavenumber ranges between 1610 and 1700 cm^{-1}, which confirmed the existence of the carbonyl group (C═O) including aldehydes, ketones and esters. The absorption peaks constructed by FTIR spectra of green synthesized SeNPs at 2364.81 substituted for the alkyne group and at 2924.18 for alkenes.

3.1.3. SEM Spectrum Analysis. Scanning electron microscopy (SEM) revealed that the shape of SeNPs was spherical. The size of phytosynthesized SeNPs by powder extraction of dry cloves of *A. sativum* was found to be 100 nm. Results of SEM are shown in Figure 1.

3.2. Germination Parameters. The germination percentage shows the seed viability and effects of different treatments on seeds. In the present research study, the highest percentage germination rate of 96.7% was observed for TS-5 and Til-18 at 40 mg/L, while it was 93.7% for TH-6. The germination percentage was lowered among all three varieties of *S. indicum* when seeds were treated with selenium salt (5 mg/L). Germination rates of 66.7% in TH-6, 70% in TS-5, and 73.33% in Til-18 were recorded. The least germination percentage of 66.7% was noticed in TS-5 and Til-18 varieties when treated with Aspergillus (Figure 2). The outcomes of the

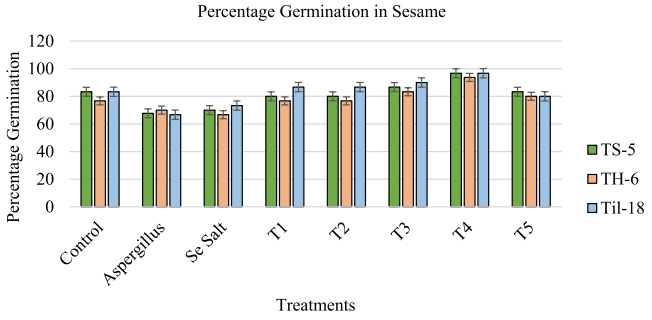

Figure 2. Green synthesized SeNP effects on the germination of *Sesamum indicum* varieties.

current study were opposite to the finding of ref 23 study conducted in 2022, in which seed germination activity was evaluated utilizing various (SeNPs) concentrations ranging from 0 to 50 mg/L; however, 25 mg/L exhibited the greatest percentage of germination. The current and prior findings suggest that SeNPs mediated by various plants have variable optimal concentrations that affect seed germination.

The highest seed vigor index observed in T4 (40 mg/L) for sesame varieties such as TS-5, TH-6, and Til-18 was 531, 505, and 490 respectively, whereas the lowest SVI was found for *Aspergillus,* which was 204.1 as compared to 333 for the control, as shown in Figure 2. The SVI was found to be the maximum for SeNPs followed by untreated plants, Se salt, and Aspergillus (Figure 3). The current study concluded that 40 mg/L SeNPs shown significant results for the seed vigor index in *S. indicum* varieties, correlating with a previous study on Labrador tea that produced the best results at 20 mg/L. The comparison made between both studies stated that the optimal concentration for the seed vigor index is found to be between

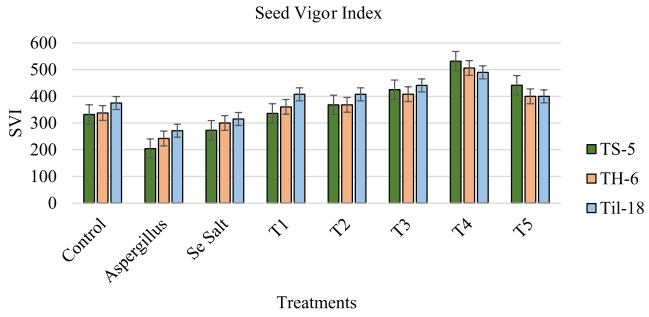

Figure 3. Green synthesized SeNPs effects on the seed vigor index of *Sesamum indicum* varieties.

10 and 50 mg/L, which is a significantly lower concentration yet extremely effective.

3.3. Morphological Parameters. Application of SeNPs caused a remarkable increase in plant height. Seed priming along with foliar treatment of SeNPs has shown greater effectiveness on plant height of *S. indicum* varieties as compared to seed priming and foliar applications. The highest percentage of plant height was recorded at 40 mg/L SeNPs as 52.7, 51.2, and 50.3% for Til-18, TS-5, and TH-6, respectively, as compared to untreated varieties. The noticeable decrease in percentage height was recorded as 17.03, 13.9, and 12.3% for Til-18, TH-6, and TS-5, respectively, when seeds were treated with selenium salt (5 mg/L) only (Figure 3). Sesame varieties treated with *Aspergillus* show stunt growth for the Til-18 variety recorded a height of 31.96 cm. Similarly, a substantial increase in root length of 77% was seen for the TS-5 variety upon treatment with SeNPs. 24% reduction in root length was noticed in the Til-18 variety as seed soaking and foliar treatment of Aspergillus were performed. The earlier study outcome of ref 23 concluded that photosynthesized SeNPs at 25 mg/L on *Allamanda cathartica* L. increased 92% in shoot length and 78% in root length, while at 50 mg/L, a significant decrease in growth parameters under salinity stress was noticed (Table 2). The comparison of both studies shows that low dosages of SeNPs enhanced root and shoot length, so that in such a way, the previous study supports the outcome of current investigation.

Similarly, the highest increase of 164% was observed in leaf number at T5 (50 mg/L) treatment using spt + foliar spray of SeNPs when compared to the control. Considerable increase of 70% and 109% was recorded for T5 (50 mg/L) in the TH-6 variety using both foliar application and seed priming. Among NP treatments, seed pretreatment shows the lowest number of average leaves per plant. Sesame plant treated with Se salts and Aspergillus shows (10) per plant in the TH-6 variety, while it shows the highest average leaves (49 leaves per plant) in Til-18 at 50 mg/L. A high value for the plant stem diameter indicates efficient plant development and health. Similarly, TH-6 and Til-18 increased percentage stem diameter by 38%, whereas reduction cause in Til-18 (22.9%), TH-6 (17.2%), and TS-5 (16%) by applying foliar application of selenium salt (5 mg/L) on sesame plant. Minimal and non-significant variations were found in different concentrations of SeNPs, aspergillus and Se salt (Figure 4). Prior to this,[24] discovered that foliar spraying Se NPs at 5 mg/L leads in an increase of leaf number in *Brassica napus*. When the current research study and the prior study were compared, a substantial difference was found.

