Egypt. J. Chem. **Vol 68** , N. 8 pp. 97 - 111 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

The Biochemical Response of *Ocimum basilicum* cv. Grand Vert Plants to Silver Nanoparticles and Green Algae as Biofertilizer

Heba M. Elbanna[1], Osama K. Ahmed[2], Sayed A. Fayed[2], Khaled A. Hammam[1], Rania S. Yousef *[2]

[1] *Medicinal and Aromatic Plants Research Department, Hort. Res. Inst., Agric. Res. Centre, Egypt*
[2]*Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt*

Abstract
Using a modern strategy of nanoparticles as nanofertilizer combined with green algae as biofertilizers in foliar spray application on medicinal plants seems to be a hopeful strategy. This study evaluated the effects of foliar spraying of biofertilizer spirulina extract at concentrations of 0.5, 1.0, and 1.5 g/L, silver nanofertilizer (AgNPs) at concentrations of 50, 100, and 150 mg/L , and the combination of AgNPs solution and microalgae extract (0.5+50, 0.5+100, 0.5+150, 1.0+50, 1.0+100, 1.0+150, 1.5+50, 1.5+100, and 1.5+150) compared to control plants in *Ocimum basilicum* cv. Grand vert plants. Our data revealed that the treatment of various concentrations of AgNPs or spirulina extract significantly promoted growth, plant height, number of leaves, fresh and dry weight, essential oil percentage, and yield. The highest promotion was with the treatments of 100 mg/L AgNPs or 1.5 g/L spirulina extract. The highest concentration of AgNPs showed a negative effect on basil plant growth and a significant accumulation of malondialdehyde content. Whereas all spirulina treatments reported synergistic effects in combination with AgNPs to mitigate the negative overproduction of reactive oxygen species (ROS) to the levels that synergistically induced essential oil accumulation, the spirulina extract supported nutritional and bioactive compounds promoting plant growth parameters and indirectly suppressed the oxidative damage by activating the antioxidant defense enzymes and directly antioxidant against ROS formation. The results highlighted that the combination of the silver nanoparticle and green algae at a concentration of 100 mg/L AgNPs + 1.5 g/L spirulina extract could collaborate as a biofertilizer to improve the quality and quantity of French basil, which is better than spraying nanofertilizer (AgNPs) individual. Thus, spirulina plays an important role in avoiding the harmful effects of nanoparticles to become a promising agrochemical fertilizer.

Keywords: French basil; Essential oils; Silver nanoparticles; Biofertilizers; Antioxidant activity

1- Introduction

Medicinal plants are increasingly used as sources of bioactive compounds required for drug development, so breeders aim to increase the area cultivated with medicinal and aromatic plants to 250 thousand feddan (180%) by 2030. The World Health Organization (WHO) estimates that approximately 80% of the population in developing countries depends on medicinal plants and traditional medicine for their primary health care [1]. A report issued by the Horticulture Research Institute of the Egyptian Ministry of Agriculture confirmed that the area of medicinal and aromatic plants amounts to 120,000 feddan. The report indicated that the total national income from exports and cultivation of medicinal plants is about 8 to 10 billion pounds annually, and they also generate remunerative income locally; for example, the price of a ton of basil is 20 thousand pounds in the local markets and higher in exports. Egypt is considered one of the major basil-exporting countries [2], with cultivated areas reaching about 10.685 thousand feddans [3].

Within the largest family of plants that yield essential oils, the Lamiaceae, is home to the French basil (*Ocimum basilicum* L.), one of the major genera [4]. Flavonoids and phenolic chemicals found in *Ocimum basilicum* L. exhibit anti-inflammatory, antioxidant, and anti-cancer properties. In traditional medicine, the plant has primarily been used to treat respiratory conditions [5,6]. The volatile organic chemicals that are highly sought after by the food and pharmaceutical industries are present in the essential oils extracted from the sections of basil; consequently, these volatile organic compounds have been demonstrated to possess biological activity. Therefore, linalool, 1,8 cineole, methyl chavicol (estragole), and eugenol are the main constituents of basil essential oil. As a result, basil essential oil has hypoglycaemic and anticancer effects on tuberculosis. Furthermore, the basil ethanolic extracts containing eugenol [7-10]. Nowadays, nanotechnology is an extremely developed and rapidly evolving scientific and technological field [11]. Therefore, nanotechnology applications have many uses in the environment and plant ecosystems [12]. Nanofertilizers significantly improve soil quality, plant growth performance, and crop

*Corresponding author e-mail:dr.raniasaber@cu.edu.eg; (Rania Saber Yousef).
Received date: 23 April 2024, Revised Date: 22 July 2024, Accepted Date: 28 December 2024
DOI: 10.21608/EJCHEM.2024.284543.9626

production with quality fruits/grains [13]. Hence, nanofertilizers control the flow of nutrients using nanoparticles, which makes them more effective as well as cheaper compared with traditional fertilizers [14]. Nanoformulations, particularly metal compound-based nano-fertilizers with sizes in the range of 10 to 100 nm, have been developed for a variety of modes of action and applications in agriculture [15]. The synthesized nanoparticles are designed with a special shape, surface properties, size, and chemistry [16]. To synthesize nanoparticles on a large scale, the reviewed articles found that using a chemical reduction process produced a relatively high yield, a low cost, and an easy practice [17]. The reviewed articles studied the influence of different metal nanoparticles such as copper, silver, zinc, gold, and titanium on various crop plants, and it was observed there were significant changes in plant morphology and enhanced antioxidant enzyme activity [18-20]. AgNPs are considered the most noticeable nanoparticles used in a wide range of applications. Scientific interest in AgNPs has proven maximum efficacy in agriculture and plant biotechnology through promoting seed germination, growth of plants, and enhancing the efficiency of the photosynthetic process, so AgNPs play a vital role in improving crop production and securing food security in the agriculture sector [21]. This is because AgNPs have a larger surface area and a higher fraction of surface atoms than bulk silver [22]. Also, AgNPs are marked by their particular features, e.g., desired magnetic, electrical, and optical properties [23]. Biofertilizers produce sustainable plants with eco-friendly microalgae. Cyanobacteria increases crop production, environmental quality, and plant performance; hence, it contributes greatly to sustainable agriculture. Also, it enhances plant growth (shoot, root, and leaf length, fresh and dry weight) and pigment yield [24,25]. Spirulina (*Spirulina platensis*) is a green-blue algae that is a discovery in bio-fertilizers for organic plant production; hence, spirulina is rich in organic and inorganic nutrients [26]. Additionally, researchers affirmed that nanofertilizer appears highly efficient in basil plants [27,28,29]. Likewise, the significant effects of AgNPs on the growth and oil production of lavender were approved. Additionally, foliar application of AgNPs achieved significant enhancements for growth variables of the fenugreek plant, such as shoot dry weight, shoot length, and number of plants or leaves, increasing plant yield and photosynthetic pigments [30, 31]. According to the findings of [32], foliar applications of several cyanobacterial species extracts can have a highly significant stimulation effect on basil growth. As well as improve the growth parameters of mint [33]. Furthermore, the promoting effect of spirulina extract on fennel (*Foeniculum vulgare*) plants [34]. On the other hand, treatments of AgNPs with Spirulina achieved a double positive impact on sugar quality, value, and inhibition of the soft rot disease in sugar beet (*Beta vulgaris* L) [35]. Furthermore, the combinations of spirulina extract and compost tea as biostimulants with 75% of the recommended dose of nitrogen for economical fennel production had the best growth and yield [34].

However, our study was conducted to measure the most promising interaction of the nanofertilizer silver nanoparticles and the biofertilizer spirulina extract at different concentrations and also the effect of both separately on improving the growth, yield quantity, the chemical composition of essential oil, and quality of French basil plants and antioxidant enzymes for a better understanding of plant responses and to accomplish the maximum benefit of nanofertilizer, which is considered inexpensive when applied on a large scale. Additionally, mixing nano fertilizer with bio fertilizer solves many future problems that resulted from using nano fertilizers in the agricultural sector and improves the productivity of medicinal and aromatic plants, with a promising future for export abroad.

1. Materials and Methods

Our pot experiment was conducted in an open field during two successive seasons (2020 and 2021) on the farm of the Medicinal and Aromatic Plants Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

1.1. *Preparation of silver nanoparticles*

Silver nitrate is reduced by the excess amount of sodium borohydride, using an ice bath to facilitate the slowdown of the reaction rate and remove secondary reactions during nanoparticle agglomeration as shown in Fig. 1 [16].

1.2. *Characterization of synthesized silver nanoparticles*

The examination of the prepared AgNPs solution was through two steps, the first one was via Transmission electron microscope to specify the size of AgNPs and their morphology throughout carbon-coated copper grids (CCG), a drop of AgNPs was applied, and the grids were exposed to infrared light for 30 minutes by (TEM, JEOL GEM-1010 transmission electron microscope at 70 kV) in the Regional Center for Mycology and Biotechnology (RCMB) of Al-Azhar University, the

Second one was XRD analysis at Central Metallurgical Research Institute (CMRDI) using a specialized X-ray diffractometer (XRD-Model-D8 advance, BRUKER Germany) which provides the crystallographic structure, and properties of AgNPs.

