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Research Article

Impact of Nitrogen, Phosphorous, Potassium and Foliar Feeding on Total Lipids and Fatty Acids of *Nigella sativa* L. Grown in Arid Zones

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Abstract

Background and Objective: Fatty Acids (FA) extracted from *Nigella sativa* L. have an antimicrobial role. Arid zones in Egypt are characterized by poor nutrients, which negatively affect productivity of medicinal and aromatic plants, so the effect of nitrogen, phosphorous and potassium (NPK) with or without Foliar Feeding (FF) on the Total Lipids (TL) and Fatty Acids (FA) content of *N. sativa* were examined during two successive seasons. The objective of study was to investigate the effect of NPK and FF on TL and FA content in *N. sativa* plant. **Materials and Methods:** Plots were divided into two main groups. The first group was subjected to different levels of NPK combinations: $N_0P_0K_0$, $N_1P_1K_1$, $N_2P_2K_2$ and $N_3P_3K_3$. $N_0 = 0 \text{ kg N ha}^{-1}$, $N_1 = 100 \text{ kg N ha}^{-1}$, $N_2 = 150 \text{ kg N ha}^{-1}$, $N_3 = 200 \text{ kg N ha}^{-1}$; $P_0 = 0 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, $P_1 = 37.5 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, $P_2 = 56.3 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, $P_3 = 75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$; $K_0 = 0 \text{ kg K}_2\text{O ha}^{-1}$, $K_1 = 37.5 \text{ kg K}_2\text{O ha}^{-1}$, $K_2 = 56.3 \text{ kg K}_2\text{O ha}^{-1}$, $K_3 = 75 \text{ kg K}_2\text{O ha}^{-1}$. The second group was subjected to the same NPK treatments but FF was added at 1.5 mL L^{-1} as FF. Averages of data were statistically analyzed using 2-ways analysis of variance (ANOVA). Significant values determined according to p-values ($p < 0.05$ = significant, $p < 0.01$ = moderate significant and $p < 0.001$ = highly significant). **Results:** The highest amounts of TL were obtained with $N_2P_2K_2$ which recorded the values 31.5 and 33.8%; 3.7 and 3.5 g plant⁻¹ of both seasons. Greatest values on main FA constituents {oleic (25.6%), linoleic (47.8%) and linolenic (14.4%)} were obtained from $N_3P_3K_3 \times \text{FF}$. **Conclusion:** Cultivated *N. sativa* plants with NPK \times FF is very important especially in arid zones for increasing the TL yield and main constituents of FA.

Key words: *Nigella sativa* L., NPK, foliar feeding, total lipids, fatty acids, oleic, linoleic, linolenic

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Total Lipids (TL) of *Nigella sativa* L. seeds have a long history of folklore usage in different pharmaceutical and food industries¹. The TL of *N. sativa* seeds used for various cases such as chronic cough and bronchial asthma². Fatty Acids (FA) of linoleic (59.6%), oleic (23.8%), palmitic (12.4%) and stearic acid (<1%) were detected in the TL extracted from *N. sativa*³. Also, *N. sativa* FA have an antimicrobial role⁴.

Arid regions in Egypt are characterized by poor nutrients, such as nitrogen (N), phosphorus (P), potassium (K) and trace elements which negatively affect productivity of medicinal and aromatic plants⁵. Nutrition is the most important factor that promotes crop productivity.

Accordingly, N plays a necessary role in synthesis of the chemical constituents of crops through the action of various enzymes⁶. The TL production require N during the seed development⁷. The FA constituents are affected by N fertilization⁸. Application of N resulted in a significant effect in TL and FA (oleic and linoleic) of cotton seeds⁹. Thirty kg N ha⁻¹ resulted in a significant increase ($p \leq 0.05$) in TL extracted from *N. sativa* seeds¹⁰. Application of N affects TL and FA contents of sunflower¹¹. It was found that the TL and FA of canola and peanut were increased with different levels of N^{12,13}. Boydak *et al.*¹³ reported that N resulted in significant increases ($p \leq 0.05$) in TL, palmitic, stearic, oleic, linoleic, linolenic, arasidic and behenic but insignificant effects ($p \leq 0.05$) were found in oleic and linoleic of peanut. N fertilization caused a significant increase ($p \leq 0.05$) in linoleic acid of sunflower plant¹⁴. Amaranth plants treated with 50 and 100 kg ha⁻¹ produced highest amounts of FA¹⁵. The intensive N fertilizer produced an increment in the accumulation of TL isolated from anise, coriander and sweet fennel fruits, the highest values were obtained with the highest N level of 200 kg ha⁻¹, TL were 8.1, 2.7 and 0.4% higher than the control for anise, coriander and sweet fennel, respectively¹⁶. N significantly increased ($p \leq 0.05$) the TL of *N. sativa*¹⁷. Khalid and Ahmed¹⁸ indicated that N level (at 3 g pot⁻¹) resulted in the highest accumulations of TL (27.5-27.7%) of *N. sativa* compared with control.