3.4. Physiological Parameters. Plants have the ability to generate chlorophyll (a), a pigment that is ubiquitous and

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Table 2. Effect of Different Concentrations of SeNPs on Various Morphological Parameters of Three Sesame Varieties

	TS-5				TH-6				Til-18			
parameter	plant height	root length	leaf number	stem diameter	plant height	root length	leaf number	stem diameter	plant height	root length	leaf number	stem diameter
control	45.40 ± 0.91	10.13 ± 0.67	26.67 ± 1.10	0.85 ± 0.45	45.57 ± 1.10	10.70 ± 0.47	15.00 ± 1.30	0.83 ± 1.21	47.93 ± 0.55	11.27 ± 0.54	21.00 ± 1.54	0.90 ± 0.62
Se salt	40.77 ± 0.87	9.03 ± 0.82	15.67 ± 1.06	0.77 ± 0.53	39.23 ± 0.65	9.70 ± 0.86	9.67 ± 1.23	0.78 ± 0.92	39.77 ± 0.83	9.27 ± 0.77	16.00 ± 1.34	0.84 ± 0.74
T1	46.43 ± 0.74	11.10 ± 0.66	27.00 ± 1.04	0.86 ± 0.94	46.13 ± 0.78	11.17 ± 0.73	15.67 ± 1.44	0.87 ± 0.85	49.77 ± 1.13	12.10 ± 0.89	21.33 ± 1.76	0.91 ± 0.87
T2	50.80 ± 1.01	12.70 ± 0.94	28.67 ± 1.00	0.87 ± 0.81	51.80 ± 0.53	12.23 ± 0.65	19.00 ± 1.67	0.93 ± 0.77	53.57 ± 1.22	13.53 ± 0.67	24.00 ± 1.12	0.95 ± 0.66
T3	55.03 ± 1.05	13.90 ± 0.43	31.00 ± 1.57	0.94 ± 0.68	56.03 ± 0.44	14.17 ± 0.98	22.67 ± 1.57	0.97 ± 0.69	59.33 ± 0.92	14.03 ± 0.71	32.00 ± 1.43	1.00 ± 0.98
T4	63.53 ± 0.65	15.53 ± 0.54	35.00 ± 1.47	0.97 ± 1.03	61.70 ± 0.89	15.50 ± 0.59	32.00 ± 1.33	1.08 ± 1.32	66.43 ± 0.98	16.20 ± 1.2	35.33 ± 1.34	1.04 ± 0.76
T5	51.23 ± 0.98	13.73 ± 0.77	34.00 ± 1.22	0.93 ± 0.88	53.63 ± 0.52	13.40 ± 0.61	31.00 ± 1.55	0.98 ± 1.22	58.77 ± 0.66	13.80 ± 0.99	34.00 ± 1.39	0.95 ± 0.85

essential for photosynthesis. Chlorophyll (a) levels are greater in healthy plants. Comparatively to the control, sesame varieties had noticeably higher chl (a) percentage, that is, TS-5 (60.5%), TiI-18 (50.1), and TH-6 (34%) seed pretreatment at T4 (40 mg/L) utilizing SeNPs (see Figure 4F). When foliar spray of SeNPs was applied, a gradual increase in chl (a) was observed for TS-5 (67.3%) followed by TIL-18 (63.2%) and then TH-6 (59.8%) at the same NP concentration. Beside this, reduction in chl (a) contents was observed in all three varieties as treatment was carried out with sodium selenite salt and Aspergillus entirely. Finding of previous study conducted by ref 25 reported that chl a and chl b content increased in plant in lower concentration.

However, non-significant changes for chl (b) were recorded using different concentrations of SeNPs, Se salt (sodium selenite), and Aspergillus, as shown in Figure 5. A higher chl "b" content of 178% was observed in Til-18 at T4 (40 mg/L) and for TH-6 (69%) at T2 (20 mg/L) of SeNPs, when seed priming was carried out. T4 (40 mg/L) had shown the highest total chl content of 20.18 μg/mL followed by T3 (30 mg/L, 17.75 μg/mL) and T5 (50 mg/L, 17.18 μg/mL), utilizing seed priming and foliar application of SeNPs (see Figure 5). However, Til-18 which was highly influenced by Aspergillus shows the least total chl content of 7.31 μg/mL. Study found that Ag NPs result in a decrease in total chlorophyll contents. Current investigation conducted on sesame varieties results in an increase in total chlorophyll contents, which shows a contradictory outcome to previous study.

SeNP applications had shown a positive impact on sesame varieties, which improved plant fresh and dry biomass/weight. In contrast, selenium salt and *Aspergillus* had a negative impact on plant fresh and dry biomass/weight. Maximum values recorded were 20.33 and 19.37 g for the TS-5 variety at 50 mg/L followed by 40 mg/L as compared to the untreated plant, 14.43 g (Figure 4). The TH-6 variety had shown 17% reduction in plant fresh biomass. In the same way, a marginal decrease of 9% was found in TH-6 when foliar interaction of Se salt was carried out. The Til-18 had the highest dry biomass of 4.39 g as compared to untreated plant that was 3.0 g. A concentration of 50 mg/L of SeNPs was the most optimum followed by 40 and 30 mg/L that significantly result in higher plant biomass in each of the three different approaches, that is, seed priming, foliar, and combination of both (Figure 5). Previous finding of ref 26 previously observed an increase in fresh weight (38.6%) and dry weight (78.3%) at T4 (100 mg/L) in cluster bean plants utilizing SeNPs.

