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Research Article

Impact of Excessive Nitrogen Fertilization on the Biochemical Quality, Phenolic Compounds, and Antioxidant Power of *Sesamum indicum* L Seeds

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Nitrogen is an essential nutrient for plants life cycle. However, the excessive use of this element causes serious problems in agriculture. This can disrupt the development of many important plants. *Sesamum indicum* or sesame represents one of the most economically important and ancient oil crops in the world. In fact, its seeds are used for many biological activities. The aim of the present study is to evaluate the effect of high levels of nitrogen on some primary and secondary metabolites and the antioxidant activity under different levels of N supply. Results analysis of our investigation showed that the excessive nitrogen supply had a serious effect on the quality of sesame seeds. In fact, N fertilizer promoted the accumulation of proteins until 57.16%, in detriment of oil and sugars that decreased with 49.43% and 16.27%, respectively. Also, total phenolic, flavonoids content, and antioxidant activity showed a significant decrease.

1. Introduction

Sesamum indicum L. is a Pedaliaceae of *Sesamum* genus; it is one of the oldest oilseed crops. Archeological studies showed that sesame cultivation dates back to 5500 BC [1]. Sesame seeds are rich in oil (44–58%), proteins (18–25%), and carbohydrates (13.5%); oil fraction contains about 90% unsaturated fatty acids, including oleic acid and linoleic acid. As protein fraction, sesame seeds are rich in arginine and leucine with about 140 $mg{\cdot}g^{-1}$ and 75 $mg{\cdot}g^{-1}$, respectively [2]. Recently, many studies highlighted some health-promoting activities of sesame seeds, such as the prevention of high plasma cholesterol [3] and diseases related to sex hormones in postmenopausal women [4], and also the seeds are considered as a strong antioxidant [5].

Nitrogen (N) is a vital nutrient for plants; it is a component of a large number of organic compounds such as amino acids, proteins, coenzymes, nucleic acids, and chlorophyll [6]. It is well known that N-deficiency causes many biochemical and physiological disturbances leading to the reduction in cell division rates and perturbation in process of photosynthesis [7]. In addition, in the last years, the use of

fertilizers has increased considerably in the objective to increase the mass of crop per land area for ensuring the increased need of world population. However, excessive nitrogen fertilization is a major problem for both agriculture and environment. Overmuch accumulation of N in plants may cause toxicity problems for human health like methemoglobinemia [8], nitrous oxide emissions, and groundwater's nitrate pollution [9]. Moreover, Jeppsson [10] has shown the negative effect of high fertilization on the composition and quality of some plants. The rational use of nitrogen supply can prevent pollution and ensure the quality and sustainability of agriculture. In this logic, farmers need to understand the importance of optimization of the mineral fertilization.

Otherwise, there are no reports, on the effect of excessive N supply on biochemical composition of *Sesamum indicum* seeds, whose global production is 3.15 mn tonnes per year. The aim of this study is to determine the effect of different N levels supply on nutritious and medicinal quality of sesame seeds by measuring some primary metabolites, total phenolic and flavonoids contents, and evaluate the antioxidant power under all of the used N levels.

2. Materials and Methods

2.1. Culture Conditions. This experiment was conducted in the natural conditions of controlled greenhouse in Laboratory of Functional Ecology and Environment (34° 33′ North, 4° 39′ West). The seeds were disinfected in 70% ethanol for 15 min, rinsed enough with distilled water, and placed to germinate on filter paper impregnated with 10 mL of distilled water at temperature of 25°C, on June, 2015. The uniform seedlings were then transplanted into 30 L plastic pots (42.0 cm × 35.5 cm) containing 3 : 1 soil/sand mixture. Physicochemical characteristics of the soil used were 14% clay, 49% silt, and 37% sand, with N 1.3 g·kg^{-1}, P 4.14 mg·kg^{-1}, and K 189.37 mg·kg^{1} and pH 7.87. The average temperatures during the period of cultivation were between 26 and 38°C. All pots were arranged in a randomized complete block design replicated four times, using five nitrogen rates applied in the form of urea (46% N), as follows: 0, 40, 120, and 160 kg·N per ha (i.e., 0, 26, 52, 78, and 104 mg·N per kg soil); these levels are noted as N1, N2, N3, and N4, respectively. The harvest was performed, in October 2015, when color of the leaves changed from green to yellow. The mature grains were collected and stored at 4°C.

