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Effect of Pollination and Different Amendments on Seed Oil, Phenolics and Antioxidants of *Sesamum indicum* L. Grown in Southern Chad

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Abstract

Sesame (*Sesamum indicum* L., Pedaliaceae) is a one of the earliest oilseed plants highly prized in tropical and subtropical countries. In Chad, its yield remains low, averaged 500 kg.ha^{-1} and organic and/or mineral fertilization are used to increase its grain yield. The low production would be linked ether to lack of crop knowledge, changing agronomic conditions and probably to the lack of pollination services. The seeds contain very high oil contents and are rich in total phenolic compounds and antioxidants which give it unprecedented stability for its oils. Thus a two-year experiment (rainy season 2022-2023) was conducted at Bébédjia in Chad to assess the effect of different types of amendments and pollination by entomophilous insects on the oil content, total phenols and antioxidant activity of sesame seeds. The study was based on a split plot design with three replications. The two studied factors were studied: fertilization with six types of amendments (control, 0.050 t.ha-^{1} of mineral NPK fertilizer , 5 t.ha^{-1} and 10 t.ha^{-1} of compost, 5 t.ha^{-1} and 10 t.ha^{-1} of cow-dung manure) and pollination with two modalities (free pollination plot and insects protected plot. The results of the combined analysis of variance showed that fertilization highly ($p < 0.001$) affected all the three studied biochemical parameters while the effect of pollination was significantly only for the antioxidant potential ($p < 0.05$). The effect of the interaction between fertilization and pollination was significant only for polyphenols content ($p < 0.05$). Among treatments, the highest seed oil contents were obtained for compost 10 t.ha^{-1} (52.77%) and cow manure10 t.ha^{-1} (51.34%). Highest rates of polyphenols and antioxidants were recorded for unfertilized control indicating that amendments reduced these biochemical traits. This study showed that insect pollination had little effect on these biochemical traits in contrast organic fertilizers at 10 t.ha^{-1} increased the seed oil content of sesame but reduced the polyphenols rates and the antioxidant potential.

Keywords: Compost, Cow Dung, NPK Fertilizer, Pollination, Biochemical Parameters, *Sesamum Indicum*, Chad.

1-INTRODUCTION

Sesame (*Sesamum indicum* L.) is cultivated in almost all tropical and subtropical countries of Asia and Africa for its highly nutritious and edible seeds [1]. Sesame is an oilseed crop with a booming international trade in seeds, oil and meal. Its demand is growing worldwide [2]. Globally, world sesame production area is estimated at more than 14 million hectares in 2020 for a total production of about seven million tons, an increase of 55.5% compared to 2010, which totaled a production of more than four million tons [3]. This increase in production is mainly due to the increase in areas from 7.4 million to 14.2 million hectares [3]. The majority of global production comes from Africa (69% of the area and 63% of the production), followed by Asia with 33% of the production and America with 4% of the production [3]. Sesame seed is among the oilseeds richest in oil and its content varies from 44% to 58% depending on the variety of sesame seeds. The oil has excellent stability due to the presence of natural antioxidants such as sesamolin and sesamin, and these lignans protect the oil from oxidative rancidity [4]. Sesame cake or meal obtained as a byproduct of oil milling industry is rich in protein, carbohydrate, vitamins, minerals and considered as it is eaten mixed with sugar by poor people [4]. Studies have shown that sesame seeds contain 21.9% protein, 61.7% fat, and are rich in minerals such as Fe and Ca [4]. Chad, which ranked 10th in 2020, has a predominantly rural population living mainly from agricultural and livestock activities [5, 6]. Sesame seeds can be eaten as is, or added to confectionery and pastries. Sesame cultivation has experienced renewed interest in recent years thanks to the recovery in market prices and an increasingly growing demand.