Proline is one of the major osmo-protectants, which protects the plant under stress conditions. SeNPs when interacting with sesame varieties have shown minimal variation in proline accumulation, whereas higher accumulation of proline contents was found as sesame varieties (TS-5, TH-6, and Til-18) were under stress situation treated with Aspergillus using three different methods such as seed priming, foliar spray, and combination of both. The highest proline accumulation of 111.1% was observed in the TS-5 variety followed by TH-6 (92.6%) and Til-18 (89.2%), as shown in Figure 5. Se salt (sodium selenite, 5 mg/L treatment) resulted in 92% increase in the TS-5 variety compared to untreated sesame plants.[24] Badri et. al found that bio-synthesized SeNPs and sodium selenite had opposite effects on *B. napus* under normal and stress conditions.

3.5. Impact of SeNPs on Antioxidant Potential of Sesame. Plants are particularly vulnerable to free radicals and

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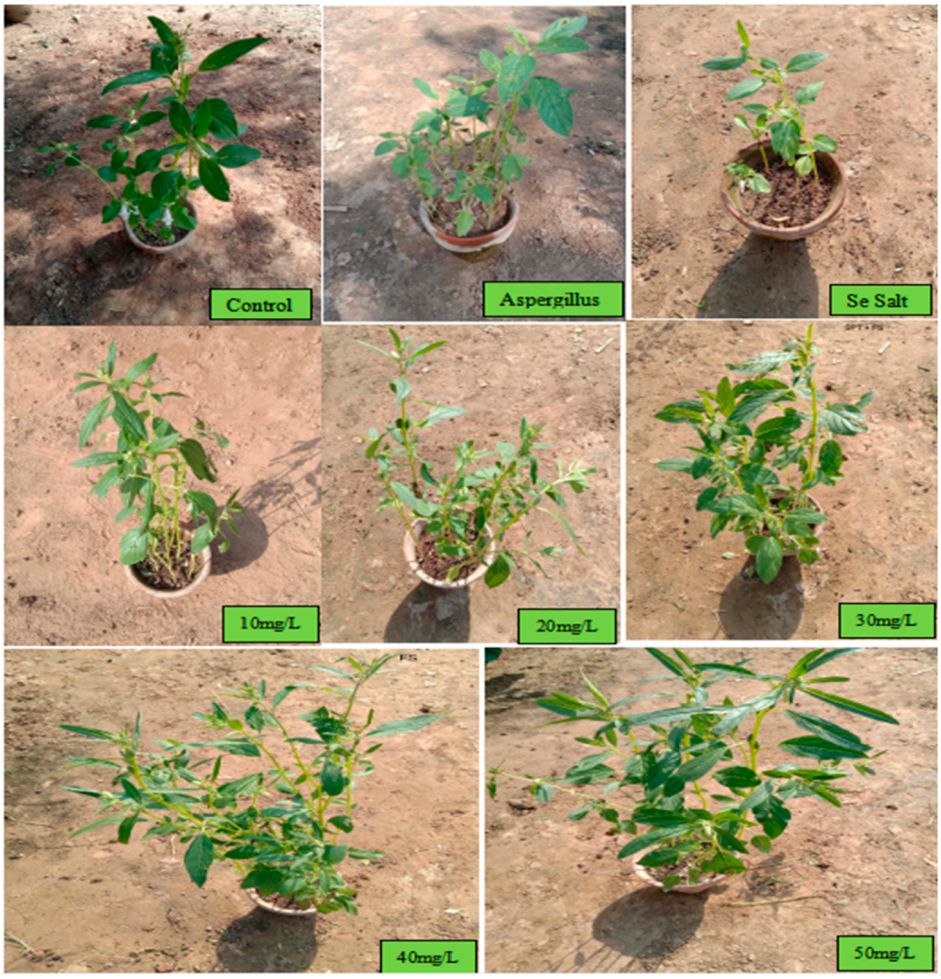

Figure 4. Variation in the phenotypic status of sesame using different concentrations of SeNPs, Se salt, and Aspergillus applications.

reactive oxygen species (ROS). The antioxidant enzyme superoxide dismutase aids plants in dealing with various stressors. T4 (40 mg/L) was the optimum concentration of SeNPs via seed soaking addition to foliar delivery resulted in significant increases of 147% in (TH-6), 145% in (Til-18), and 137% for (TS-5) in superoxide dismutase enzyme units. A 60% reduction in the superoxide dismutase (SOD) enzyme unit was observed for the TS-5 type treated with selenium salt (5 mg/L), as shown in Figure 5. Peroxidase is an enzyme that catalyzes the breakdown of hydrogen peroxide into water and molecular oxygen. Similarly, peroxidase levels increased by 140% for TH-6, 131% in Til-18, and 128% for TS-5 at 40 mg/L treatments by utilizing seed priming and foliar spray of SeNPs (see Figure 6). Reduction in peroxidase was noticed when sesame plant was introduced to Aspergillus and salt treatment.

In the current research investigation, it was found that catalase enzyme units became higher when sesame plant was fed with SeNPs. Higher CAT enzyme units were recorded for seed priming coupled with foliar spray of NPs followed by foliar and seed priming methods. An increase of 76% catalase in TS-5 and 74.54% in Til-18 at 40 mg/L was recorded using treatment (spt + fs), whereas 69.1% increase for TS-5 was recorded by utilizing the foliar approach, as shown in Figure 6. Noticeably, 27.54% reduction in catalase units was observed in the TH-6 variety treated with selenium salts. Aspergillus treatment on sesame plant results in 39.5% decrease in catalase enzyme units using the seed soaking technique as compared to control plants. Previous work by ref 27 showed an enhancement in antioxidant enzymes (SOD and CAT) when tomato plants were fed with SeNPs. The present research comparison made with earlier study results found that SeNPs highly involved in antioxidant enzyme enhancement in vegetable and oil-seed crops.