2.2. Chemicals. All standards and chemicals used in this study were analytical grade. Ethanol, methanol, hexane, Folin–Ciocalteu reagent, boric acid, bromocresol green methyl reed, buffer sodium phosphate, Lowry reagent, bovine serum albumin, anthrone reagent, glucose, sodium carbonate solution, gallic acid, quercetin, aluminium chloride, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, sulfuric acid, sodium, ammonium molybdate, and ascorbic acid were obtained from Sidi Mohamed Ben Abdellah University, Fez, Morocco.

2.3. Biochemical Composition

2.3.1. Total Nitrogen Concentration. For determination of total nitrogen concentration, the Kjeldahl method was used [11]. 1 g of ground seeds, per treatment, was digested with 8 mL of concentrated H_2SO_4 in the presence of a catalyst. Then, to distill the sample, sodium hydroxide was added using a semiautomatic unit. 4% boric solution was used to collect nitrogen as ammonia (NH_3). The titration was conducted with H_2SO_4 in the presence of an indicator (bromocresol green and methyl reed). The following equation was used to estimate the total nitrogen concentration:

$$\mathrm{N}(\%) = \frac{\mathrm{V}(\mathrm{H_2SO_4}) \times \mathrm{N}(\mathrm{H_2SO_4}) \times 1.4}{\mathrm{DW}}, \quad (1)$$

where $\mathrm{V}(\mathrm{H_2SO_4})$ is the volume of H_2SO_4 used for titration, $\mathrm{N}(\mathrm{H_2SO_4})$ is the normality of H_2SO_4 used for titration, and SW is the sample dry weight.

2.3.2. Total Soluble Proteins. 0.2 g of ground seeds per treatment was dissolved in 5 mL of buffer sodium phosphate, and then the mixture was centrifuged at 4000 g. 2 mL of Lowry reagent was added to the supernatant. After incubation, the absorbance was measured at 750 nm. BSA (bovine serum albumin) was used, as standard, for determination of total soluble proteins [12].

2.3.3. Oil Content. Samples of 40 g of ground seeds were subjected to extraction with 400 mL of *n*-hexane, for 8 hours with constant shaking. The extracts were filtered, and then the solvent was removed using rotary evaporator apparatus at 40°C.

2.3.4. Total Soluble Sugars. 100 mg of ground seeds per treatment were extracted with 4 mL of ethanol (80%). The mixture was centrifuged for 10 min at 4500 rpm. Anthrone reagent was added in the supernatants, and then the absorbance was measured at 625 nm, to be converted after into glucose equivalent (mg·g^{-1}) [13].

2.3.5. Extracts Preparation. Extraction was carried out using the maceration method. Methanol: water (70 : 30 v/v) was added, for 8 hours with constant shaking, to ground seeds of each treatment. After filtration, the extracts were concentrated under reduced pressure and stored at 4°C in dark.

2.3.6. Total Phenolic Content. Total phenolic content of each extract was determined according to the method of Iqbal et al. [14]. The appropriate dilution of each extract was mixed with 1 mL of the diluted Folin–Ciocalteu reagent. Then, 2 mL of 5% sodium carbonate solution was added. After incubation, the absorbance was measured at 750 nm. Results are expressed as gallic acid equivalents (GAE).

2.3.7. Total Flavonoid Content. Total flavonoid content was measured by the aluminium chloride colorimetric method. 0.1 mL of hydromethanolic extracts was mixed with aluminium chloride methanolic solution (10%). After 5 min, 0.1 mL of sodium acetate was added and mixed thoroughly. After 1 h of incubation at room temperature, the absorbance was measured at 415 nm. Then, the total flavonoid content was calculated in terms of equivalent quercetin as reference to standard curve [15].