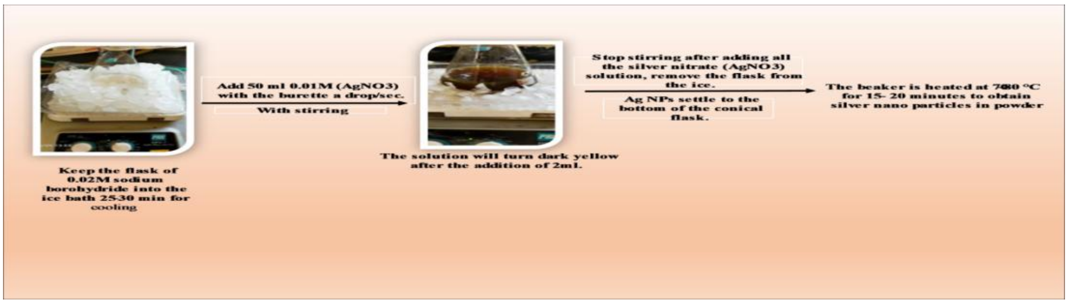

Fig. 1. Scheme of preparation of silver nanoparticles (AgNPs)

2.3. Experimental design and treatments

On 15th February, the seeds of French basil (*Ocimum basilicum* L var. Grand vert) were sown in the plant nursery at the farm of the Medicinal and Aromatic Plants Department then, seedlings were transplanted into pots in April in the two seasons. The pots that were used in our study were Pottery (40 cm) in diameter. Each pot was filled with a soil mixture of clay: silt: sandy (1: 1: 1) and pH 7.33. Sand and silt were washed with water and fertilized with 16 g of ammonium sulphate (20.5 %N) and 5 g of potassium sulphate (48 % K_2O_2). Half a dose of the nitrogen and potassium fertilizer was applied 45 days after transplanting while; the second one was applied a month after the first one. 5 g of Calcium superphosphate (15.5 % P_2O_5) was mixed with the soil before transplanting. Each pot contained four seedlings and was placed in full sunlight under the natural conditions in the Giza governorate. Plants were irrigated to field capacity using tap water for about 3 weeks until the basil establishment.

Spirulina extract was obtained from the Algae Department, National Research Centre. Spirulina extract was applied to the plants with distilled water in three concentrations in addition to three treatments of AgNPs, all combined treatments of spirulina and AgNPs concentrations and Control plants (only treated with distilled water) which, were a total 16 treatments in this study.

The treatments as follow:
Control plants (0.0).
(0.5, 1 and 1.5) g/L spirulina extract.
(50, 100 and 150) mg/L AgNPs.
50 mg/L AgNPs + 0.5 g/L spirulina.
100 mg/L AgNPs + 0.5 g/L spirulina.
150 mg/L AgNPs + 0.5 g/L spirulina.
50 mg/L AgNPs + 1 g/L spirulina.
100 mg/L AgNPs + 1 g/L spirulina.
150 mg/L AgNPs + 1 g/L spirulina.
50 mg/L AgNPs + 1.5 g/L spirulina.
100 mg/L AgNPs + 1.5 g/L spirulina.
150 mg/L AgNPs + 1.5 g/L spirulina.

These treatments were arranged as a factorial based on the completely randomized design with three concentrations of AgNPs, three concentrations of spirulina extract, and the combined treatments as well as control (untreated plants). All treatments were in three replications (each with one pot including four plants). The basil plants were sprayed with different concentrations of AgNPs, spirulina, and their interaction. The foliar spraying of basil was performed twice every 10 days after planting all over the growth stages and cultivation seasons. The first spraying of every factor separately and the mixed solution was when the plant reached a height of 10-15 cm with about 10 leaves and the second spraying was after 10 days from the first one. Sprays were passed out for both sides of the leaves and stem using an atomizer sprayer in a way that all above-ground parts of basil plants were covered. To increase the plant's absorption of solutions, we have to apply foliar spraying before sunrise due to the stomata of the plant being open and in conditions lacking wind and rain. Within the two seasons, the plants were irrigated regularly beside other agricultural processes as usual. All measurements were taken at the blooming stage. All growth parameters, oil %, and oil yield were calculated for all treatments over three replicates of each treatment in both seasons. The second season recorded basil oil analyzed by gas chromatography, chemical characterization of the basil plant, and Antioxidant activity for the best treatments that were recorded over three replicates of each treatment.

2.4. Morphological growth characteristics

Plant fresh and dry weights (g) were recorded before the blooming stage and at the vegetative growth termination in both seasons.

2.5. Essential oil percentage and Chemical constituents by Gas chromatography-mass spectrometry

To determine the essential oil percentage (%); 100 g of fresh leaves were placed to the hydro-distillation according to [36], oil yield (mL)/plant. Analysis of the essential oils was conducted by using gas chromatography (Hewlett–Packard model 5890), coupled to a mass spectrometer (Hewlett–Packard-MS model 5970), and equipped with a DB5 fused silica capillary column. The oven temperature was maintained initially at 50 ºC for 5 min and then programmed from 50 to 250 ºC at a rate of 4 ºC/min. Helium was used as the carrier gas, at a flow rate of 1.1 mL/min. The temperature of the injection was 220 ºC. Mass spectra in the electron impact mode (EI) were obtained at 70 eV and scan m/z range from 39 to 400 amu. The isolated peaks were identified by matching them with data from the mass spectra library (National Institute of Standards and Technology, NIST [37].

2.6. Chemical characterization of Basil plant

The percentage of moisture content was determined in addition to the ash, protein, lipid, and carbhydrate was calculated according to [38].

2.7. Determination of Lipid Peroxidation

Malondialdehyde (MDA) activity was determined as the method designed by Satoh [40], 0.2 mL sample with 1.0 mL thiobarbituric acid detergent stabilizer 25 mmol/L, heat for 30 min in boiling water bath, at 534 nm, compare the absorbance of a sample to the blank and standard.

2.8. Antioxidant Activity Analysis

The effect of foliar application on Basil plants' defense enzymes was studied on treated and untreated Basil leaves. Samples extract of the reaction performed according to [39], after homogenization of fresh French basil leaves in 10 ml cold mixed buffer/1g tissue. The buffer consists of 50 mM potassium phosphate with 1 mM EDTA, 1 mL/L Triton X-100, and adjusted pH 7.4. The homogenized solution Centrifuged for 15 minutes at 4 °C by 4,000 rpm. At that time, the supernatant was ready for assay.

Catalase (CAT) activity was determined according to Aebi [39]. Catalase reacts with a known volume of H_2O_2, after one minute, the reaction was stopped by catalase inhibitor, read sample against blank and standard at 510 nm.

Superoxide dismutase activity (SOD) was estimated by Nishikimi and Yogi [42], recording the increase in absorbance at 560 nm, the change in absorbance over 5 min following the addition of Phenazinemethosulphate (PMS) to the reaction mixture in the absence of sample, Purified SOD was used to inhibit the initial rate of phenazine methosulfate mediated reduction, then reduced nitro blue tetrazolium.

Glutathione peroxidase activity (GPx) was described by Paglia and Valentine [41], who measured the decrease of absorbance at 340 nm/ min within 3 min against deionized water.

2.9. Statistical analysis

This factorial experiment tested the significance of two main factors with different concentrations, and the significance of interactions. After recording the data analysis with variance in a randomized complete design then, the mean comparison using analysis of variance (ANOVA) using the Statistical Analysis System [43] (SAS Institute, inc, 1996). Significant differences were tested according to the LSD range test at $P \leq 0.05$ levels.

2. Results and Discussion

2.1. *Characterization of synthesized silver nanoparticles*

Several techniques, including the transmission electron microscope (TEM) and the X-ray diffraction method (XRD), were used to characterize the synthesized particles. The size and shape of the synthesized AgNPs were ascertained by TEM analysis. When produced in small sizes, nanoparticles display superior physical properties, making them an excellent fertilizer. Nearly spherical nanoparticles with a mean diameter size of 24.444 nm were visible in the micrograph in Figure 2.

The crystalline nature of silver nanoparticles was confirmed by comparing the results of the X-ray powder diffractometer (XRD) spectrum with standards. Four intense peaks were seen in the spectrum of 2 theta values 38.15, 44.335, 64.443, and 77.392, formed peaks at 2µ values corresponding to 2.35706, 2.04066, 1.4468, and 1.23211 of Bragg reflections for silver metal (Fig. 3). From XRD data, the crystallite size of AgNPs can be computed. It was discovered that the mean size of the generated AgNPs was 50.9 nm. A face-centred cubic phase of crystalline silver was indicated by the presence of Bragg reflections that matched card code 174091 from the ICSD card, which corresponded to the (0 1 1), (1 11), and (0 0 2) planes.