Phosphorus (P) is required in large amounts in young cells, where metabolism is high and cell division is rapid. P has been found to improve the quality of certain crops¹⁹. It is involved many metabolic processes required for normal growth such as FA synthesis²⁰. Adding P resulted in a significant effect ($p \leq 0.05$) in accumulation of TL extracted from anise, coriander and sweet fennel²¹. Opposite trend was found by Brennan and Bolland¹², they reported that application of P resulted in no significant effect in TL extracted from canola plant.

Potassium (K) is an important nutrient and it has essential role for enzyme activation such as enzyme of TL synthesis^{22,23}. Soybean plants treated with 380 mg of K produced the highest amounts of TL compared with untreated plants²⁴. K treatments resulted in a significant effect ($p \leq 0.05$) in TL and FA constituents (oleic, palmitic, stearic and myristic acid) of sunflower^{25,26}. Under K treatments, different changes were found in TL and FA composition of *Jatropha curcas* L. plants²⁷.

The soils of Egypt are generally sandy with low Cation Exchange Capacity (CEC) values. This means that the soil does not have the ability to hold on to many of the nutrients allowing them to be easily leached out of the rooting zone during irrigation. Foliar feeding is a technique of feeding plants by applying liquid fertilizer directly to their leaves²⁸. Foliar Feeding (FF) is an effective method for correcting soil deficiencies and overcoming the soils inability to transfer nutrients to the plant under moisture conditions. The FF caused a positive effect in TL and FA (oleic and linoleic acid) cotton seeds⁹. The TL and oleic acid isolated from soybean seeds were significantly increased ($p \leq 0.05$) under FF while linolenic acid was decreased²⁹. The TL and FA composition of *N. sativa* and *N. damascena* were significantly increased ($p \leq 0.05$) with FF³⁰. The FF caused a significant effect ($p \leq 0.05$) in TL of sunflower and insignificant effect in oleic acid³¹. The FF produced the greatest values of TL compared with untreated plants of soybean²⁴. Application of FF \times soil nutrition resulted in higher accumulations in TL of anise, coriander, sweet fennel and *N. sativa* than soil nutrition without FF^{5,18}. In the future, results of this study will be introduced to who benefit such as, Ministry of Agriculture, farmers and producers to help them in production TL and FA from *N. sativa* under reclaimed lands in Egypt.

In this study, the possible effects of NPK with or without FF on the TL and FA of *N. sativa* were investigated under arid zones in Egypt.

MATERIALS AND METHODS

Experimental: Experiments were carried out in arid zone at the Experimental Farm of National Research Centre (NRC) located in Nubaria city, Egypt, during two successive seasons, 2013/2014 and 2014/2015. Physical and chemical properties of the soil used in this study were determined according to Jackson³² and Cottenie *et al.*³³ which are: Sand (81%), silt (13.5%), clay (3.5%) and gravel (2%); pH (7.9), EC (1.2 ds m⁻¹) and OM (0.3%). Anions and cations (mg 100 g⁻¹ Soil): SO₄²⁻ (1.1), Cl⁻ (19.1), HCO₃⁻ (0.2), Na⁺ (12.2), Mg⁺⁺ (0.5), Ca⁺⁺ (0.1) and K⁺ (0.4). Seeds of *N. sativa*, which were kindly provided by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. *N. sativa* seeds were sown directly in

the open field in the third week of October during both seasons. The experimental design was a complete randomized block (RCBD) with four replicates. The experimental area (plot) was 4 m² (2×2 m) containing 4 rows; the distance between hills was 25 and 50 cm apart. Thinning for three plants per hill was made 45 days after cultivating the plants in the open field. All agriculture practices operations other than experimental treatments were performed according to the recommendations of the Ministry of Agriculture, Egypt.