Antioxidants prevent the destruction of biologically significant macromolecules by interrupting the chain reaction that creates oxidative damage. A number of *S. indicum* cultivars (TS-5, TH-6, and Til-18) were evaluated for their antioxidant potential using green synthesized SeNPs and ascorbic acid as control and standard samples. In the present study, the influence of Se salt and Aspergillus applications on sesame varieties were also under consideration. A DPPH reagent is an electrophilic reagent used to extract hydrogen from *A. sativum* SeNPs. The highest levels of radical scavenging were achieved with T4 (40 mg/L) treatments employing seed priming and foliar application of SeNPs applied on *S. indicum* varieties. Percentage inhibition for the TS-5 variety with seed priming and foliar application of SeNPs, the peak was 83.05% at T4 (40 mg/L) as in Figure 6. After that, it was found that the Til-18 variety comprised 80% and TH-6 comprised 75.8% inhibition as compared to the standard sample. Aspergillus and salt treatments on sesame showed the least percentage inhibition of 42 and 34%, respectively. Current research investigation was compared to the outcome of Morales-Espinoza et. al study that

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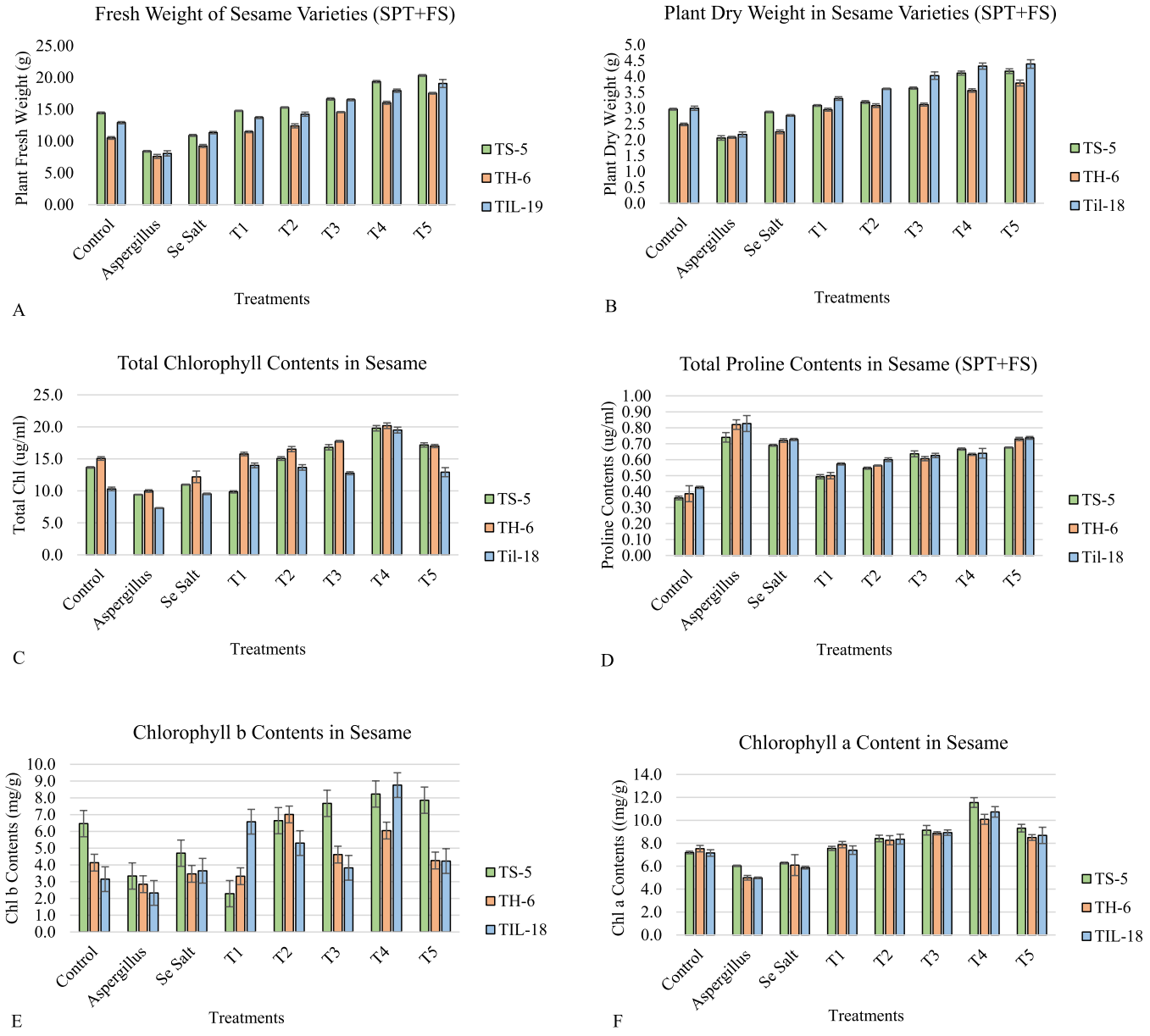

Figure 5. Impact of SeNPs on physiological parameters of sesame varieties (TS-5, TH-6, and Til-18) (A) impact of SeNP fresh weight of sesame plants, (B) dry weight of plants, (C) total chlorophyll contents in sesame, (D) total proline contents in sesame, (E) chlorophyll *a* contents in sesame varieties, and (F) chlorophyll *b* contents in sesame varieties.

documented mild antioxidant potential employing the ginger extract, with a calculated IC_{50} value of SeNPs of 125 μg mL^{-1}, whereas that of ascorbic acid was 250 μg mL^{-1}.

3.6. Identification of Total Metabolites in Oil Samples. A total of 58 metabolites were detected from 11 samples of sesame oil, belonging to 15 different classes of chemical compounds. Fatty acids, both saturated and unsaturated, have dominated the metabolites. Many unsaturated fatty acids such as linoliec acid, oleic acid, and α-linoliec acid were detected in oil samples. It was noticed that the Till-18 variety is more diverse in metabolites as compared to TS-5 and TH-6. In control samples of all these three varieties highest metabolite, 23 were found in Till 18, followed by 20 metabolites in TH-6 and 19 metabolites in TS-5 control of these three varieties (Table 3, Figure 7). Furthermore, GCMS results suggested that SeNPs have not greatly increased the number of metabolites; rather, they have increased the concentration of various metabolites found in these three controls. Like in control samples, concentration of these fatty acids was low in all the three varieties (TS-5, TH-6, and Till-18), as compared to seed + foliar treatment of SeNPs at a concentration of 40 mg/L. This suggests that SeNPs have elicited the biosynthesis of these fatty acids. Moreover, bioactive compounds such as sesamin and sesamol were also greatly affected by the SeNP treatment, as their retention time was 9.66 in control which was found 14.68 in T4 sample of seed + foliar treatments in Till-18. Vernolic acid content was also increased in T4 (seed + foliar) treatment in Till-18. It can be concluded that SeNPs have induced the expression of certain metabolites along with the increase in concentration of already present metabolites in these varieties, which suggests the potential of SeNPs for enhancement of metabolites .