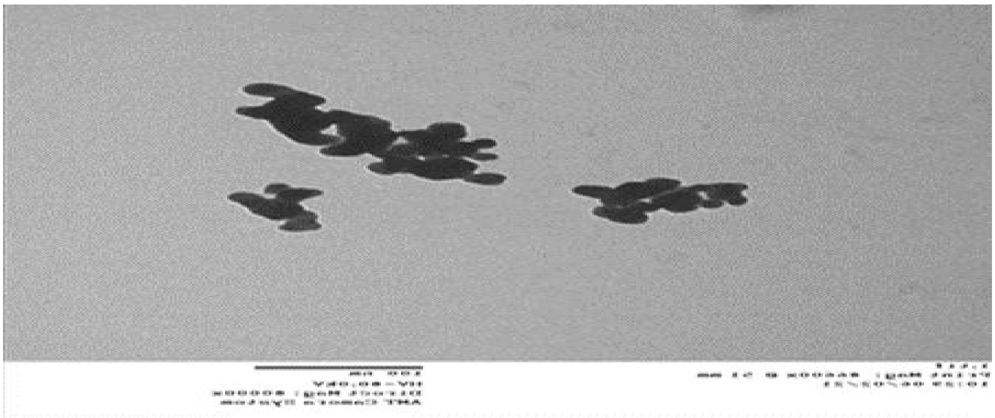

Fig. 2. Transmission electron microscope (TEM) image of spherical silver nanoparticles.

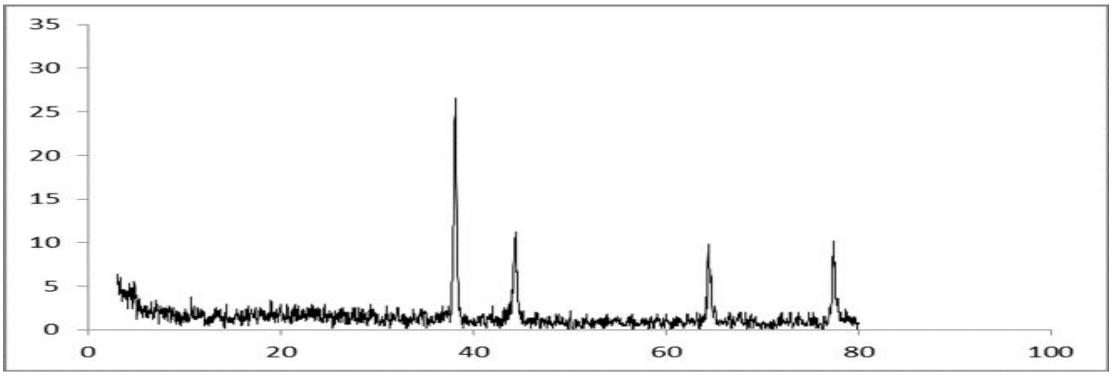

Fig. 3. X-ray diffraction plot of silver nanoparticles (AgNPs).

2.2. *Effect of spraying silver nanoparticles, spirulina extract, and its combination on the fresh and dry weight of French basil plants*

Fresh and dry weight: regarding the effect of foliar spray fertilization. As shown in Table 1 the foliar spray of AgNPs and spirulina extract had a marked effect on fresh and dry weight during the two seasons compared with control plants (91.8, 87.5 g) (14.98, 15 g), respectively at the two seasons. The highest fresh and dry weight achieved with AgNPs concentration 100 mg/L as follow (142.43, 109.25 g) (28.80, 19.63 g) at the two seasons, respectively, then decrease with increasing of AgNPs concentration.

Concerning the enhancing effect of spirulina foliar spray, the fresh and dry weight increased with increasing spirulina extract concentration; hence, a better effect on the fresh and dry weight of basil was found with 1.5 g/L spirulina (141.6, 106.1g) (21.4, 18.17g) at the two seasons, respectively. The significantly superior treatment was foliar spraying of AgNPs combined with foliar spray spirulina extract which produced the best values of fresh and dry weight over control. So, the treatment of 100 mg/L of AgNPs combined with 1.5 g/L spirulina extract achieved the best fresh and dry weight in both seasons (178.6, 118.9 g) (28.7, 20.5g) at the two seasons, respectively.

3.3. Essential oil percentage and yield

Foliar application of AgNPs significantly improved the essential oil content % and yield of *O. basilicum* cv. Grand vert plants compared with control (0.067, 0.060 %) (6.16 and 5.50 ml/plant) in both seasons, respectively. Concerning treatment with AgNPs at 100 mg/L was the most effective concentration of essential oil (%) and yield (0.132, 0.113%) (18.71, 11.31ml/plant) in both seasons, respectively, compared with the other used concentrations. Data in Table 3 shows that the increase in the concentration of spirulina extracts from 0.5 to 1.5 g/L has led to an improvement in the essential oil (%) and yield; thus,1.5g/L spirulina extract recorded the highest oil percentage and yield followed by (1 g/L). The highest improvement in the essential oil content % and yield occurs with foliar application of combination AgNPs and spirulina extract in general, the best treatment ever 100 mg/L AgNPs +1.5 g/L spirulina extract (0.140, 0.147%) (22.09, 16.07 mL/plant). The combination was the most effective when the AgNPs and spirulina were sprayed separately.

Table 1: Effects of AgNPs, spirulina extract, and their combination at different concentrations on the fresh and dry weight of basil plant in both seasons

AgNPs (mg L^{-1})	Fresh Weight (g)									
	First season Algae extract (Spirulina) g/L					Second season Algae extract (Spirulina) g/L				
	0	0.5	1	1.5	Mean of AgNPs	0	0.5	1	1.5	Mean of AgNPs
0	91.8±1.1^{j}	100±0.1^{i}	134.2±0.1^{c}	135.3±1.1^{c}	123.17	87.5±1.1^{g}	90.2±0.9^{f}	91.7±0.95^{e}	109.8±0.1^{b}	94.8
50	104.4±1.2^{hi}	121.9±1^{d}	116.7±0.9^{ef}	138.9±1^{bc}	120.48	98±0.5^{d}	90.5±0.76^{ef}	87.7±1.1^{g}	97.8±1.1^{d}	93.5
100	123.4±1^{d}	124.7±1.1^{d}	143±1^{b}	178.6±1.1^{a}	142.43	107.8±1.05^{bc}	100.8±0.1^{cd}	109.5±0.1^{b}	118.9±1^{a}	109.25
150	102.4±0.2^{i}	120.4±1de	108.9±1^{gh}	113.6±1.1^{fg}	111.33	101±1^{c}	86.3±1.05^{gh}	85±1^{h}	97.8±1.1^{d}	92.53
Mean ofAlgae	105.75	116.75	125.7	141.6		98.575	91.95	93.475	106.075	
L.S.D 0.05 AgNPs			2.4276					0.7363		
L.S.D 0.05 Algae			2.4276					0.7363		
LSD Ag xSp			**					**		

AgNPs (mg L^{-1})	Dry weight (g)									
	First season Algae extract (Spirulina) g/L					Second season Algae extract (Spirulina) g/L				
	0	0.5	1	1.5	Mean of AgNPs	0	0.5	1	1.5	Mean of AgNPs
0	14.98±1.11^{g}	17.08±1.01^{fg}	17.56±1.11^{f}	18.28±1.11^{f}	17.15	15±0.5^{g}	15.25±1.11^{g}	15.25±1.11^{g}	16.75±1.11^{efg}	15.56
50	17.04±1.01^{fg}	17.54±0.89^{f}	20.55±0.89^{e}	19.72±1.05^{e}	18.71	16.023±1.01^{efg}	16.25±1.11^{efg}	15.25±1.11^{g}	17.55±1.12^{cdef}	16.27
100	26.59±1^{c}	27.02±1.01^{bc}	28.7±1.1^{b}	32.88±1.05^{a}	28.80	19±1.53^{abc}	19.25±1.11^{abc}	19.75±0.89^{ab}	20.5±1^{a}	19.63
150	17.01±1^{fg}	23.89±1^{d}	20.62±1.11^{e}	25.66±1.11^{c}	21.80	16±1.5^{efg}	16±1.5^{efg}	17.25±1.11^{def}	17.88±1.1^{bcde}	16.78
Mean of Algae	20.65	21.275	21.4375	23.09		16.56825	16.8125	16.6875	18.17	
L.S.D 0.05 AgNPs			2.2679					0.954		
L.S.D 0.05 Algae			2.2679					0.954		
LSD AgNPsx Sp			**					NS		

The data are expressed as mean values± standard deviation; LSD refers to the least significant difference test. In each column, the same letter means non-significant difference, while different letters mean significant difference at $p \leq 0.05$.