Sesame is autogamous crop and reported to have outcrossing between 0.5% to 65% depending on insect activity, environmental conditions, variety and availability of other vegetation [7-9]. The cross-pollination is effected due to insects as honey bees [8-10]. Declining in soil fertility, inappropriate use of fertilizers, low productivity, lack of pollination and monocropping are among the major production constraints [7-11]. In the south of the country, these generally sandy-clayey soils have low organic matter and phosphorus contents, a continuous decline in fertility, a tendency towards acidification. It is thus accepted that improving agricultural production must necessarily involve improving soil fertility [11]. Insect pollination plays an essential role in flowering plants reproduction, supporting a wide share of cultivated food crops. Previous studies showed that insect pollination increases seed yield of sesame from 22% to 33% compared to pollinators -protected treatment [9-13]. Research on the nutrition of sesame in the tropics showed significant yield increase due to application of mineral fertilizers in Nigeria, Vietnam, India and Pakistan, [14-16]. Shathi *et al*., [17], outlined that vermicompost and inorganic fertilizers improve sesame plant growth and seed nutrient content. The beneficial effect of organic fertilizers on sesame production and seed quality was widely reported [18-21]. Fertilizer application to sesame in either organic or inorganic form is a key component to good growth, high yield, high seed quality, and oil content [15-22]. The use of fertilizers for sesame is a notorious issue in most parts of the developing countries [23]. The rates of N fertilizer vary with soils, climate conditions, and local farming practices. Increases in N supply within limits are associated with increase in leaf area, carboxylases, and chlorophyll content, all of which determine the photosynthetic activities of leaf and ultimately dry matter production and allocation to the various organs of a plant [23]. In the current Chadian context, organic amendments are necessary to improve soil fertility and increase yields while its application remains low or even insufficient [11]. As food quality can affect market prices and the behavior of many actors along the food supply chain, it is crucial to assess the role of pollinators and fertilization. Polyphenols, nutraceutical ingredients, are products of secondary metabolism of plants and their role is diverse: assimilation of nutrients, photosynthesis, enzyme activity, protein synthesis, photorecptors and antioxidant activity [23]. Thus, the investigation highlighted the beneficial effects of fertilization and insect pollination in improving the seed quality namely the oil content, polyphenols and antioxidant capacity of sesame in sustainable agriculture.

2-MATERIALS AND METHODS

2.1. Materials

Sesame variety S42 was taken as test crop. This improved variety with white seeds and 90 days cycle was obtained from the Chadian Institute of Agronomic Research for Development (ITRAD) at the Bébédjia station [5]. The animal material is represented by insects naturally present in the environment.

2.2. Experimental Details

Fields trials were conducted during the rainy season 2022 and 2023 at the Bébédjia Agronomic Research Station (latitude 8°40' 09" N and longitude 16°54'65" E) [5]. Bébédjia (Department of Nya, Logone oriental region in Southern Chad) belongs to the savannah Sudano-Guinea belt with an annual average rainfall ranges between 900 to 1300 mm [11]. The climate is tropical semi-humid with a single rainy season that ranges from April to November. The mean annual temperature is between 25 - 30°C, while the annual humidity is about 60%. The soil is sandy-clay with 8.2 $mg.kg^{-1}$ of organic matter and pH of 5.5 [24]. The vegetation is a clear forest tree savannah [24].

The seeds of entry were sown in a 750 m^2 (25 m length x 30 m broad) experimental plot arranged in a split plot design (RCBD) with three replications, consisting of six treatments and two sub-treatments (free pollinated and closed plots). The main factor is six types of amendments (control without fertilization; $50kg.ha^{-1}$ NPK fertilizer containing 20% nitrogen, 10% phosphorus, 10% potassium; $5t.ha^{-1}$ compost; $10t.ha^{-1}$ compost; $5t.ha^{-1}$ cattle manure; $10t.ha^{-1}$cattle manure). The field was divided into three blocks (8 m x 25 m) spaced 2 m apart. Within each block, there are two main plots (3 m x 25 m) corresponding to non-opened and opened pollination treatments. Within each main plot, there are six sub-plots of 3 m x 3 m corresponding to the different amendments. Each plot unit consisted of five rows of 3m length, and observations were made on the three central lines. Three seeds were sown at an intra-row spacing of 30 cm and thinned to one per hill, 20 days after sowing (DAS). All amendments were made at 15 DAS. The plots were manually weeded 15, 35 and 60 DAS. For non-opened pollination treatments selected plants were covered before the start of the flowering period with performed net bag, to allow the air to pass through and prevent insects from approaching the plants. At maturity, harvesting was done at five-day intervals and seeds were separated to dry pods.