Plots were divided into two main groups. The first group was subjected to different levels of NPK combinations: N₀P₀K₀, N₁P₁K₁, N₂P₂K₂ and N₃P₃K₃. N₀ = 0 kg N ha⁻¹, N₁ = 100 kg N ha⁻¹, N₂ = 150 kg N ha⁻¹, N₃ = 200 kg N ha⁻¹; P₀ = 0 kg P₂O₅ ha⁻¹, P₁ = 37.5 kg P₂O₅ ha⁻¹, P₂ = 56.3 kg P₂O₅ ha⁻¹, P₃ = 75 kg P₂O₅ ha⁻¹; K₀ = 0 kg K₂O ha⁻¹, K₁ = 37.5 kg K₂O ha⁻¹, K₂ = 56.3 kg K₂O ha⁻¹, K₃ = 75 kg K₂O ha⁻¹. The second group was subjected to the same NPK treatments but FF was added at 1.5 mL L⁻¹. N source was ammonium sulfate {(NH₄)₂SO₄} (20% N). The P₂O₅ source was calcium superphosphate (15% P₂O₅). The K₂O was potassium sulfate (48% K₂O). The FF source was commercial solution (Agronal), which consists of the following minerals: N (0.12 g L⁻¹), P₂O₅ (0.04 g L⁻¹), K₂O (0.04 g L⁻¹), Mg (0.002 g L⁻¹), S (0.002 g L⁻¹), Fe (1.2 g L⁻¹), Zn (1.2 g L⁻¹), Mn (1 g L⁻¹), Cu (0.5 g L⁻¹), Ni (0.001 g L⁻¹) and Co (0.001 g L⁻¹).

Harvesting: At fruiting stage, plants were harvested. Seed yields (g plant⁻¹) were recorded.

Extraction of TL: The seeds 10 g were powdered mechanically and extracted with light petroleum ether (40-60°C) for 4 h in a Soxhlet apparatus. Removal of the solvent under reduced pressure gave the crude lipid³⁴. In addition, TL yield (% and g plants⁻¹) were calculated by using the dry seeds of both seasons. The TL extracted from black cumin seeds was collected in both seasons from each treatment to identify the FA.

Gas Chromatography (GC): The FA content of the TL was investigated by GC analysis (Shimadzu GC-9) of their methyl esters. A TL (0.5 g) was dissolved in 20 mL light petroleum ether (60-80°C), 2 mL methanol and 2 mL KOH was added. The mixture was shaken for 2 min and allowed to stand for 10 min. The upper layer was removed, washed with water and 1 mL used for GC analysis³⁵. The GC analyses were performed using an HP 6890 gas chromatograph with a Supelco SP23 80 capillary column (60 m×0.25 mm×0.20 µm) and helium as the carrier gas. The oven temperature was kept

at 140°C for 5 min, programmed to 165°C at 5°C min⁻¹ and kept at 165°C for 10 min, then programmed to 190°C at 5°C min⁻¹ and kept at 190°C for 20 min. Inject or/and detector (FID) temperatures were kept at 250°C. The split ratio was 70:1. Relative percentage amounts were calculated from the total area under its peaks by the software of apparatus (MON 2000, version 4.25).

Gas Chromatography Mass Spectrometry (GC-MS): The GC-MS analyses of the TL were carried out on HP GC-MS 6890-5 973 model instruments. The GC column used SP23 80 capillary column 60 m×0.25 mm×0.20 µm. The oven temperature was as above; transfer line temperature 280°C; ion source temperature 230°C; carrier gas helium; splitting ratio 1:10; ionization energy 70 eV; scan range 15-550 amu.

Qualitative and quantitative analyses: Compounds were identified by comparison of their GC retention times with those of reference solutions of 1% w/v of the methyl esters of the FA and also by comparison of their mass spectra with either known compounds or published spectra (Wiley 275.L). Quantified ion of FA methyl esters was obtained directly from GC peak area using Chemstation 8.02 software and expressed as percent ages.

Statistical analysis: In this experiment, 2 factors were considered: NPK: N₀P₀K₀, N₁P₁K₁, N₂P₂K₂ and N₃P₃K₃ and FF (with and without). For each treatment there were 4 replicates. The experimental design followed a RCBD. According to Snedecor and Cochran³⁶ the averages of data were statistically analyzed using two way analysis of variance (ANOVA). Significant values determined according to p-values (p<0.05 = significant, p<0.01 = moderate significant and p<0.001 = highly significant). The applications of that technique were according to the STAT-ITCF program, version 10³⁷.