Table 2: Influence of AgNPs, spirulina extract at various concentrations, and its combination on essential oil in basil plant during two seasons

AgNPs (mg L^{-1})	Oil%									
	First season					Second season				
	Algae extract (Spirulina) g/L					Algae extract (Spirulina) g/L				
	0	0.5	1	1.5	Mean of AgNPs	0	0.5	1	1.5	Mean of AgNPs
0	0.07±0.01^{ef}	0.09±0.01^{cd}	0.09±0.001^{cd}	0.1±0.01^{c}	0.087	0.06±0.01^{h}	0.06±0.01^{h}	0.07±0.01^{gh}	0.07±0.01^{fg}	0.065
50	0.08±0.01^{de}	0.08±0.01^{de}	0.09±0.001^{cd}	0.09±0.01^{cd}	0.087	0.07±0.011^{gh}	0.09±0.002^{de}	0.09±0.01^{cd}	0.09±0.01^{cd}	0.086
100	0.12±0.01^{b}	0.13±0.01^{ab}	0.14±0.01^{a}	0.14±0.01^{a}	0.132	0.113±0.01^{b}	0.09±0.001^{cd}	0.1±0.01^{cc}	0.15±0.01^{a}	0.113
150	0.06±0.01^{f}	0.07±0.01^{ef}	0.09±0.01^{cd}	0.1±0.01^{c}	0.08	0.07±0.01^{gh}	0.09±0.011^{de}	0.08±0.01^{ef}	0.09±0.001^{cd}	0.082
Mean of Algae	0.0818	0.0918	0.1033	0.108		0.0777	0.0828	0.085	0.102	
L.S.D 0.05 AgNPs	0.102					0.1019				
L.S.D 0.05 Algae	0.102					0.1019				
LSD AgNPs x Sp	**					**				
AgNPs (mg L^{-1})	Oil Yeild									
	First season					Second season				
	Algae extract (Spirulina) g/L					Algae extract (Spirulina) g/L				
	0	0.5	1	1.5	Mean of AgNPs	0	0.5	1	1.5	Mean of AgNPs
0	6.16±1.01^{g}	8.83±1.1^{f}	8.90±1.1^{ef}	9.84±1.11^{ef}	8.431	5.50±1.1^{h}	6.71±1.11^{fgh}	6.77±1.11^{efgh}	8.73±1.11^{cd}	6.928
50	8.35±1.1^{f}	11.54±1.1^{de}	11.19±1.01^{de}	12.94±1.01^{cd}	11.004	6.53±1.11^{gh}	7.33±1.12^{defg}	7.16±1.01^{defgh}	9.13±1.01^{c}	7.538
100	12.34±1.11^{cd}	19.83±1.1^{b}	20.09±1^{b}	22.60±1.1^{a}	18.713	12.22±1.11^{b}	8.43±1.11^{cdef}	8.50±1.1^{cde}	16.07±1^{a}	11.306
150	11.99±1^{cd}	12.63±1.1^{cd}	12.92±1.01^{cd}	13.52±1.11^{c}	12.764	6.72±1.11^{efgh}	8.04±1.01^{cdefg}	8.18±1.01^{cdefg}	9.15±1^{c}	8.025
Mean of Algae	9.7087	13.2052	13.2750	14.7236		7.7439	7.6294	7.6526	10.7713	
L.S.D 0.05 AgNPs	0.8881					0.8913				
L.S.D 0.05 Algae	0.8881					0.8913				
LSD AgNPs x Sp	**					**				

The data are expressed as mean values ± standard deviation; LSD refers to the least significant difference test. In each column, the same letter means non-significant difference, while different letters mean significant difference at $p \leq 0.05$.

3.4. Chemical analysis of the essential oil

The GC–MS analysis of the volatile oil samples taken from the second season of the experiment indicated the chemical composition of essential oils of *Ocimum basilicum* L var. Grand vert plants sprayed with AgNPs and spirulina extract foliar applications are presented in Table 3. In total, 18 components were identified in the essential oil of all tested plant samples.

Table 3: Effect of spraying AgNPs, spirulina extract, and its combination at various concentrations on the composition of the essential oil in the second season

Components (%)	Control	Spirulina (1.5 g/L)	AgNPs (100 mg/L)	AgNPs (100 mg/L) + Spirulina (1.5 g/L)
Linalool	29.93	23.86	32.3	19.61
Eugenol	12.09	7.94	24.53	11.42
Methyl chavicol	1.22	1.04	1.69	0.97
Methyl Eugenol	2.08	1.82	2.72	2.2
Geranylpropanoate	0.89	0.74	1.44	1.05
Bornyl acetate	1.29	0.9	1.01	0.79
Carvacrol	0.52	4	----	3.27
α-Cubebene	0.49	0.4	---	0.44
β –Elemene	5.02	28.43	-----	26.39
α-*trans*-Bergamotene	3.92	3.44	4.94	2.84
β-Farnesene	0.42	0.3	---	0.46
γ-Muurolene	0.51	0.4	0.86	0.64
α-selinene	2.22	2.09	3.78	2.57
β-Bisabolene	1.55	1.23	2.53	1.99
γ-Cadinene	0.59	0.56	----	0.7
δ-Cadinene	2.98	1.91	3.72	2.83
cis-Nerolidol	0.59	0.3	---	0.69
γ-Eudesmol	0.99	0.85	1.28	1.43

Our study demonstrated that the major compounds in the essential oil in all tested samples were linalool, methyl chavicol, eugenol, Methyl Eugenol, and α-trans-Bergamotene. Linalool and eugenol were the major constituents of essential oil in plants treated with the different treatments of AgNPs and algae extract, as detected by GC-analysis. The GC-MS analysis of French basil volatile oils as shown in (Table 3) proved the presence of linalool and eugenol as the main dominant components in volatile oils composition. The highest values of linalool (32.3 %) which obtained with (100 mg/L AgNPs) followed by control, (1.5g/L spirulina extract) then (100 mg/L AgNPs+1.5 g/L spirulina) which resulted in (29.93, 23.86 and 19.61 % linalool), respectively. Moreover, the highest accumulation of eugenol (24.00 %) was obtained from plants treated with 100 mg/L AgNPs followed by control, 100 mg/L AgNPs+1.5 g/L spirulina and 1.5 g/L spirulina which recorded (12.09, 11.42, 7.94% eugenol), respectively. It seems that the foliar application of these compounds had positive effects on the biosynthesis and accumulation of these high–valued compounds. Additionally, the percentage of eugenol in treated plants with 100 mg/L AgNPs was 24.00% compared to 12.09% in the untreated plants while the least value of eugenol was 1.5 g/L spirulina extract. The highest methyl chavicol was obtained with 100 mg/L AgNPs (1.69%) followed by control, 1.5 g/L spirulina (1.41, 1.04%), respectively. The lowest content of methyl chavicol was with 100 mg/L AgNPs+1.5g/L spirulina (0.97% methyl chavicol). The highest value of methyl eugenol accumulated with 100 mg/L AgNPs (2.72%) followed by 100 mg/L AgNPs+1.5 g/L spirulina extract which accumulated (2.2%) methyl eugenol then control (2.19%) while the least content of methyl eugenol was accumulated with (1.5 g/L algae) (1.82% methyl eugenol). From the above results, the highest percentage of eugenol, methyl chavicol, and methyl eugenol in treated plants with 100 mg/L was (24.53%, 1.69%, and 2.72%) compared to the untreated plants (12.09, 1.22 and 2.08%), respectively. The highest significantly superior treatments of carvacrol and β –Elemene were with the spraying of 1.5 g/L algae (4, 28.43 %), respectively. followed by 100 mg/L AgNPs with 1.5g/L spirulina extract (3.27, 26.39%), respectively while the control was (0.52,5.02%). Furthermore, the highest amount of γ-Cadinene, cis-Nerolidol, and γ-Eudesmol resulted in the essential oil of plants treated with a combination (100 mg/L AgNPs+1.5g/L spirulina extract) (0.7, 0.69, 1.43%), respectively. Not only the treatment 100 mg/L AgNPs accumulated the highest value of the first main component (linalool) but also the highest significant values of the second, third, and fourth main components (methyl chavicol, eugenol, and methyl eugenol) over the control treatment. Additionally, increased amount of γ-Muurolene, α-selinene, and β-Bisabolene (0.86, 3.78, and 2.53%) compared with control, followed by 100 mg/L AgNPs + 1.5 g/L spirulina extract.

3.5. Chemical characterization of Basil plant

The chemical composition of *Ocimum basilicum* L. var. Grand vert plants was evaluated and the results are given in Table 4, carbohydrates in the herb were the most abundant macronutrients. Total carbohydrates showed significant decreases with spraying AgNPs, spirulina extract, and the combination AgNPs+ spirulina extract. Therefore, the amount of total carbohydrates was 67.145 g /100g DW achieved with 1.5 g/L spirulina+100 mg/L AgNPs followed by 100 mg/L AgNPs and 1.5 g/L (65.803 and 59.512 g /100g DW), respectively compared with control (68.388 g /100g DW). Moreover, ash levels had no significant increase with 1.5 g/L spirulina (13.939 g/100 g DW) compared with control (13.679 g/100 g DW) also; total ash decreased with 100 mg/L and the combination. The levels of protein were significantly increased with all treatments, there was a significant increase with 1.5g/L spirulina+100 mg/L AgNPs (15.621g/100g DW), also total protein increased with 100 mg/L AgNPs and 1.5g/L spirulina (13.341, 12.801 g/100g DW), respectively compared with control (9.649 g/100g DW). Also, data showed that the total lipid content had a significant increase with 1.5g/L spirulina (13.994 g/100g DW) and then a decrease with 1.5g/L spirulina+100 mg/L AgNPs and 100 mg/L AgNPs (9.111, 8.637 g/100g DW), respectively compared with control (8.284 mg/L AgNPs).

Table 4: Effects of AgNPs, spirulina extract, and its combination on the chemical composition of French basil plants

Treatment	Protein (%)	ASH (%)	Lipid (%)	Carbohydrate (%)
Control	9.649±0.06^{c}	13.679±0.46^{a}	8.284±0.11^{c}	68.388±0.63^{a}
1.5 g/L spirulina	12.801±0.06^{b}	13.939±0.08^{a}	13.994±0.13^{a}	59.512±0.13^{c}
100 mg/L (AgNps)	13.341±0.51^{b}	12.219±0.20^{b}	8.637±0.37^{bc}	65.803±1.08^{b}
100 mg/L (AgNps)+1.5 g/L spirulina	15.621±0.36^{a}	8.114±0.07^{c}	9.111±0.10^{b}	67.154±0.13^{ab}
LSD 0.05	0.6024	0.687	0.8515	1.854

The data are expressed as mean values± standard deviation; LSD refers to the least significant difference test. In each column, the same letter means non-significant difference, while different letters mean significant difference at $p \leq 0.05$.