2.4. Biochemical Analysis

The oil content was determined by the Soxhlet method, a reference method used for the determination of fat in dehydrated solid foods. Dried *Sesamum indicum* whole seeds were ground for continuous extraction in a Soxhlet apparatus using hexane as solvent, as described by Salghi [25]. The oil was recovered by evaporating the solvent using a rotary vacuum evaporator and the percentage of oil content was calculated.

The total phenolic compound (TPC) of sesame flours were analyzed using the method described by Gao *et al.,* [26], using the Folin-Ciocalteu reagent. Diluted ethanolic extracts, 0.02 ml was introduced into a test tube, then 1.38 mL of distilled water and 0.2 mL of 1:16 diluted Folin-Ciocalteu phenol were added. The whole was mixed thoroughly and after 3 min, 0.4 mL of sodium carbonate (Na2 CO3, 20%) was added. The mixture was incubated at 45°C for 20 min and cooled to room temperature before reading the absorbance at 760 nm using a UV spectrophotometer. The calibration was carried out using a freshly prepared aqueous solution of gallic acid (0.2 g/l) and the results were expressed as mg GAE/100g of dry matter (DM).

The antioxidant activity (AOA) of the defatted flour was measured by 2,2- diphenyl-2-picrylhydraxyl hydrate (DDPH) free radical scavenging assay as described by Brand-Williams *et al.,* [27]. Extract (100 µL) was added to 500 µL of methanolic DDPH solution, vortexed and keep for one hour at room temperature. The absorbance of resulting solution was read at 517nm with lower absorbance indicating a higher DDPH scavenging activity. The standard used was a DDPH solution (5 mg/100 mL), and the AOA was expressed as percentage of inhibition as [27, 28]:

AOA (%) = 100 x (DRc – DRs) / DRc

Where DRc was the degradation rate of control and DRs the degradation rate of the sample.

2.5. Statistical Analysis

All data were subjected to descriptive statistics and analysis of variance using computer program STATGRAPHICS Plus. The pollination effect (PE) in percentage was calculated (PE) as [29]:

PE (%) = [(Yp – Yc)/Yp] x 100 where Yp is the average of a treatment in free pollinated plot and Yc is it values in closed plot.

When the F-test was significant at $p<0.05$ for a parameter, the treatments means were tested by Least Significant Difference (LSD) at 5% level of probability. Plots' means were compared using t-Student test. The combined analysis of variance to evaluate the effect of amendments and pollination, and also to determine whether their interaction was significant was done using the GEST 98 microcomputer program [30].

3- RESULTS AND DISCUSSION

3.1. Effect of Fertilizers, Insect Pollination and Their Interaction on Sesame Seed Soil Content

The combined analysis of variance for oil content (Table 1) revealed that the effect of fertilization was highly significant ($p < 0.001$) while the effects of pollination and the interaction were not significant (p = 0,337 and p = 0,837 respectively). Sesame seed oil was significantly affected by application of different fertilizers which explained 98.16% of the total variation, while insect pollination and the interaction captured respectively 0.59% and 1.25% of the total sum of square. The fertilization variance was significantly higher compared to other sources of variation, suggesting that only the application of tested fertilizers affected the rate of sesame seed oil.