RESULTS

Effect of NPK, FF and their interactions on TL contents: Data presented in Table 1 revealed that NPK treatments resulted in a positive increment in TL contents (% and yield) during both seasons. The highest amounts of TL were obtained with N₂P₂K₂ during both seasons while the lowest values were recorded with N₀P₀K₀ treatment. The increases in TL were highly significant (p<0.001) for NPK treatments.

The FF caused highly significant increases (p<0.001) in TL (%) or yield compared with the treatments of without FF.

Table 1: Effect of NPK, FF and their interactions on TL contents

		Total lipids			
Treatments		Percentage		Yield (g plant ⁻¹)	
FF	NPK	1st seasons	2nd seasons	1st seasons	2nd seasons
Without FF	N ₀ P ₀ K ₀	14.9±0.9	17.8±0.8	0.9±0.1	1.7±0.3
	N ₁ P ₁ K ₁	23.6±0.6	24.7±0.7	1.9±0.1	2.0±0.1
	N ₂ P ₂ K ₂	29.5±0.5	32.7±0.7	2.7±0.3	2.9±0.1
	N ₃ P ₃ K ₃	27.9±0.9	31.1±0.1	2.2±0.2	2.5±0.5
Overall without FF		24.0±5.9	27.0±6.2	2.0±0.7	2.2±0.7
With FF	N ₀ P ₀ K ₀	16.9±0.9	19.6±0.6	1.5±0.5	1.8±0.2
	N ₁ P ₁ K ₁	24.8±0.8	26.7±0.7	3.0±0.1	3.2±0.2
	N ₂ P ₂ K ₂	33.5±0.5	34.9±0.9	4.7±0.7	4.9±0.1
	N ₃ P ₃ K ₃	30.8±0.8	32.7±1.8	4.0±1.0	3.1±0.1
Overall with FF		27.0±6.9	28.5±6.4	3.3±1.4	3.3±1.2
Overall NPK	N ₀ P ₀ K ₀	15.9±1.4	18.7±1.2	1.2±0.5	1.8±0.2
	N ₁ P ₁ K ₁	24.2±0.9	25.7±1.3	2.5±0.8	2.5±0.9
	N ₂ P ₂ K ₂	31.5±2.2	33.8±1.4	3.7±1.2	3.5±1.1
	N ₃ P ₃ K ₃	29.4±1.8	31.9±1.6	3.1±1.2	3.0±0.5
F-value					
NPK		505.5***	350.6***	19.1***	25.9***
FF		67.0***	31.5***	31.4***	31.4***
NPK×FF		3.8*	0.1	1.7	5.5**

FF: Foliar feeding, N: Nitrogen, P: Phosphorous, K: Potassium, *p<0.05, **p<0.01 and ***p<0.001, values are given as Mean±SD

The TL contents (% and yield) were affected by NPK×FF treatments during both seasons (Table 1). Thus the various TL contents in general were increased under NPK×FF treatments compared with NPK levels without FF. Greatest contents of TL were recorded with the treatment of N₂P₂K₂×FF. Lowest TL amounts were recorded with N₀P₀K₀ without FF treatment. The changes in TL (%) were significant (p<0.05) during first season but it was insignificant during second season. The variations in TL yield were insignificant at first season while it was moderate significant (p<0.01) during second season.

Effect of NPK, FF and their interactions on FA constituents:

The GC-MS analysis revealed the presence of nine different FA identified under NPK, FF and their interactions (Table 2, 3), oleic, linoleic and linolenic were detected as the major FA which gave the highest percentages (more than 80%). The FA constituents were identified in TL isolated from *N. sativa* seeds belong to two chemical classes. Unsaturated FA (UFA) was the major one, the remaining fraction as saturated FA (SFA) formed the minor class. The SFA were caprylic, capric, lauric, myristic, stearic and arachidic acid while UFA were oleic, linoleic and linolenic acid.

Different changes were found in FA under various NPK treatments (Table 3). The SFA i.e. caprylic, capric and myristic acid were gradually increased as NPK increase while lauric, stearic and arachidic acid were decreased. Regarding to UFA (oleic, linoleic and linolenic acid) were increased with increasing NPK dose. Meanwhile TSFA decreased but TUFA

increased with increasing NPK amount. The N₃P₃K₃ treatment produced the highest values of caprylic, capric, myristic, oleic, linoleic, linolenic acid and TUFA while N₀P₀K₀ produced greatest values of lauric, stearic, arachidic and TSFA. Changes in all FA were highly significant (p<0.001) except caprylic, capric (insignificant) and linoleic (significant, p<0.05).