3.6. Malondialdehyde (MDA) levels

Data in Fig. 4 showed a significant decrease in MDA with 1.5 g/L spirulina extract followed by 100 mg/L AgNPs +1.5 g/L spirulina extract (42.787 and 47.59 n mol/g tissue), respectively. On the contrary, an increase in MDA (78.67 n mol/g tissue) was observed with AgNPs application at 100 mg/L. However, the untreated French basil plants recorded 56.88 n mol/g tissues MDA.

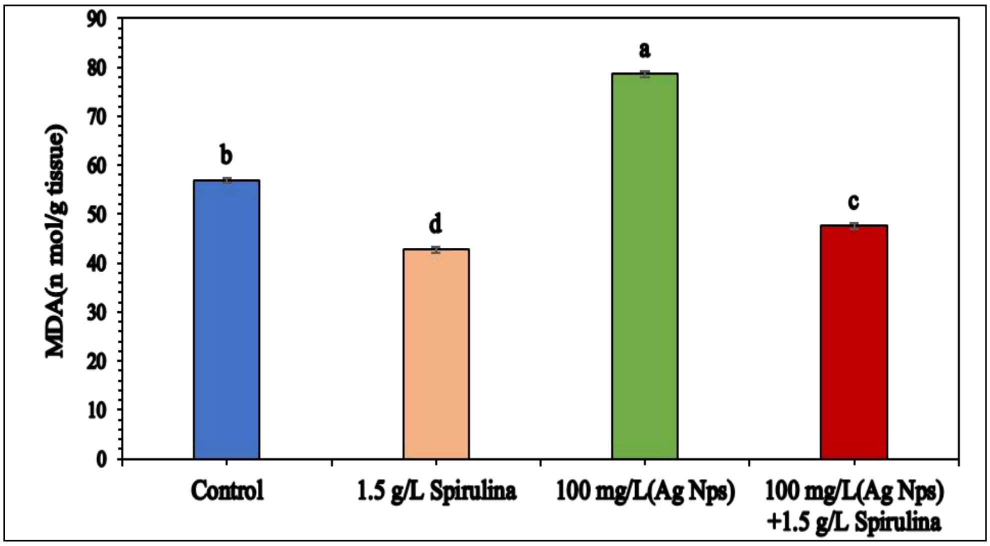

Fig. 4. Effects of application of AgNPs, spirulina extract, and its combination on plant MDA levels in French basil plants.

3.7. Antioxidant Activity Analysis

Regarding the enzymatic antioxidant compounds in French basil, statistically significant differences were observed in all evaluated enzymes. Foliar application of AgNPs, spirulina extract, and the interaction had a positive effect on CAT, GPx, and SOD compared with the control (Fig. 5). The highest value of CAT enzyme was observed with 100 mg/L AgNPs (29.45 IU/g tissue) compared with control (19.99 IU/g tissue) followed by 100 mg/L AgNPs+1.5 g/L spirulina extract and 1.5 mg/L AgNPs (26.048, 22.65 IU/g tissue), respectively.

The SOD enzymatic activity also increased significantly with foliar application of 100 mg/L Ag NPs, spirulina extract, and the interaction; hence, the highest value generated with 100 mg/L AgNPs (16544.12 IU/g tissue) followed by 100 mg/L AgNPs + 1.5 g/L spirulina extract and 1.5 g/L spirulina which recorded (11029.4, 11026.4 IU/g tissue), respectively compared with the control (5429.99 IU/g tissue).

While The GPx enzyme showed a similar effect since 100 mg/L AgNPs generated the highest value (22.623 IU/g tissue) followed by 100 mg/L AgNPs + 1.5 g/L spirulina (20.711 IU/g tissue) since 1.5 g/L spirulina extract treatment generated the low value (19.342 IU/g tissue) compared with control (12.919 IU/g tissue).

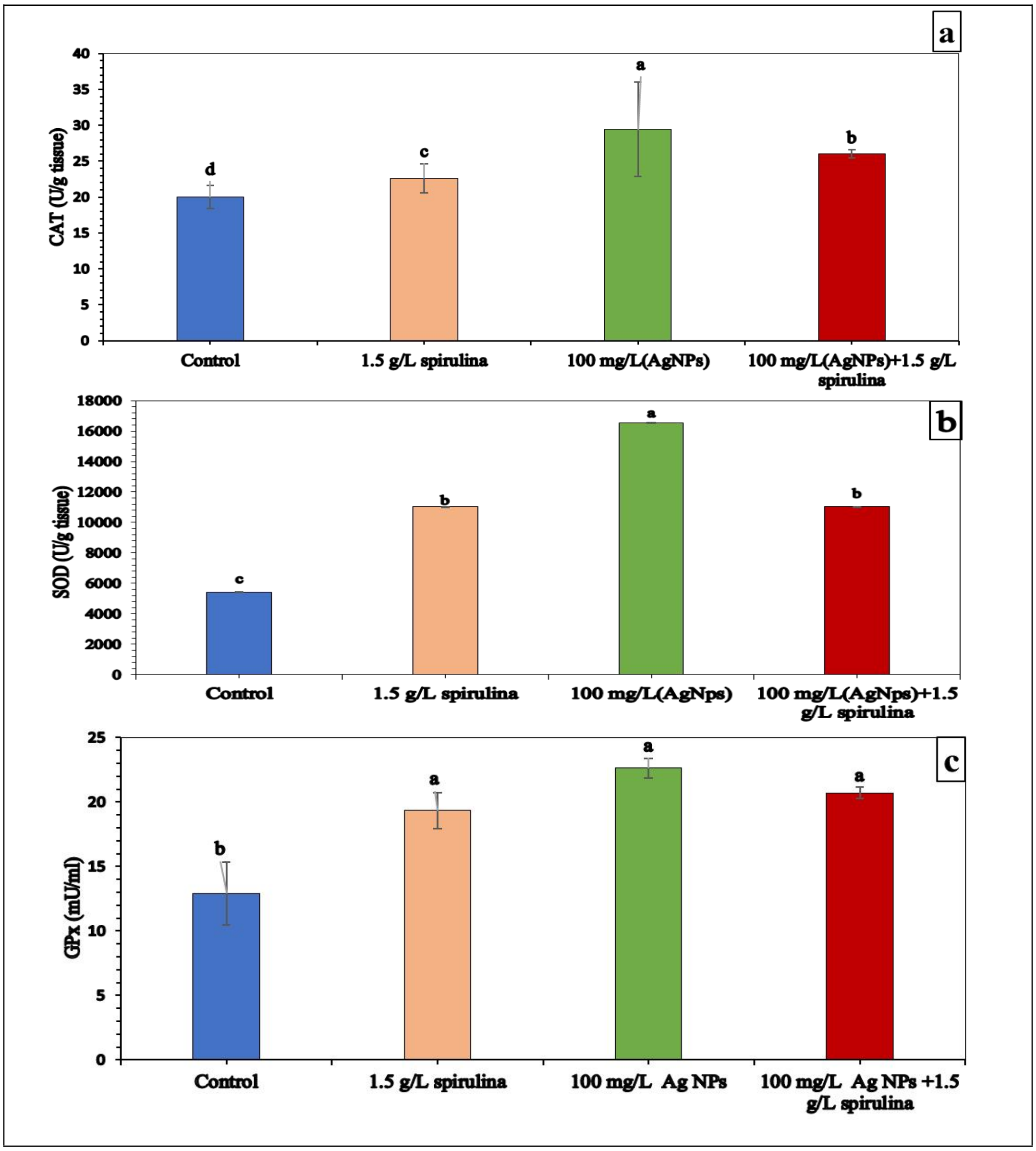

Fig. 5. The enzymatic antioxidant activity (a) Catalase (CAT), (b) Superoxide dismutase's activity (SOD) and (c) Glutathione peroxidase activity (GPx) levels in French basil with the application of AgNPs, spirulina extract, and its combination. Vertical bars represent the means of three independent determinations ± standard error (SE). The different letters are significantly different between treatments at the 0.05 level.

3. Discussion

In the present study, the results clearly illustrated the effect of separated or combined foliar applications of silver nanoparticles or green algae as bio-fertilizers in French basil. First, we characterized the synthesized AgNPs by TEM which exhibit small spherical nanoparticles. The minimum diameter was 20.7 nm and the maximum diameter was 46.2nm, and Dev (rms) =10.89 nm [44,45]. As well as the crystallite size of AgNPs shown by X-ray diffractometer (XRD) agreed with [46,47].