The oil contents of different treatments in open pollinated and insect excluded plots varied between 45.36 to 52.11% with an average of 48.57% (Table 2). The comparison of opened and protected plots showed that the effect of pollination was not significant for different treatments. The simple analysis of variance revealed very highly significant differences among amendments for oil content ($p < 0.001$). Among the fertilizers, the compost at 10t/ha and the cow manure at 10t/ha highly increased the oil content while the poorest rate of seed oil was noted in the control, the cow dung at 5t/ha and the compost 5t/ha. In this study, insect pollination had no significant effect on oil content. In contrast, Mahfouz *et al*., [32], noted that sesame oil content was higher (6.46%) in opened pollination and the difference compared to the content of non-opened pollination was significant. Blal *et al.,* [13], studying the impact of pollination and fertilization on sesame production in Egypt, concluded that yield parameters and oil content significantly increased with open pollination and with the increase of nitrogen fertilizer level up to 95 kg N/ha. It is well known fact that the incorporation of organic residual in the soil plant system improves the physical, chemical and biological properties of soils and helps in sustaining the fertility and crop production. The range of oil content reported in this study was in agreement with many reports [1-34], who noted the oil content of sesame varied from 24 to 59%. Choudhary *et al*., [22], also noted in Egypt, that the application of organic manure and vermicompost on sesame significantly increase the seed oil content. In Bangladesh, Shathi *et al.,* [17], observed that the application compost and inorganic fertilizers in combination has pronounced effects on sesame seed nutrient concentration. The significant increase in oil content of seed with amendments may be due to enhanced availability of nutrients to plant that in turn may have resulted in higher synthesis of metabolites responsible for increased oil content [34].

Table 1: Combined analysis of variance for seed oil content

Source of variation	df	SS	%SS	MS	F	Probability
Pollination	1	1.629	0.59	1.629	0.96^{ns}	0.3378
Fertilization	5	273.243	98.16	54.648	32.07***	<0.0001
Interaction	5	3.484	1.25	0.697	0.41^{ns}	0.8378
Residual	24	40.894		1.704		

Source of variation	df	SS	%SS	MS	F	Probability
Total	35	278.356				

df: degree of freedom; SS: Sum of square; %SS: Percentage of the sum of square; MS: mean square; F: Fisher value; ns: not significant; ***: highly at 0.1% level of probability.

Table 2: The mean of seeds oil content (%) among treatments in open and insect closed plots

Treatments	Opened plot	Closed plot	Treatment (mean)	Pollination effect (%)
Control	45.36 ± 1.38^{b}	45.40 ± 0.71^{c}	45.38^{c}	-0.088^{ns}
NPK fertilizer 50kg.ha^{-1}	50.30 ± 1.52^{a}	49.90 ± 2.06^{b}	50.10^{b}	0.792^{ns}
Compost 5t .ha^{-1}	46.53 ± 1.26^{b}	46.60 ± 1.63^{c}	46.56^{c}	-0.150^{ns}
Compost 10t.ha^{-1}	52.11 ± 1.78^{a}	53.43 ± 0.68^{a}	52.77^{a}	-2.533^{ns}
Cow manure 5t.ha^{-1}	46.40 ± 0.62^{b}	46.76 ± 0.58^{c}	46.58^{c}	-0.776^{ns}
Cow manure 10t.ha^{-1}	50.76 ± 1.02^{a}	51.93 ± 1.76^{ab}	51.34^{ab}	-2.305^{ns}
Plot mean	48.57 ± 2.81^{A}	49.00 ± 3.26^{A}	48.78	-0.885^{ns}
CV (%)	5.78	6.65	6.20	
LSD (5%)	2.35	2.47	2.33	

CV: Coefficient of variation; LSD: Least significant difference; Means of treatments with the same subscript within the same column are not significantly different at 5%

3.2. Effect of Fertilizers, Insect Pollination and Their Interaction on Sesame Phenolic Compounds Content

The combined analysis of variance for total phenols revealed that the fertilization effect was highly significant ($p < 0.001$) capturing 93.88 % of the sum of square (Table 3). In contrast, the effect of insect pollination on sesame polyphenols content was not significant ($p = 0.213$) and captured only 0.64% of the total variance. The interaction effect was also significant ($p = 0.0411$) accounting for 5.44% of the total variance (Table 3). The polyphenols content was greatly affected by different fertilizers application and weakly influenced by interaction of fertilization and insect pollination. In many food crops, pollinators strongly improve several important attributes related to appearance and shelf live, whereas they have smaller effects on nutritional value [29].