It was found that FF resulting in various changes in FA (Table 3). All USF and TUFA were increased with FF treatment. FF caused an increment in SFA such as caprylic, capric, lauric and myristic acid while produced a reduction in stearic, arachidic and TSFA. The FF treatment recorded the highest values for caprylic, capric, lauric, myristic, stearic, oleic, linoleic and linolenic and TUFA. Treatments without FF recorded the highest values for stearic, arachidic and TSFA. Changes in lauric, myristic, arachidic, TSFA, oleic, linolenic and TUFA were highly significant (p<0.001) for FF treatment. Variations in caprylic, capric, myristic and linoleic were insignificant but it was significant (p<0.05) in arachidic.

The interactions (FF×NPK) resulted in different changes in FA isolated from *N. sativa* seeds. The FF×NPK caused different increases in caprylic, capric, myristic, all USFA and TUFA but resulted in different reductions in lauric, stearic, arachidic and TSFA. The N₃P₃K₃×FF produced the highest values of caprylic, capric, myristic, oleic, linoleic, linolenic and TUFA. The N₀P₀K₀ produced greatest values of lauric, stearic, arachidic and TSFA. Changes in caprylic, capric, myristic, arachidic and linoleic acid were insignificant for FF×NPK but the variations in lauric, stearic, TSFA, oleic and

Table 2: Effect of the interactions between NPK and FF on FA constituents

		NPK×FF								
		Without FF				With FF				
Fatty acids	RT	N ₀ P ₀ K ₀	N ₁ P ₁ K ₁	N ₂ P ₂ K ₂	N ₃ P ₃ K ₃	N ₀ P ₀ K ₀	N ₁ P ₁ K ₁	N ₂ P ₂ K ₂	N ₃ P ₃ K ₃	F-value
SFA										
Caprylic (C8:0)	7.2	1.2±0.2	1.3±0.3	1.4±0.2	1.5±0.5	1.3±0.1	1.4±0.2	1.6±0.2	1.7±0.3	0.1
Capric (C10:0)	11.6	2.9±0.1	3.2±0.2	3.3±0.3	3.5±0.5	3.0±0.3	3.3±0.3	3.4±0.4	3.7±0.3	1.0
Lauric (C12:0)	14.2	4.7±0.2	3.2±0.2	2.9±0.1	2.7±0.2	4.3±0.2	2.8±0.2	2.2±0.2	1.3±0.3	6.8***
Myristic (C14:0)	19.4	2.1±0.1	2.5±0.5	2.6±0.1	2.7±0.2	2.2±0.2	2.6±0.1	2.7±0.2	2.9±0.1	0.0
Stearic (C18:0)	22.6	6.9±0.9	5.2±0.2	4.5±0.5	3.9±0.1	5.7±0.2	5.1±0.1	4.7±0.3	2.1±0.1	8.8***
Arachidic (C20:0)	24.8	0.8±0.2	0.7±0.3	0.6±0.1	0.5±0.1	0.9±0.1	0.5±0.1	0.3±0.1	0.2±0.1	2.3
TSFA		18.6±0.1	16.1±0.1	15.3±0.3	14.8±0.2	17.4±0.2	15.7±0.3	14.9±0.1	11.9±0.1	12.2***
UFA										
Oleic (C18:1)	28.2	22.7±0.3	22.8±0.2	22.9±0.9	23.4±0.4	22.9±0.3	23.1±0.1	23.3±0.3	25.6±0.4	7.9***
Linoleic (C18:2)	33.4	44.9±0.1	45.1±0.1	45.2±0.2	46.6±0.4	45.0±0.4	45.4±0.4	46.3±0.3	47.8±0.2	0.4
Linolenic (C18:3)	37.6	13.1±0.1	13.2±0.2	13.5±0.5	14.1±0.1	13.8±0.1	13.8±0.2	14.7±0.3	14.4±0.2	3.2*
TUFA		80.7±0.1	0.1±0.1	81.6±0.4	84.1±0.1	81.7±0.1	82.3±0.3	84.3±0.4	87.8±0.4	9.7***
TFA		99.3	97.2	96.9	98.9	99.1	98.0	99.2	99.7	

TL: Total lipids, FF: Foliar feeding, N: Nitrogen, P: Phosphorous, K: Potassium, FA: Fatty acids, SFA: Saturated fatty acids, TSFA: Total saturated fatty acids, UFA: Unsaturated fatty acids, TUFA: Total unsaturated fatty acids, TFA: Total fatty acids, RT: Retention time, *p<0.05, **p<0.01 and ***p<0.001, Values are given as Mean±SD