Nanoparticles derived from natural sources that are more acceptable and effective, environmentally safe, and efficient plant bio-stimulant [48]. Separated foliar sprays of AgNPs specifically at 100 mg/Lor spirulina at 1.5 g/L increased fresh and dry weight in both seasons. These findings are matched with some studies [49,50] that confirmed these results on lily growth and flowering which were content of greenness index and the highest fresh weight with the best positive AgNP concentration at 100 ppm. There is a decrease in varied germination percentages on *Lupinus termis* L. seeds with increasing the AgNPs concentration over 100 mg/L [47]. In addition, increasing concentrations of spirulina extract in soil fertilization up to 4000 mg/L with a simper florence begonia plant led achieved a great improvement in the growth parameters, phosphorus, potassium, and chlorophyll yield of leaves and the plant quality [26]. Furthermore, Anitha [51] reported that *Spirulina platensis* has a stimulatory effect in improving the growth of Bayam Red plants cultivated in soil supplemented with spirulina showed increased plant height by 58.3%, as well as greater fresh and dry weights by 110.1% and 155.8% respectively when compared with the control group. The application of foliar spray with *Spirulina platensis* extract on plants achieved great enhancement, this effect might be owed to its high content of free amino acids, and elements, as well as its high content of plant hormones [52,53]. According to the results presented by Toaima [54] who confirmed that the best dry weight per plant occurred with spirulina extract at 1.5 liter/feddan. Our results demonstrated a great effect with the combination of spirulina and AgNPs, the significantly superior treatment was spraying 100 mg/L of AgNPs combined with 1.5 g/L spirulina extract which produced the most plant height, and number of branches in both seasons. These results were in agreement with a study concluded that the maximum increases in growth, fruits, and oil yields were obtained when Dutch fennel plants were treated with the combination of sowing dates, organic fertilization, and foliar spray of *Spirulina platensis* algae extract [55]. The same trends of results are obtained by Anitha et al. [52], who combined spirulina with organic manure in 50:50 proportions (54.4 ± 1.69 ppm) have recorded highly significant zinc uptake by *Phaseolus aureus* which has a good effect on yield and grain zinc concentration also, improves the shoot growth. Furthermore, Sadak [56] also indicated that using a foliar application of AgNPs combined with folic acid at different concentrations resulted in a great increase in flax plant growth (shoot fresh, dry weight, and shoot length).

Fertilization treatments in foliar spray had a significant effect on the essential oil content %, yield, and chemical composition in *O. basilicum* cv. Grand vert plants compared with control, we observed that the foliar spraying of AgNPs had a positive impact in increasing oil seed and fatty acid contents of sunflower plants [46]. In addition, Hatami [57] suggested that the application of AgNPs might preserve and improve the quality and amount of essential oil of *Pelargonium graveolens* plants. Also studies confirmed that using a foliar spray of *Spirulina platensis* extract made great improvement in essential oil accumulation in Dutch fennel plants compared with untreated plants [55]. Spirulina contains not only a great number of amino acids, and macro and micro elements but also several plant hormones, all these cause the plant to increase vital production of the plants and improve the survival ability in different stressful surroundings.

The combination was the most effective when the AgNPs and spirulina were sprayed separately. These results were in line with Abd El-Aleem [55] which combined the highest level of compost manure with spirulina extract foliar application with early sowing date recorded best increases in yield/plant and yield/feddan of Dutch fennel plant essential. Additionally, Sadak [56] showed that the potential effect of AgNPs foliar treatments on flax plants could be valuable for plant growth, photosynthetic pigments, indol acetic acid, phenolics contents and yield quantity and quality; on the other hand, Hatami [57] obtained maximum Citronellol/geraniol ratio (C/G) in Pelargonium species oils at 80 mg/L AgNPs treatment and beneficial positive influence of silver nanoparticles on essential oil quality of Pelargonium plants. Also, Silveira [58] showed that linalool and eugenol are present in variable volumes and strongly impact the plant flavor.

The chemical composition of *Ocimum basilicum* L. var. Grand vert plants were evaluated and the results were in good harmony with Elbanna [59] showed that, the stimulatory effect of nano fertilizer on enhancing plant functions, chemical and biological properties, and plant growth and production because nano-fertilizers deliver nutrients easily in a nano form [60,61]. Moreover, Jameel[62] interpreted the increase of total protein by 1% as a considerable increase in proline contents by using nano fertilizers at the recommended level on sunflower plants. Improvement in the growth of spinach enhanced the content of chlorophyll and protein. Yang [63] evaluated that application with nano fertilizer can significantly enhance nitrogen accumulation and its use efficiency and also induce the expression of genes related to nitrogen metabolism [64]. Furthermore, Rady [65] confirmed that spirulina extract acts as a biofertilizer when used as a foliar application hence 40 mg spirulina /plant improved significantly the NPK as well as protein content. Also, data showed that the total fat content had a significant increase. In the same line, Godlewska [66] reported that spirulina extract application can increase the plant's micro- and macro elements amounts. In addition, Osman [67] stated that spirulina suspension can protect plant roots and shoots from the risky effects of herbicides because spirulina has a great influence on the enhancement of synthesis protein in roots. Dowom [68] investigated that the application of chitosan nanoparticles improved total chlorophyll, flavonoids, total soluble sugar, proline, and protein in *S. abrotanoides* plants.

MDA is a product of polyunsaturated fatty acids peroxidation in the cells. It is caused by the increase in free radicals and is known as a marker of oxidative stress. Data showed a significant decrease in MDA with 1.5 g/L spirulina extract followed by 100 mg/L AgNPs +1.5 g/L spirulina extract. On the contrary, an increase was observed with AgNPs application at 100 mg/L. As well as Iqbal [69] stated that AgNPs application on wheat plants under heat stress increases protein and normalized MDA levels via the prevention of proteolysis and the degradation of the membrane. Furthermore, using a high concentration of CuNPs or AgNPs with seedlings of turnip led to increasing production of H_2O_2 and lipid peroxidation [59,70]. Additionally, Selem [71] indicated that super influence of foliar spray *spirulina platensis* at 100 mg/L on *Vicia faba* L. plants under salt stress reduced the activity of the plant MDA but also loosen Salinity effect inside the plant via decrease the high activity of antioxidant enzymes by improving plant physical and chemical properties. Rady [65] proved that using foliar spraying spirulina extract at high concentrations with bean plants under stress led to the best growth and bean production by preventing oxidative and osmotic stresses and their effects.

The results strongly suggested that the use of spirulina in combination with AgNPs has reduced the toxicity of separated foliar spray of AgNPs within the plant by altering the physicochemical properties. Consequently, the levels of antioxidant enzymes were decreased by using the spirulina foliar application with AgNPs. The highest value of CAT, GPx, and SOD enzyme was observed with 100 mg/L AgNPs followed by the combination 100 mg/L AgNPs+1.5 g/L spirulina extract. These results coupled with Selem [71] showed that using the combination of spirulina with salinity on *Vicia faba* L. Plants decreased the enzyme activity values of the SOD and catalase compared with plants treated with salinity without spirulina. A previous study by Manaf [72] observed that the antioxidant enzyme activity showed a significant correlation with all growth Parameters in the wheat plant. Mehrian [73] found that applying AgNPs at high concentrations on *Lycopersicon esculentum* had a great increase in peroxidase and catalase levels. As well as Barbasz [74] studied the influence of increasing of AgNPs concentration at 20:60 mg/L which enhances the catalase and peroxidase activity in the sugar cane callus on the media supplied with AgNPs. Dowom et al. approved that chitosan nanoparticles treatment enhanced the activity of antioxidant enzymes; superoxide dismutase (86%), guaiacol peroxidase (75.7%), and polyphenol oxidase (72.8%) [68]. As well as, Hassan [75] confirmed that applying spirulina extract at 5% as a foliar spray on the *Eruca sativa* plant improved considerably all growth parameters, quality, quantity, and antioxidant activity. Furthermore, copper nanoparticles combined with spirulina decrease the activity of antioxidant enzymes. Nanochitosan has the ability to activate the antioxidant enzymes in plants against different pathogens [59,76].

4. Conclusions

Our data suggested an association nano fertilizer (AgNPs) with specific biofertilizer blue green algae spirulina and significant increase the main components of essential oil (linalool and methyl chavicol) of French basil plants. Foliar application of all treatments increased significantly growth, pigments essential oil percentage, and yield of French basil at the addition of spirulina extract or AgNPs at different concentrations. Whereas 100 mg/L AgNPs was the greatest among AgNPs treatments, in addition, 1.5 g/L spirulina extract was the best moreover, 100 mg/L AgNPs +1.5 g/L spirulina extract was the superior one compared with the control treatment. Moreover, nano fertilizer-treated plants appeared more antioxidant activities (CAT, SOD, and Gpx activities) and malondialdehyde contents. AgNPs foliar application is recommended for high quantity and quality of basil based on its concentration and the type of used application so, AgNPs impact will be improved when combined with natural fertilizer spirulina which reduces possible hazardous effects of nanoparticles. Therefore, the super attention would be paid to applying nanomaterials with biofertilizers for large-scale production of medicinal plants for sustainability with low environmental cytotoxicity. And then encourage export abroad in the medicinal and aromatic plants sector.

5. Conflicts of interest

The authors declare that there are no conflicts to declare.

6. Formatting of funding sources

This research did not receive any external funding.

7. Acknowledgments

Not applicable.