Among the different fertilizers used, the rates of total phenolic compounds in pollinated plots varied from 399 mg EAG/100g DM (NPK fertilizer at 50kg/ha) to 501.66 mg EAG/100g DM (control) °with an average of 447.83 mg EAG/100g DM; while in insect protected plots, these values ranged from 398 mg EAG/100g DM (NPK fertilizer 50t/ha) to 511.66 mg EAG/100g DM (control without fertilizer) with an average of 454.19 mg EAG/100g DM (Table 4). Sesame seeds from the unfertilized control and from plots with cow manure at 5t/ha produced higher rates of phenolic compounds and the lowest values were noted in plots fertilized with NPK mineral fertilizer, compost at 10t.ha^{-1} and cow manure r at 10t.ha^{-1}. In this study, insect pollination had no significant effect on total phenols content but the interaction with fertilization had a significant effect. The total phenolic compounds contents of *Sesamun indicum* seeds reported in this study were in the range of 260.6 to 598.2 mg EAG/100g DM previously reported in this crop [35-37]. In maize, Alynad et *al.,* [38], also noted that the applications of higher amounts of organic and synthetic fertilizers nether in monoculture nor in competition with weeds, caused a statistically reduction in the content of total phenols and antioxidant activity. Similar changes caused by application of N fertilizers were observed by Amarovicz *et al*., [39], for the content of polyphenols in *Helianthus tuberosum* tubers. The effect of amendments on polyphenols content is mostly related to the amount of nitrogen but also those of phosphorus, potassium or sulfur present in the organic or inorganic fertilizers [29]. According to Heimler *et al*., [40], soil nitrogen affects both flavonoids and anthocyanin content, and generally, higher polyphenolic content is observed when less nitrogen fertilizer is added to the soil. Reshma *et al*., [40], highlighted that total polyphenol contents vary quantitatively and qualitatively with several factors such as climatic or environmental factors; genotype, plant development stage, harvest period and extraction method.

Table 3: Combined analysis of variance for sesame seeds polyphenols content

Source of variation	df	SS	% SS	MS	F	Probability
Insect pollination	1	373.78	0.64	373.78	1.64^{ns}	0.2130
Fertilization	5	54208.70	93.88	10841.70	47.55***	<0.0001
Interaction	5	3162.56	5.47	632.51	2.77*	0.0411
Residual	24	5480.00		228.33		
Total	**35**	**57745.04**				

df: degree of freedom; SS: Sum of square; %SS: Percentage of the sum of square; MS: mean square; F: Fisher value

Table 4: The mean of seeds polyphenols content (mg GAE.100g^{-1} DM) among treatments in open and insect closed plots

Treatments	Opened plot	Closed plot	Treatment (mean)	Pollination effect (%)
Control	501.66 ± 5.55^{a}	511.66 ± 22.67^{a}	506.66^{a}	2.000^{ns}
NPK fertilizer 50kg.ha^{-1}	399.00 ± 25.56^{d}	397.33 ± 29.29^{d}	398.16^{c}	0.418^{ns}
Compost 5t .ha^{-1}	458.33 ± 16.22^{bc}	427.66 ± 7.50^{d}	443.00^{b}	6.691*
Compost 10t.ha^{-1}	409.00 ± 9.23^{d}	419.66 ± 32.00^{d}	414.33^{c}	-2.606^{ns}
Cow manure 5t.ha^{-1}	480.00 ± 17.33^{ab}	506.50 ± 15.00^{a}	493.25^{a}	-5.521*
Cow manure 10t.ha^{-1}	439.00 ± 9.11^{c}	462.33 ± 10.80^{c}	450.66^{b}	-5.314*
Plot mean	447.83 ± 22.55^{A}	454.19± 25.55^{A}	451.01	-1.420^{ns}
CV (%)	5.04	5.62	7.58	
LSD (5%)	28.22	30.10	22.0	

CV: Coefficient of variation; LSD: Least significant difference; Means of treatments with the same subscript within the same column are not significantly different at 5%

3.3. Effect of Fertilizers, Insect Pollination and Their Interaction on Sesame Seed Antioxidant Capacity

Combined analysis of variance for antioxidant capacity of sesame seeds using the DDPH method revealed that both effects of pollination and fertilization were significant ($p < 0.05$) but the effect of their interaction was not significant (Table 5). Pollination effect accounted for 8.57% of the total variance; fertilization effect for 89.84% of the sum of square, and the interaction effect captured only 1.59% of the total variance. Fertilization variance appeared as the main sources of variation for the antioxidant capacity which is weakly influenced by insect pollination.