3.7. Kegg Pathway Annotation of Differential Metabolites. To scrutinize the differential metabolites in

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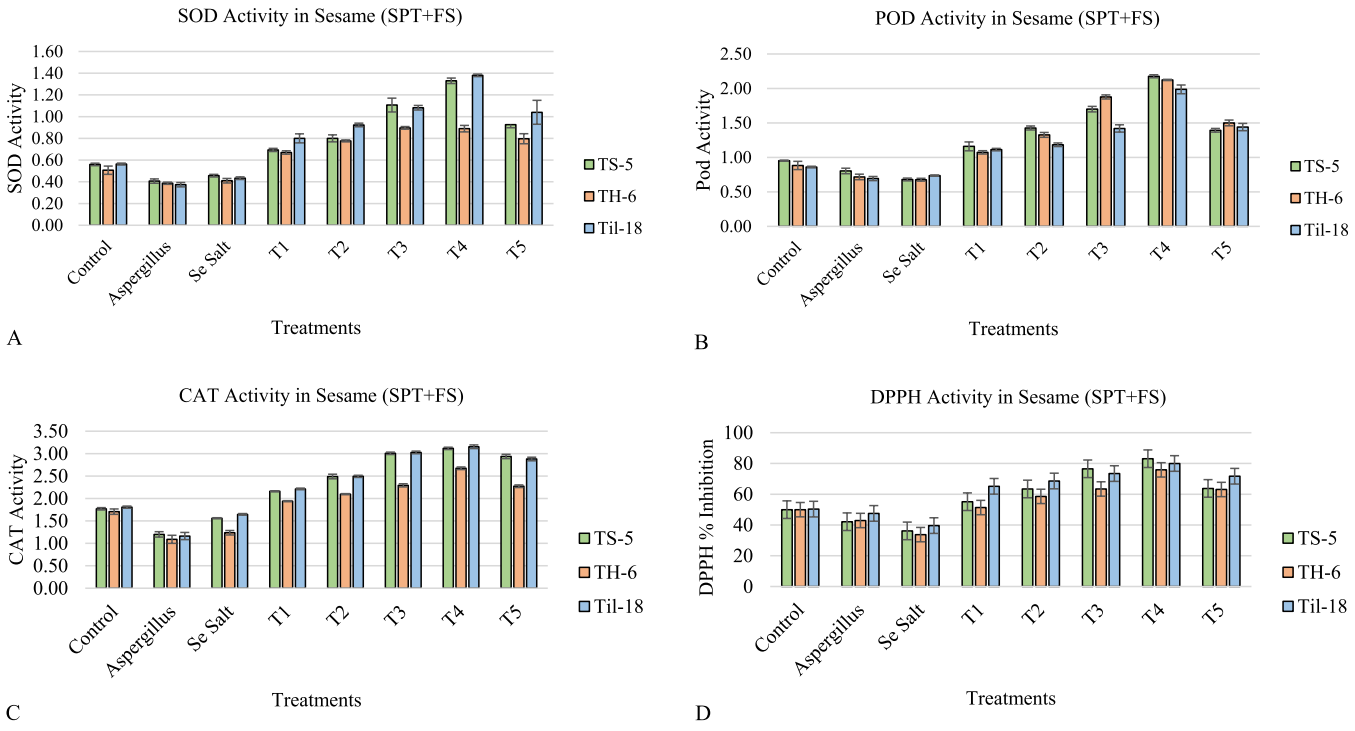

Figure 6. Green synthesized SeNP effect on antioxidant activities, (A) superoxide dismutase enzyme of sesame, (B) influence of the SeNP effect on peroxidase enzyme potential in sesame, (C) plant-based SeNPs alleviate catalase accumulation in sesame, and (D) DPPH % inhibition of different treatments (SeNPs, Aspergillus, and Se salt) on sesame varieties.

Table 3. Key 22 Metabolites Revealed by GCMS Analysis of Sesame Oil Treated With Different Concentrations of SeNPs along With Their HMDB ID, Pubchem ID, and KEGG ID

S. No	metabolite name	HMDB ID	PubChem ID	Kegg ID
1	palmitic acid	HMDB0000220	985	**C00249**
2	linoleic acid	HMDB0000673	5280450	**C01595**
3	stearic acid	HMDB0000827	5281	**C01530**
4	tricosane	HMDB0061866	12534	**C17433**
5	gamma-tocopherol	HMDB0001492	14986	**C02483**
6	(+)-sesamin	HMDB0034256	5204	**C10882**
7	sesamol	HMDB0033812	68289	**C10832**
8	p-anisic acid	HMDB0001101	7478	**C02519**
9	campesterol	HMDB0002869	5283637	**C01789**
10	stigmasterol	HMDB0000937	5281330	**C05442**
11	clionasterol	HMDB0000649	457801	**C19654**
12	phthalic acid	HMDB0002107	1017	**C01606**
13	petroselinic acid	HMDB0002080	5461010	**C08363**
14	catechol		3390	**C00090**
15	*N*-phenyl-N		17396093	**C15096**
16	cyclohexanecarboxylic acid	HMDB0031342	7413	**C09822**
17	vernolic Acid		6449780	**C08368**
18	acetophenone	HMDB0033910	7410	**C07113**
19	ricinoleic acid	HMDB0034297	643684	**C08365**
20	L-phenylalanine	HMDB0000159	6140	**C00079**
21	nonacosane	HMDB0034288	12409	**C08384**
22	cholest-5-ene	HMDB0000941	440663	**C05416**

the gene expression level, we carried out KEGG pathway analysis. The results of Kegg annotation showed that most of the metabolites were involved in secondary metabolite biosynthetic pathways. The KEGG pathway enrichment analysis indicated that the main enrichment of the metabolites occurred in biosynthesis of unsaturated fatty acids, phenylalanine, tyrosine, and tryptophan metabolism, linoliec acid metabolism, steroid biosynthesis, phenylalanine metabolism, fatty acid elongation, fatty acid degradation, fatty acid biosynthesis, and amino-acyl t-RNA biosynthesis (Figures 8, 9). Metabolite set enrichment analysis revealed that most of the metabolites were involved in phenylalanine, tyrosine, and tryptophan metabolism, followed by linoliec acid metabolism. Certain metabolites which were involved in amino-acyl t-RNA