2.4. Evaluation of the Antioxidant Activity

2.4.1. DPPH Scavenging Activity. The effect of extracts on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was estimated according to the method of Manzocco et al. [16]. Different concentrations of extracts (10, 50, 250, and 500 μg/mL) were added to a recently prepared DPPH solution (0.5 mL). The mixture was vigorously shaken and incubated at room temperature for 30 min in a dark room. The absorbance of the solution was measured using a UV spectrophotometer at 517 nm against methanol as a blank. Ascorbic acid was used as a positive control. The proportion of DPPH radical scavenging is calculated using the following equation:

$$\%\ \text{inhibition of DPPH radical} = \frac{\text{Ac} - \text{Ae}}{\text{Ac}} * 100, \quad (2)$$

where Ac is the absorbance of the control and Ae is the absorbance of the extract.

The % inhibition of DPPH radical was then used to calculate IC50, which is the antiradical concentration required to cause 50% of inhibition.

2.4.2. Total Antioxidant Capacity. Total antioxidant capacity was carried out using the phosphomolybdenum method according to Prieto et al. [17]. The tubes, containing a mixture of extract solutions of each treatment and reaction solution (0.6 M sulfuric acid, 28 mM sodium, and 4 mM of ammonium molybdate), were incubated at 95°C for 90 min. After cooling, the absorbance of the solution was measured at 695 nm. The antioxidant activity was expressed as ascorbic acid equivalents.

2.5. Statistical Analysis. Statistical analysis was performed using SYSTAT 12. Data were subjected to one-way analysis of variance (ANOVA) in order to determine significant differences among the treatments. The results were considered significant at $p < 0.05$.

3. Results

3.1. Biochemical Composition

3.1.1. Total Nitrogen Concentration. The application of N4 level caused an important increase of 44.31% in the concentrations of total nitrogen content in seeds, and it dropped from 18.6 mg·g^{-1} DW in control seeds to 33.4 mg·g^{-1} DW in N4 fertilized seeds ($p < 0.05$) (Figure 1).

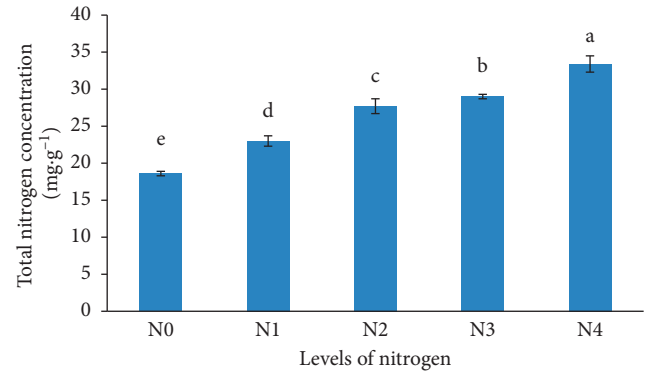

Figure 1: Effect of different levels of N on total nitrogen concentration. Data labeled with different letters are significantly different at $p < 0.05$.

3.1.2. Total Soluble Proteins. The content of total soluble proteins is reported in Figure 2; after exposure to croissant nitrogen's levels, a greater total soluble proteins content was observed in N4 by 327.8 mg·g^{-1} DW compared to the control treatment (140.4 mg·g^{-1} DW) ($p < 0.05$).

3.1.3. Oil Content. The content of oil is reported in Figure 2. Oil concentration does not exceed the value of 442.2 mg·g^{-1} for N1, which is not statistically significant compared to the values obtained with N0 and N2. Application of N3 and N4 decreased significantly the oil content (with 18.06% and 22.36%, respectively) compared with the control ($p < 0.05$).

3.1.4. Total Soluble Sugars. As shown in Figure 2, total soluble sugars content in sesame seeds was negatively influenced by nitrogen supply. The highest value was recorded in N2 with 93.4 mg·g^{-1} DW. After exposure to N3 and N4, a significant decrease of 10.70% and 16.27%, respectively, was recorded in the content of total soluble sugars in sesame seeds ($p < 0.05$).

3.1.5. Total Phenolic and Flavonoids Contents. The effect of different N levels on total phenolic and total flavonoids content is represented in Table 1. N4 reduced the total phenolic content by 19.07%, when compared to the control. Similar observation was noted in total flavonoids content which decreased by 21.13% at N4 ($p < 0.05$) compared to the control.