In pollinated and protected plots, significant variability was noted among the different amendments for the antioxidant potential (Table 6). Fertilization highly significantly influenced ($p < 0.001$) the antioxidant activity in open pollination with rates ranging from 63 to 87.33% and an average of 73.05%. In protected pollination, the average of 70.55% was recorded while in opened plots the mean value was 75.53 %. The treatments with 50kg.ha^{-1} NPK and 10t.ha^{-1} cow manure produced seeds with lowest antioxidant activity while the highest values were noted in control, compost at 5t .ha^{-1} and cow manure at 5t .ha^{-1}. In this study, insect pollination had significant effect on antioxidant activity with an increase of 6.6% in insect pollinated plots compared to closed plots. The observed reduction of antioxidant activity in insect excluded plots suggested that the management of pollinators contributed to maximize the seed quality. Gazzea *et al.,* [29], observed that in fruits and vegetables, pollinators strongly improve several attributes whereas they have smaller effects on nutritional values.

Previous reports [42-44], observed significant differences in sesame for antioxidant capacity with range of 40 and 94.9%. As noted for the polyphenols, the applications of higher amounts neither of organic and synthetic fertilizers, caused a statistically reduction in the antioxidant activity. This is in agreement with previous studies [18-40]. Barreca *et al.,* [45], noted that he antioxidant activity is mainly based on the polyphenols content.

Table 5: Combined analysis of variance for sesame seed antioxidants

Source of variation	df	SS	% SS	MS	F	Probability
Insect pollination	1	225.00	8.57	225.00	9.18*	0.0058
Fertilization	5	2357.22	89.84	471.44	19.24***	<0.0001
Interaction	5	41.67	1.59	8.33	0.34^{ns}	0.8835
Residual	24	588.00		24.50		
Total	**35**	**2623.89**				

Df: degree of freedom; SS: Sum of square; %SS: Percentage of the sum of square; MS: mean square; F: Fisher value

Table 6: The mean of seeds antioxidant capacity (%) among treatments in open and insect closed plots

Treatments	Opened plot	Closed plot	Treatments (mean)	Pollination effect (%)
Control	87.33 ± 2.08^{a}	79.00 ± 5.56^{a}	83.16^{a}	9.538*
NPK fertilizer 50kg.ha^{-1}	63.00 ± 2.85^{d}	58.00 ± 2.33^{d}	60.50^{d}	7.935*
Compost 5t .ha^{-1}	80.33 ±3.55^{ab}	78.00 ± 3.89^{a}	79.16^{ab}	2.901^{ns}
Compost 10t.ha^{-1}	74.33 ± 2.11^{bc}	69.67 ± 3.00^{bc}	72.00^{bc}	6.283*
Cow manure 5t.ha^{-1}	82.00 ± 1.95^{a}	75.00 ± 2.85^{ab}	78.50^{ab}	8.537*
Cow manure 10t.ha^{-1}	66.33 ± 4.06^{cd}	63.66 ± 2.85^{cd}	65.00^{cd}	4.025^{ns}
Plot mean	75.53 ± 7.85^{B}	70.55± 6.67^{A}	73.05	6.593*
CV (%)	10.40	9.45	9.77	
LSD (5%)	8.41	7.17	9.20	

CV: Coefficient of variation; LSD: Least significant difference; Means of treatments with the same subscript within the same column are not significantly different at 5%

4. CONCLUSION

The results obtained revealed that fertilization had significant effect on the studied biochemical attributes while the insect pollination showed a significant effect only for the antioxidant potential. The treatments 10t.ha^{-1} compost and 10t.ha^{-1} manure produced seeds with higher oil content but with a decrease in polyphenols content and antioxidant activity. Sesame seeds obtained in organic plots without any fertilization are rich in phenolic compounds and antioxidants. A part of the research demonstrated that maintaining optimal pollination services is essential not only to achieve high crop yield but also to improve the quality. As concern the kind of agricultural management, in order to recommend a viable agro-economic production at growers' level, additional research with different sesame varieties and combination of amendments may be necessary for different agro-ecological zones of Chad.

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