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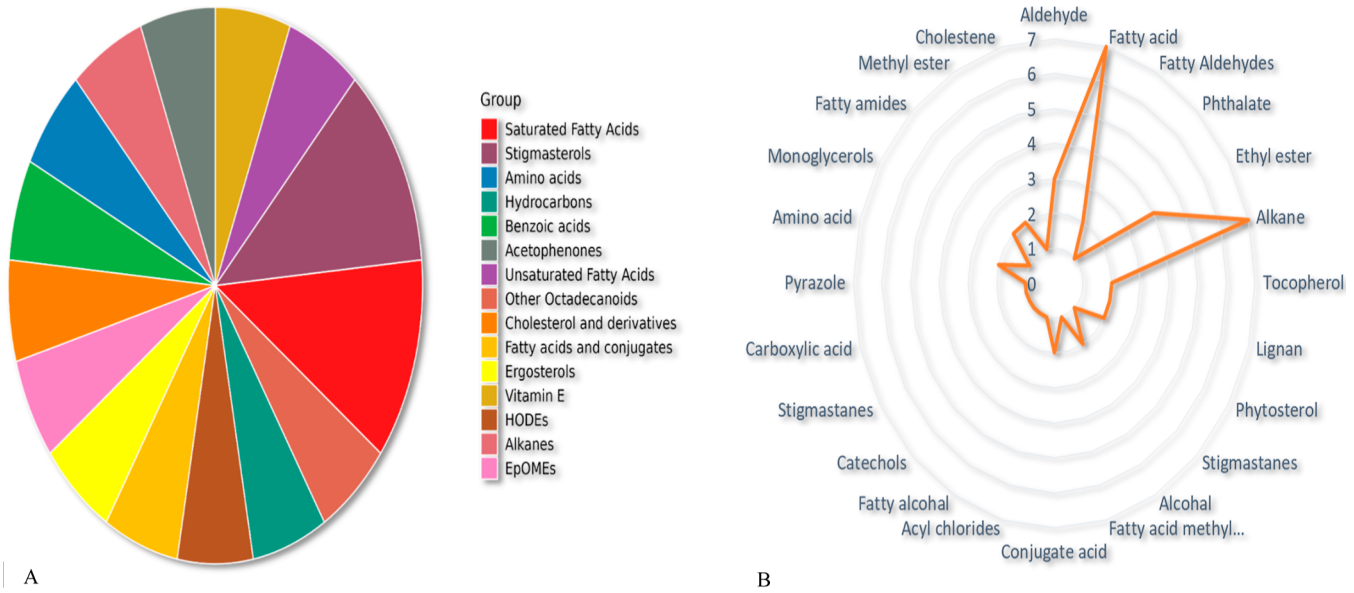

Figure 7. (A) Pie chart showing classes of all the metabolites detected in GCMS analysis. (B) Radar chart showing the concentration of various metabolites in all the oil samples in which fatty acids had the highest concentration followed by alkanes, tocopherols, and conjugated acids.

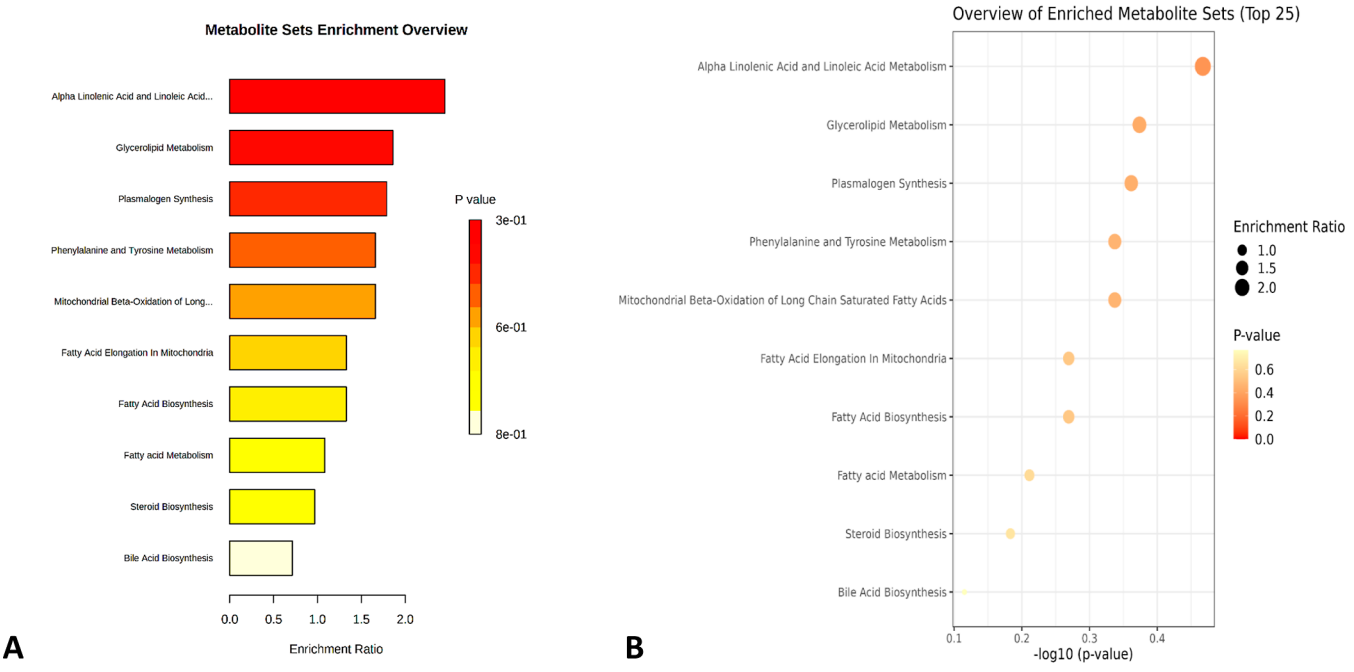

Figure 8. (A) Metabolite set enrichment analysis in which 10 important pathways were detected including fatty acid metabolism, fatty acid elongation, and α-linoleic acid and linoleic acid metabolism, and the color change is dependent upon the p value and (B) enrichment's ratio of all metabolites detected.