8. References and Bibliography

[1] WHO (2018) WHO guidelines on good herbal processing practices for herbal medicines. In WHO technical report series; World Heal Organization: Geneva, Switzerland, pp 81–152.
[2] FAO (2017) Proposal for new work on codex standard for basil. Codex committee on spices and culinary herbs, 1-6.
[3] Abdel-Hamid NS, Makled SMS, Abd Elmonem SMA (2019) Determinants of production and export for some medicinal and aromatic plants in Egypt. Arab Univ J Agric Sci 27:1351-1369.
[4] Mrabti HN, Bouyahya A, Ed-Dra A, Kachmar MR, Mrabti NN, Benali T, Shariati MA, Ouahbi A, Doudach L, Faouzi MEA (2021) Polyphenolic profile and biological properties of *arbutus unedo* root extracts. Eur J Integr Med 42:101266.
[5] Alkhateeb MA, Al‐ Otaibi WR, AlGabbani Q, Alsakran AA, Alnafjan AA, Alotaibi AM, Al‐ Qahtani W S (2021) Low‐ temperature extracts of Purple blossoms of basil (*Ocimum basilicum L.*) intervened mitochondrial translocation contributes prompted apoptosis in human breast cancer cells. Biol Res 54:2.
[6] Aminian AR, Mohebbati R, Boskabady MH (2022) The Effect of *Ocimum basilicum* L. and its main ingredients on respiratory disorders: An experimental, preclinical, and clinical review. Frontiers in Pharmacology 12:1-14.
[7] Tangpao T, Chung HH, Sommano SR (2018) Aromatic profiles of essential oils from five commonly used Thai Basils. Foods 7:175.
[8] Amer A, Ghoneim M, Shoala T, Mohamed H I (2021) Comparative studies of eco-friendly compounds like humic acid, salicylic, and glycyrrhizic acids and their nanocomposites on French basil (*Ocimum basilicum* L. cv. Grand verde). Environmental Science and Pollution Research. https://doi.org/10.1007/s11356-021-14022-1
[9] Verma S (2016) Chemical constituents and pharmacological action of *Ocimum sanctum* (Indian holy basil-Tulsi). J Phytopharmacol 5: 205-207.
[10] Hiremath C, Sundaresani V, Baskaran K, Kumar A, Nd Y, Yadav MK, Verma RS, Srinivas KS, Kumar JK, Kumar N, Kalra A, Tripath AK (2020) A linalool rich variety-CIM-Sukhda of *Ocimum basilicum*. Journal of Medicinal and Aromatic Plant Sciences 42(1-2), 68-72.
[11] Khan S, Zahoor M, Khan R S, Ikram M, Islam N U (2023) The impact of silver nanoparticles on the growth of plants: The agriculture applications. Heliyon. 9(6)16928.

[12] Mehmood A (2018) Brief overview of the application of silver nanoparticles to improve growth of crop plants. IET Nanobiotechnol 12:701-705.
[13] Verma K AK, Song X P, Joshi A, Tian D D, Rajput V D, Singh M, Arora J, Minkina T, Li YR (2022) Recent Trends in Nano- Fertilizers for Sustainable Agriculture under Climate Change for Global Food Security. Nanomaterials 12, 173.
[14] Akhilraj B C, Suresh J, Rajamani K, Kuma M (2023) Exploring the therapeutic potential of volatile bioactive compounds from different parts of *Tinospora cordifolia*: a gas chromatography-mass spectrometry (GC-MS) study: Therapeutic potential of bioactive compounds from Tinospora.. Journal of Spices and Aromatic Crops. 32 (1): 90-100 (doi:10.25081/josac.2023.v32.i1.8592.
[15] Bhaskar M, Kumar A, Rani R (2023) Biocatalysis and Agricultural Biotechnology. Application of nano formulations in agriculture. https://doi.org/10.1016/j.bcab.2023.102934.
[16] Banne SV, Patil MS, Kulkarnic RM, Patilb SJ (2017) Synthesis and characterization of silver nano particles for EDM applications. Materials Today 4:12054–12060.
[17] Zhang XF, Liu ZG, ShenW, Gurunathan, S (2016) Silver nanoparticles: Synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci 17(9):1534.
[18] Jadczak P, Kulpa D, Drozd R, Przewodowski W, Przewodowska A (2020) Effect of AuNPs and AgNPs on the antioxidant system and antioxidant activity of lavender (*Lavandula angustifolia* Mill.) from in vitro cultures. Molecules 25(23):610-624.
[19] Satti SH, Raja NI, Javed B, Akram A, Mashwani ZUR, Ahmad MS, Ikram M (2021) Titanium dioxide nanoparticles elicitedagro-morphological and physicochemical modifications in wheat plants to control *Bipolaris sorokiniana*. PLoS ONE 16:1-19.
[20] Zare Z, Pishkar L, Iranbakhsh A, Talei D (2020) Physiological and molecular effects of silver nanoparticles exposure on purslane (*Portulaca oleracea* L.). Russ J Plant Physiol 67:521–528.
[21] Khan S, Zahoor M, Khan R S, Ikram M, Islam N U (2023) The impact of silver nanoparticles on the growth of plants: The agriculture applications. Heliyon. 9(6)16928.
[22] Kale SK, Parishwad GV, Husainy A S N, and Patil AS (2021). Emerging Agriculture Applications of Silver Nanoparticles.ES Food Agrofor. 3:17-22.
[23] Parveen A, Rao S (2015). Effect of nanosilver on seed germination and seedling growth in Pennisetum glaucum. J Clust Sci 26(3):693–701.

[24] Win TT, Barone GD, Secundo F, Fu P (2018) Algal biofertilizers and plant growth stimulants for sustainable agriculture. Indust Biotechnol 14(4):203–211.
[25] Shedeed ZA, Gheda S, Elsanadily S, Alharbi K, Osman MEH (2022) *Spirulina platensis* biofertilization for enhancing growth, photosynthetic capacity and yield of *Lupinus luteus*. Agriculture 12: 781.
[26] Jowkar A, Bashiri1 K, Golmakani MT (2017) The effect of soil fertilization and foliar spray of semperflorens begonia (*Begonia semperflorens*) by Spirulina cyanobacterium biomass. J. Sci. & Technol. Greenhouse Culture 8(3)65-74.
[27] Sami MKH, Ali MA, Gaber MK, Sidky MMA,and Farroh KY (2022) Growth, Productivity and Quality of Sweet Basal in relation to Minerals, Nanoparticles of Chitosan for NPK Fertilization.. Journal Biology. Sci. 13(2):179-193.`
[28] Abbasifar A, Shahrabadi F, ValizadehKaji B (2020) Effects of green synthesized zinc and copper nanofertilizers on the morphological and biochemical attributes of basil. plant Journal of plant nutrition. https://doi.org/10.1080/01904167.2020.1724305.
[29] Genady, EA, Ahmed, SS, Fahmy, AH and Ashour, RM (2017) Copper sulfate nanoparticles enhance growth parameters, flavonoid content and antimicrobial activity of *Ocimum basilicum* Linnaeus . Journal of American Science;13 (4).
[30] Jadczak P, Kulpa D, Drozd R, Przewodowski W, Przewodowska A (2020) Effect of AuNPs and AgNPs on the antioxidant system and antioxidant activity of lavender (*Lavandula angustifolia* Mill.) from in vitro cultures. Molecules 25(23):610-624.
[31] Sadak MS, Bakry BA, El-Karamany MF (2022) Agrophysiological variation in flax affected by folic acid and Ag nanoparticles foliar applications. Asian J Plant Sci 21:339-346.
[32] Santini G, Rodolfi L, Biondi N, Sampietro G, Tredici M R (2022) Effects of cyanobacterial-based biostimulants on plant growth and development: a case study on basil (*Ocimum basilicum* L. (34)2063–2073.
[33] Elansary HO, Mahmoud EA, El-Ansary DO, and Mattar MA (2012) Effects of water stress and modern biostimulants on growth and quality characteristics of Mint. agronomy 10(6)1-16; doi:10.3390/agronomy10010006.
[34] Shawky AA, Khalifa GS, Hegazi A, ElSherif M. Growth, Productivity, and Essential Oil Content of Fennel Plants Treated with *Spirulina Platensis* Extract and Compost Tea Under Low Nitrogen Doses. Gesunde Pflanzen. 2023. doi:10.1007/s10343-023-00870-z.
[35] Ghazy NA, Abd El-Hafez OA, El-Bakery AM, and El-Geddawy DIH (2021) Impact of silver nanoparticles and two biological treatments to control soft rot disease in sugar beet (*Beta vulgaris* L). Egyptian Journal of Biological Pest Control. 31:3.
[36] British pharmacopoeia. (1963) Determination of Volatile Oil in Drugs. The pharmaceutical press, 17, Bloomsbury square, London, WCI. England, Xiii, 112.
[37] Adams RP (2001) Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corporation, Carol Stream.
[38] A.O.A.C. (2000) Official Methods of Analysis of the Association of Official Agriculture Chemists., 17^{th} ed., Published by USA, 630P.
[39] Aebi H (1984) Methods Enzymol 105:121 –126.

[40] Satoh, KEI. (1978) Serum Lipid Peroxide in Cerebrovascular Disorders Determined by a New Colorimetric Method. Clinica Chimica Acta, 90, 37-43.

[41] Paglia DE, Valentine WN (1967) J Lab Clin Med 70:158 – 169.