3.2. Evaluation of the Antioxidant Activity

3.2.1. DPPH Scavenging Activity. Generally, the hydromethanolic extracts of sesame seeds, in all treatments, showed a low antiradical capacity recorded in ascorbic acid, as an antioxidant of reference. Increasing of nitrogen fertilization up to N2 increased consequently antioxidant activity. In fact, the antiradical capacity of control extract (N0) was 48%, 55%, 67%, and 78% for the concentrations 10, 50, 250, and 500 μg·mL^{-1}, respectively, compared to those of the ascorbic acid with 64, 75, 91%, and 92 respectively, for the

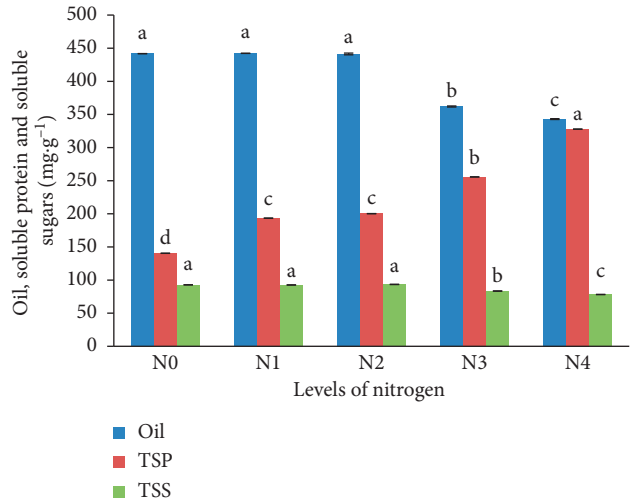

Figure 2: Effect of different levels of N on oil content, total soluble sugars, and total soluble proteins. TSP: total soluble proteins; TSS: total soluble sugars. Data labeled with different letters are significantly different at $p < 0.05$.

Table 1: Effect of different levels of N on total phenolic and total flavonoids content of hydromethanolic extracts of sesame seeds.

	Total phenolic content (μg GAE/g DW)	Total flavonoids content (μg QE/g DW)
N0	278.58 ± 0.07^{a}	124.74 ± 2.78^{a}
N1	275.43 ± 0.03^{a}	115.78 ± 0.34^{a}
N2	267.12 ± 0.12^{a}	121.98 ± 1.05^{a}
N3	246 ± 0.56^{b}	114.04 ± 0.09^{a}
N4	225.81 ± 0.1^{c}	98.35 ± 0.076^{b}

Data labeled with different letters are significantly different at $p < 0.05$; GAE : gallic acid equivalent; QE : quercetin equivalent; DW : dry weight.

same concentrations. At the level of N2 and for the same concentrations, the antioxidant activity increased by 7%, 9%, 9.5%, and 11%. However, above this level, at N3 and N4, the antioxidant activity decreased significantly as shown in Figure 3. The IC50 found for the control extract correspond to 1.30 μg·mL^{-1}, compared to ascorbic acid which has an IC50 = 0.29 μg·mL^{-1}; however, it reached 0.9 μg·mL^{-1} and 3.63 μg·mL^{-1} after the application of N2 and N4, respectively.

3.2.2. Total Antioxidant Activity. The effect of different N levels on total antioxidant activity of sesame seeds extracts is represented in Figure 4. The total antioxidant activity was negatively influenced by high level of nitrogen application ($p < 0.05$). In control seeds extract, the total antioxidant activity reached 503.77 ± 2.93 μg·g^{-1} DW, after the application of N4, this value decreased to 481.12 ± 1.9 μg·g^{-1} DW, reflecting a reduction of 16. 40%.