biosynthesis were reported for the first time in sesame oil. Furthermore, it was also noticed that a higher proportion of metabolites was involved in bio-synthetic mechanisms, which indicates that SeNPs have induced the biosynthesis of important metabolites which have activated various important pathways .

4. DISCUSSION

The results declare that SeNPs have a positive impact on overall growth of all the three varieties, that is, TS-5, TH-6, and Till-18 of sesame, by upregulating various biochemical and antioxidant systems.

Photosynthetic pigments, especially chlorophyll, play a central role in light harvesting and subsequent energy conversion to carbohydrates; thus, any alteration in chlorophyll content causes an overall effect on the overall metabolism of plants. Present investigation had showed that SeNPs have a positive effect on photosynthetic pigments as chlorophyll content has increased with increasing SeNP concentration. Moreover, it was also suggested that foliar treatment of SeNPs has resulted in more total chlorophyll content as compared to seed priming only. Similar results were also given by ref 28 who concluded that metallic nanoparticles have increased photosynthetic pigments in *Brassica juncea*. This increase in

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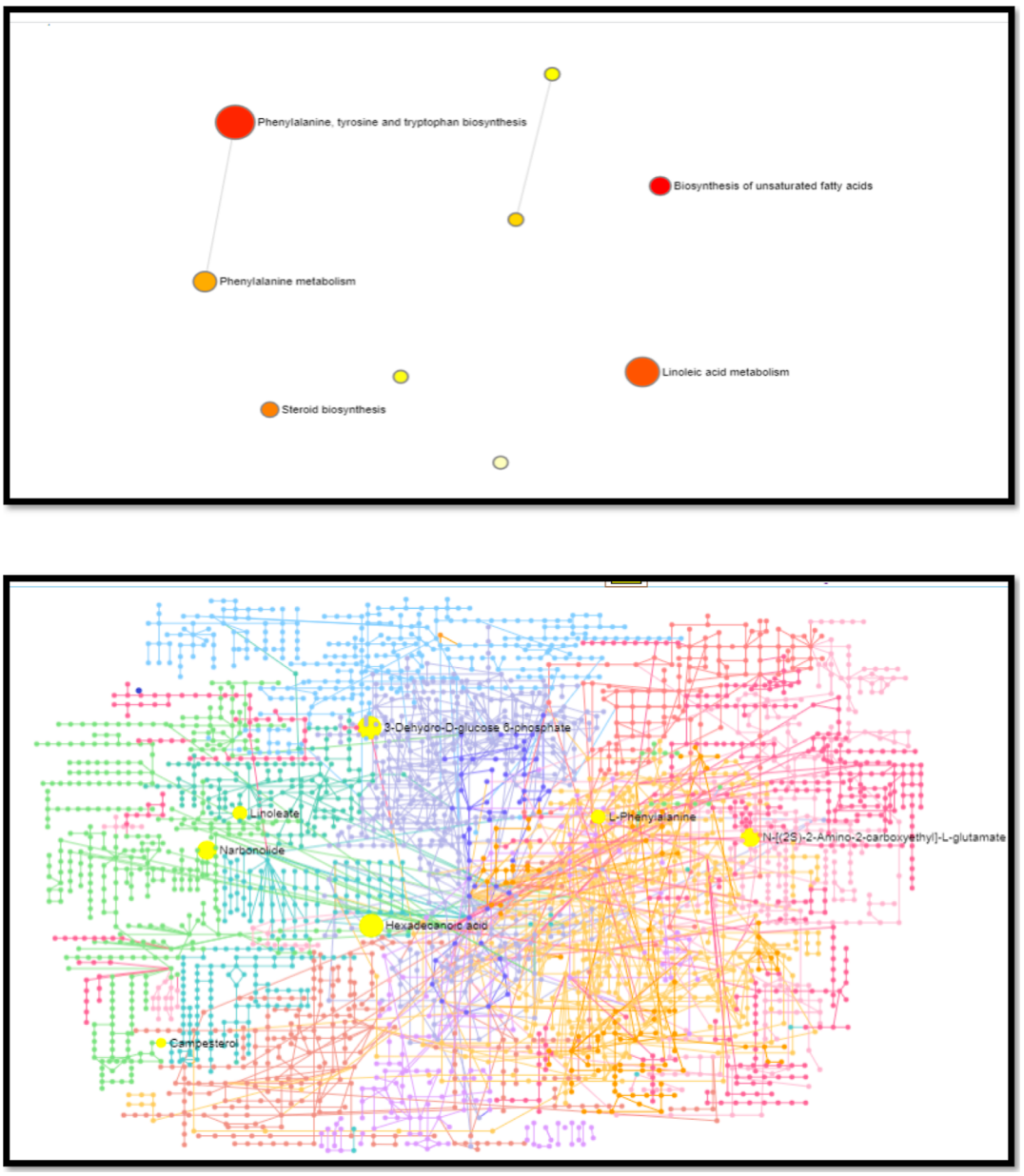

Figure 9. Mapping of important metabolites involved in various metabolic pathways in the Kegg global metabolic network. Most of the hits were found in biosynthesis of unsaturated fatty acids, suggesting that SeNPs have elicited the biosynthesis of unsaturated fatty acids such as oleic acid, linoleic acid, and α-linoleic acid.