[42] Nishikimi M, Roa NA, Yogi K (1972). Biochem Bioph Res Common 46:849 – 854.

[43] SAS/STAT Users Guide (1996) Version 6, 12th Ed. SAS Institute, Inc. Cary, North Carolina, 2: 846.

[44] Orlowski P, Tomaszewska E, Gniadek M, Baska P, Nowakowska J, Sokolowska J, Nowak Z, Donten M, Celichowski G, Grobelny J, Krzyzowska M (2014) Tannic acid modified silver nanoparticles show antiviral activity in herpes simplex virus type 2 infection. PLoS ONE 9: e104113.

[45] Abo‑ El‑ Yazid Z H, Ahmed O K, El‑ Tholoth M, Ali M A (2022) Green synthesized silver nanoparticles using *Cyperus rotundus* L. extract as a potential antiviral agent against infectious laryngotracheitis and infectious bronchitis viruses in chickens. Chem Biol Technol Agric 9:55.

[46] Batool SU, Javed B, Sohail, Zehra SS, Mashwani Z, Raja NI, Khan T, ALHaithloul HAS, Alghanem SM, Al-Mushhin AAM, Hashem M, Alamri S (2021) Exogenous applications of bio-fabricated silver nanoparticles to improve biochemical, antioxidant, fatty acid and secondary metabolite contents of sunflower. Nanomaterials 11:1750-1766.

[47] Al-Huqail AA, Hatata MMb, AL-Huqail AA, Ibrahim MM (2018) Preparation, characterization of silver phyto nanoparticles and their impact on growth potential of *Lupinu stermis* L. seedlings. Saudi Journal of Biological Sciences 25:313–319.

[48] Matthews S, Ali A, Siddiqui Y, Supramaniam CV (2022) Plant Bio-stimulant: Prospective, Safe and Natural Resources. Journal of Soil Science and Plant Nutrition 22:2570–2586.

[49] Salachna P, Byczynska A, Zawadzinska A, Piechocki R, Mizielinska M (2019) Stimulatory Effect of Silver Nanoparticles on the Growth and Flowering of Potted Oriental Lilies. Agronomy 9: 610-624.

[50] Byczyńska A., Zawadzińska A. and Salachna P. (2019) Silver nanoparticles preplant bulb soaking affects tulip production. Acta Agriculture Scandinavica, Section B — Soil & Plant Science. 69(3).250–256.

[51] Wuang SC, Khin MC, Chua PQD, Luo YD (2016) Use of spirulina biomass produced from treatment of aquaculture wastewater as agricultural fertilizers. Algal Res 15:59–64.

[52] Anitha L, Bramari GS, Kalpana P (2016) Effect of supplementation of Spirulina platensis to enhance the zinc status in plants of *Amaranthus gangeticus*, Phaseolus aureus and tomato. Advances in Bioscience and Biotechnology 7:289-299.

[53] Guerrero TM, Johnson CB (2000) Season has a major effect on the essential oil yield response to nutrient supply in *Origanum majorana*. J Hort Sci and Biotech 75:520-527 .

[54] Toaima WIM, Osman YAH, Hamed ES (2017) Comparative study for the effect of spraying with blue green algae extracts on some Russian dill varieties under El-Maghara region - North Sinai conditions. Journal of Medicinal Plants Studies 5:112-119.

[55] Abd El-Aleem W, Hendawy SF, Hamed ES, Toaima WIM (2017) Effect of planting dates, organic fertilization and foliar spray of algae extract on productivity of Dutch fennel plants under Sinai conditions. Journal of Medicinal Plants Studies 5:327-334 .

[56] Sadak MS, Bakry BA, El-Karamany MF (2022) Agrophysiological variation in flax affected by folic acid and Ag nanoparticles foliar applications. Asian J Plant Sci 21:339-346.

[57] Hatami M, Ghafarzadegan R, Ghorbanpour M (2014) Essential oil compositions and photosynthetic pigments content of *pelargonium graveolens* in response to nanosilver application. Journal of Medicinal Plants 13:5-14.

[58] Silveira JAdM, Rocha DS, De Morais GB, De Siqueira RJB, Leal- Cardoso JH, Evangelista J, Monteiro JSA (2017) Inhibitory effect of linalool in preparations of isolated smooth muscle of rat trachea with epithelium stimulated by electromechanical coupling. Ciência Anim 27:20–30.

[59] Elbanna H M, Ahmed O K, Fayed S A, Hammam K A, Yousef R S. (2024) Enhancing French basil growth through synergistic foliar treatment with copper nanoparticles and *Spirulina sp.* BMC Plant Biology 24:512. https://doi.org/10.1186/s12870-024-05153-x

[60] ElSaadony MT, Saad AM, Najjar AA, Alzahrani SO, Alkhatib FM, Selem E, Desoky SM, Fouda SS, El-Tahan AM, Hassan MAA (2021) The use of biological selenium nanoparticles in controlling *Triticum aestivum* L. crown root and rot diseases induced by Fusarium species and improve yield under drought and heat stress. Saudi J Biol Sci 28:4461-4471.

[61] Dimkpa CO, Bindraban PS (2016) Fortification of micronutrients for efficient agronomic production: a review Agron Sustain Dev 36(1):7-32.

[62] Jameel DA, Al-Tai AAS (2018) Effect of NPK compound fertilizer normal and nano on some growth Traits and Oil Content of Three Species of Apiaceae Plants. Research J Pharm and Tech 11(1):301-307 .

[63] Yang F, Hong FS (2006) Influence of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach. Biol Trace Elem Res 110:179-190.

[64] Yang M, Dong C, Shi Y (2023) Nano fertilizer synergist effects on nitrogen utilization and related gene expression in wheat. BMC Plant Biology 23:26.

[65] Rady MM, Elrys AS, Selem E, Mohsen AAA, Safaa MAI, Arnaout, El-Sappah AH, El-Tarabily KA, Desoky EM (2023) *Spirulina platensis* extract improves the production and defenses of the common bean grown in a heavy metals-contaminated saline soil. Journal of Environmental Sciences 129:240-257.

[66] Godlewska K, Michalak I, Pacyga P, Baśladyńska S, Chojnacka K (2019) Potential applications of cyanobacteria: *Spirulina platensis* filtrates and homogenates in agriculture Content courtesy of Springer Nature, terms of use apply. Rights reserved. World Journal of Microbiology and Biotechnology 35(6):80-96.

[67] Osman MEH, Abo-Shady AM, El-Nagar MMF (2016) Cyanobacterial Arthrospira (*Spirulina platensis*) as safener against harmful eff ects of fusilade herbicide on faba bean plant. Rend Fis Acc Lincei 27:455–462.

[68] Dowom SA, Karimian Z, Dehnavi MM, and Samiei L. (2022). Chitosan nanoparticles improve physiological and biochemical responses of *Salvia abrotanoides* (Kar.) under drought stress. BMC Plant Biol. 22: 364.
[69] Iqbal M, Raja1 NI, Mashwani1 Z, Wattoo FH, Hussain M, Ejaz1 M, Saira H (2018) Assessment of AgNPs exposure on physiological and biochemical changes and antioxidative defence system in wheat (*Triticum aestivum* L) under heat stress. IET Nanobiotechnology 13:230–236.
[70] Thiruvengadam M, Gurunathan S, Chung IM (2015) Physiological, metabolic, and transcriptional effects of biologically-synthesized silver nanoparticles in turnip (*Brassica rapa* ssp. rapa L.). Protoplasma 252:1031–1046.
[71] Selem E (2019) Effects of *spirulina platensis* in salt stressed *Vicia faba* L. plants. Physiological 59:1185-194.
[72] Manaf A, Wang X, Tariq F, Jhanzab HM, Bibi Y, Sher A, Razzaq A, Fiaz S, Tanveer SK, Qayyum A (2021) Antioxidant enzyme activities correlated with growth parameters of wheat sprayed with silver and gold nanoparticle suspensions. Agronomy 11:1494-1506.
[73] Mehrian SK, Heidari R, Rahmani F, Najafi S (2016) Effect of chemical synthesis silver nanoparticles on germination indices and seedlings growth in seven varieties of *Lycopersicone sculentum* Mill (tomato)plants. Clust Sci 27:327–340.
[74] Barbasz A, Kreczmer B, Oćwieja M (2016) Effects of exposure of callus cells of two wheat varieties to silver nanoparticles and silver salt ($AgNO_3$). Acta Physiol Plant 38:76-85.
[75] Hassan SM, Ashour M, Soliman AAF (2017) Anticancer activity, antioxidant activity, mineral contents, vegetative and yield of *Eruca sativa* using foliar application of autoclaved cellular extract of *Spirulina platensis* extract, comparing to N-P-K fertilizers. Journal of plant production 8(4):529-536.
[76] Imara, DA, Ghebrial, EWR, EL-Abeid, SE, Hussein, EMA, Elsayed, MIE, Yousef, RS (2024) Reduction of Oxidative Damage Caused by *Fusarium falciforme* and *Fusarium foetens* in Schefflera Plants Using Chitosan Nanoparticles Loaded with L-Proline or Indole Butyric Acid. Chemical and Biological Technologies in Agriculture 11:167. https://doi.org/10.1186/s40538-024-00680-z