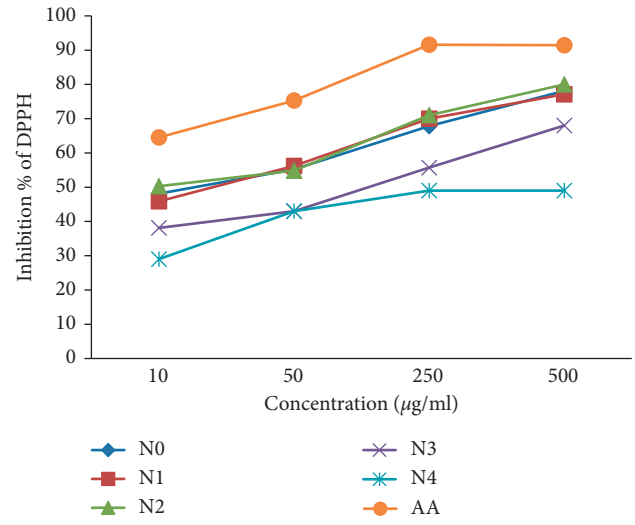

Figure 3: Effect of different levels of N on DPPH scavenging activity in hydromethanolic extracts of seeds of sesame.

4. Discussion

Results revealed that the total nitrogen concentration increased by increasing N levels. It is known that many parameters can influence the concentration of nitrogen in the plant depending on genetic and environmental conditions, age of plant tissues, and stage of plant development. According to Barker and Gretchen [18], the concentration of total nitrogen must be superior to 2% in seed. In our study, after application of nitrogen, the concentration of total nitrogen balanced between 2.3% and 3.34%. Besides this, Sheikh and Ishak [19] showed that nitrogen is an essential element for amino acids and proteins construction, but high concentration of nitrogen in the tissues may cause mineral toxicity and reduce the phenological and physiological responses of the plant.

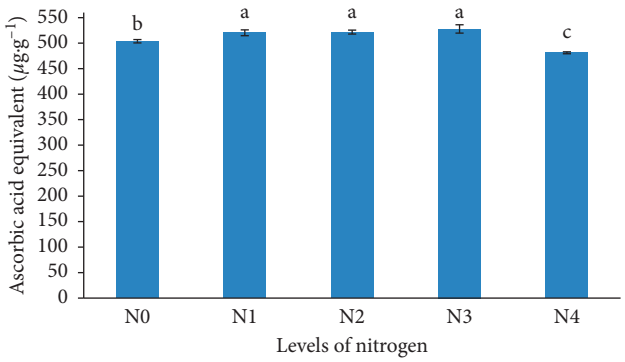

Figure 4: Effect of different levels of N on total antioxidant activity in hydromethanolic extracts of seeds of sesame. AA: ascorbic acid.

In this study, the nitrogen supply increased the total soluble proteins content in seeds. In fact, after many reactions needing energy, the assimilated nitrogen is converted to amino acids that build proteins [20]. Rathke et al. [21] reported that the increased N supply intensifies the synthesis of proteins at the expense of fatty acids synthesis. For more precision, assimilated nitrate is converted, by the enzyme nitrate reductase, to nitrite which is reconverted via metabolic pathway to ammonium. And then, this element is incorporated into amino acids via the GS/GOGAT pathways [22]. These physiological reactions are necessary to increase the protein content.

For the lipid fraction, the oil % decreased with increasing N levels, this may be a consequence of diverting more energy and resources into proteins production rather than oil [23], leading to oil concentration accumulation reduce. Similar results have been reported by Cheema et al. [24] who showed that low oil content was registered by applying a high level of N.

In our investigation, we report a significant reduction in total soluble sugars with using high doses of N. For Evans [25], the nitrogen is a direct factor which regulates carbon balance that is the basic element for sugar construction. For Xia and Cheng [26], the sugar content decreased with increasing N levels. This can be explained by the fact that sugar is involved in many chemical reactions in order to produce energy. In harmony with our results, several studies showed the negative relationship between high doses of N and carbohydrate accumulation in the grain [27, 28].

A serious decrease in total phenolic and flavonoids contents was recorded following excess N uptake; the same results were reported in several studies [29–31]. Also, Jones and Hartley [32] showed that the concentration of proteins and phenols is negatively correlated. Phenylalanine, which is a key substance in the synthesis of phenols, is preferentially applied into chain protein synthesis rather than phenol compounds under high nitrogen levels [33].

Sesamum indicum extract is known for its powerful antioxidant activity [34]. However, this capacity has been reduced as a response to high nitrogen supply. Several previous studies showed a strong and positive correlation between phenolic compounds quantity and antioxidant activity [35–37]. Thus, a reduction in phenolic compounds could reduce the plant antioxidant capacity, as clearly shown in this study.