photosynthetic pigments, especially in chlorophyll, may be attributed to SeNPs which have a protecting role in chloroplast enzymes which are involved in biosynthesis of chlorophyll.[26] Furthermore, selenium also acts as a catalytic center of various important selenoprotiens involved in the enzymatic antioxidant system such as glutathione peroxidase which by scavenging reactive oxygen species protects the photosynthetic apparatus.[29] It may also be noticed that higher concentration of SeNPs (50 mg/L) resulted in decreased chlorophyll content which may be justified by the fact that higher concentration of selenium induces certain oxidative defense systems which in turn damage the photosynthetic pigments and ultimate cause a decrease in carbon assimilation and declined food production which ultimately lead to impaired growth. It is also further reported by ref 30 that higher concentration of selenium degrades the precursor of chlorophyll, which leads to decreased chlorophyll content. Therefore, it can be concluded that selenium plays a positive role in photosynthetic pigments, but its concentration is a very important factor which cannot be ignored.

Plants have both enzymatic and non-enzymatic antioxidant defense systems which serve to protect the cellular machinery from oxidative damage, by scavenging ROS and maintaining their level to optimum. In the present investigation, SeNPs have shown an enhanced antioxidant defense system as the activity of various enzymatic POD, SOD, and CAT and non-enzymatic DPPH was increased with increasing concentrations of SeNPs.[31] These are all the enzymes which basically carry out the conversion of hydrogen peroxide (H_2O_2) to H_2O and O_2.[32] As both biotic and abiotic stress cause elevating levels of ROS, the balance between the production and scavenging of these ROS determines the fate of the cell, wither damage, or

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ACS Omega 2023, 8, 3354−3366

protection by inducing the signaling pathway.[33] Treatment with SeNPs has shown dose-dependent results as the enzymatic activity was increased, and the highest activity was shown at 40 mg/L on seed priming + foliar spray. The possible reason for this elevated oxidative defense response may be attributed to upregulation of various stress-responsive genes, which results in the enhanced enzymatic defense system along with production of various osmoprotectants such as proline, ultimately leading to better response to oxidative stress.[34] It improved maize's enzymatic activity by increasing the transcription levels of antioxidant defense-responsive genes.[35] Moreover, selenium acts as a stimulant to induce various stress-responsive antioxidant systems which subsequently result in enhanced stress tolerance. Our study has concluded that seed pretreatment with various concentrations of SeNPs has a positive impact against biotic stress, as *Aspergillus*-treated seeds which were later treated with SeNPs had showed better antioxidant response.[36] As antioxidant activity is attributed to most of the nanoparticles, by activating the antioxidant defense system, the study[35] concluded that application of selenium resulted in elevated concentration of CAT and SOD by upregulating the genes associated with the antioxidant defense system to upregulation of the antioxidant system in maize. Moreover, similar results were obtained by ref 37 who also reported selenium-mediated activation of the antioxidant defense system.

Metabolites reflect the integration of gene expression, protein interaction, and other different regulatory processes and are therefore closer to the phenotype. Metabolomics analysis plays a critical role in revealing differential expression of metabolites affected by various types of stress. Nanoparticles, due to their small size and lower surface area, have a greater capability to stimulate various biosynthetic pathways, which ultimately leads to beneficial metabolites not only for plants but also for humans who are using them for food purposes. In the present study, metabolic analysis of sesame oil treated with different concentrations of SeNPs was carried out through GCMS, which revealed that there was a greater difference in the metabolic profile of control and treated plants. The concentration of most of the fatty acids such as linoleic acids, palmitic acids, stearic acids, and petroselinic acids was increased to 40 mg/L SeNPs in treated plants as compared to the control. These unregulated levels of fatty acids show that SeNPs have altered the fatty acid profile of sesame oil. As sesame seeds eventually come to humans, these increased fatty acid contents have multiple beneficial health effects on health, such as linoleic acid (LA), an essential fatty acid, is metabolized to gamma linoleic acid (GLA), which serves as an important constituent of neuronal membrane phospholipids and also as a substrate for prostaglandin formation, seemingly important for preservation of nerve blood flow. Similarly, petroselinic acid has a moisturizing and nourishing effect on the skin and is already being used in various cosmetics products.

Moreover, enrichment analysis showed that most of the metabolites were associated with phenylalanine, tyrosine, and tryptophan biosynthesis. These are three aromatic amino acids which are the integral part of important proteins. These amino acids and their metabolism are linked to the synthesis of a variety of secondary metabolites, a subset of which are involved in numerous anabolic pathways responsible for the synthesis of pigment compounds, plant hormones, and biological polymers, to name a few. In addition, these metabolites derived from the AAA pathways mediate the transmission of nervous signals, quench reactive oxygen species in the brain, and are involved in the vast palette of animal coloration among others pathways. The AAA and metabolites derived from them also play integral roles in the health of both plants and animals.[38] Furthermore, for the bioactive compounds found in sesame such as sesamin, sesmolin, and tocopheols, concentration were also increased under 40 mg/L seed + foliar spray of SeNPs. The concentration of sesamin, the most important bioactive compound, was increased by about 11% as compared to the control, which indicates the ameliorative role of SeNPs. Sesamin enhances hepatic detoxification of chemicals, reduces the incidence of chemically induced tumors, and protects neuronal cells against oxidative stress.[39]

5. CONCLUSIONS

Present study was focused to assess the effect of SeNPs on the physiological, biochemical, antioxidant, and metabolic profile of sesame under induced biotic stress. This study has revealed that lower concentrations of SeNPs have the capability to improve the biochemical and antioxidant profile of sesame. However, higher concentration of selenium becomes toxic to plants and has a negative impact on plant growth. Moreover, our metabolic analysis revealed that sesame oil of all three varieties is rich in various important fatty acids and bioactive compounds, which were significantly affected by SeNPs as well. Therefore, it can be further concluded that there is a dire need of omics studies to further confirm the role of SeNPs in plants and also to explain the mechanism of action of SeNPs as well.

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Notes

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