Based on the results found in this article, we seek to complement other research studies [10–38], which tend to focus on the importance of rational fertilization supply. The work of Vitousek et al. [39] showed that maize crop can keep the sustained yield using only a half of the N fertilizer usually used. In this logic, it is necessary to use optimized fertilizer rate, in order to ensure the agriculture sustainability.

5. Conclusion

This study has clearly shown that excessive use of nitrogen fertilization could have a serious impact on sesame seeds characteristics. In fact, total concentration of nitrogen and proteins can be promoted by increasing nitrogen levels, in detriment of oil and sugars. Also, total phenolic and flavonoids contents with antioxidant power have decreased by increasing rates of nitrogen. Our results suggest that the application of 120 kg·N per ha is sufficient, under our conditions, to promote the parameters studied.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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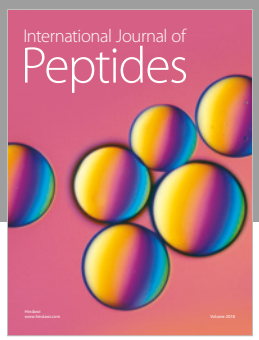

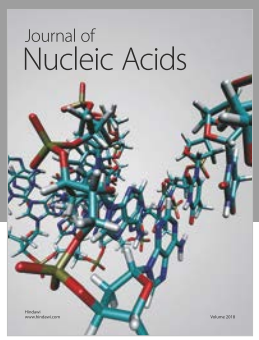

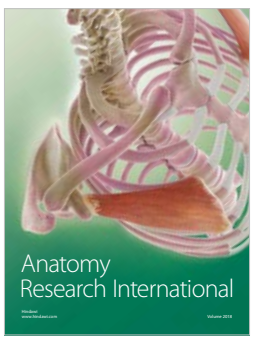

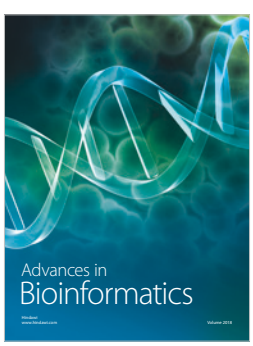

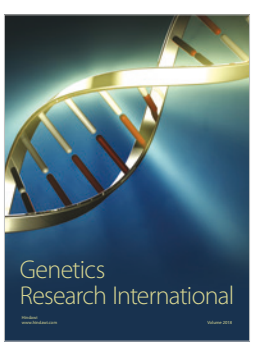

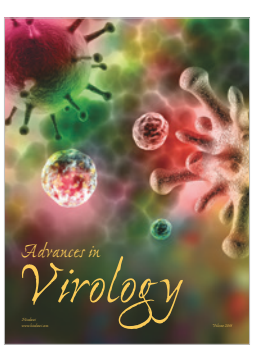

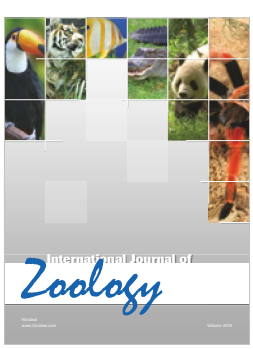

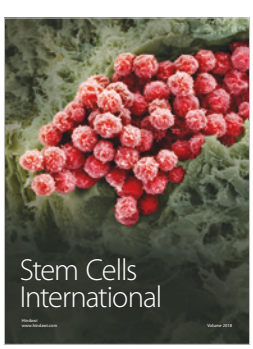

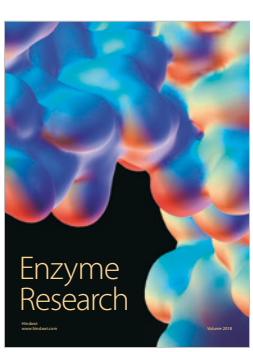

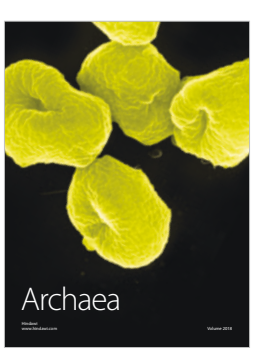


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