Table 3: Effect of NPK or FF on FA constituents

		Overall NPK				Overall FF		F-value	
Fatty acids	RT	N ₀ P ₀ K ₀	N ₁ P ₁ K ₁	N ₂ P ₂ K ₂	N ₃ P ₃ K ₃	Without FF	With FF	NPK	FF
SFA									
Caprylic _(C8:0)	7.2	1.3±0.2	1.4±0.2	1.5±0.2	1.6±0.4	1.4±0.3	1.5±0.3	1.7	1.6
Capric _(C10:0)	11.6	3.0±0.6	3.3±0.2	3.4±0.3	3.6±0.4	3.2±0.3	3.4±0.5	0.3	0.8
Lauric _(C12:0)	14.2	4.5±0.3	3.0±0.3	2.6±0.4	2.0±0.8	3.4±0.8	2.7±1.1	141.6***	64.7***
Myristic _(C14:0)	19.4	2.2±0.2	2.6±0.3	2.7±0.2	2.8±0.2	2.5±0.3	2.6±0.3	8.1***	1.2
Stearic _(C18:0)	22.6	6.3±1.0	5.2±0.2	4.6±0.4	3.0±1.0	5.1±1.2	4.4±1.4	61.7***	22.7***
Arachidic _(C20:0)	24.8	0.9±0.2	0.6±0.2	0.5±0.2	0.4±0.1	0.7±0.2	0.5±0.3	11.9***	7.7*
TSFA		18.2±0.9	16.1±0.3	15.3±0.3	13.4±1.6	16.3±1.6	15.1±2.1	129.4***	51.4***
UFA									
Oleic _(C18:1)	28.2	22.8±0.2	23.0±0.2	23.1±0.6	24.5±1.3	23.0±0.5	23.8±1.2	21.6***	21.0***
Linoleic _(C18:2)	33.4	45.0±1.9	45.3±0.3	45.8±0.6	47.2±0.7	45.5±0.7	46.1±1.7	5.0*	2.3
Linolenic _(C18:3)	37.6	13.5±0.4	13.5±0.4	14.1±0.8	14.3±0.2	13.6±0.5	14.1±0.5	15.5***	45.2***
TUFA		81.3±0.9	81.8±0.7	83.0±1.5	86.0±1.9	82.1±1.5	84.0±2.4	122.1***	124.8***
TFA		99.5	97.9	98.3	99.4	98.4	99.1		

FF: Foliar feeding, N: Nitrogen, P: Phosphorous, K: Potassium, SFA: Saturated fatty acids, TSFA: Total saturated fatty acids, UFA: Unsaturated fatty acids, TUFA: Total unsaturated fatty acids, TFA: Total fatty acids, *p<0.05, **p<0.01 and ***p<0.001, Values are given as Mean±SD

TUFA were highly significant (p<0.001) while the changes in linolenic acid were significant (p<0.05).

DISCUSSION

During this study, it was found that NPK, FF or NPK×FF resulted in different variations in TL and FA composition. The variation in TL and FA constituents under NPK and FF treatments may be due to its effects on enzymes activity and metabolism improvements³⁸. On the other hand, NPK and FF play an important role through the action of different enzymes activity and protein synthesis, cell divisions and involve many metabolic processes required for normal growth^{6,19}, that reflected on an increase in seed yield which is

storage of TL and FA composition. Also FF eliminates the effects of soil pH on the availability of soil nutrients; it means FF can decrease the soil pH that reflected an increase in soil nutrients availability, so the plants grow well and produce high amounts of TL and FA¹⁴.

The obtained results are in accordance with those obtained by previous literature i.e. application of N resulted in a significant effect (p≤0.05) in TL and FA (oleic and linoleic) of cotton and *N. sativa* seeds^{9,10}. Phosphorus (P) resulted in significant (p<0.05) effect in TL extracted from anise, coriander and sweet fennel⁵. Opposite trend was found by Brennan and Bolland¹², canola plant. Potassium (K) treatments resulted in a various changes in TL and FA constituents (oleic, palmitic, stearic and myristic) of sunflower and *Jatropha curcas* L.

plants^{26,27}. The TL and oleic acid isolated from soybean seeds were significantly increased ($p \leq 0.05$) under FF while linolenic acid was decreased²⁹. The TL and FA composition of *N. sativa*, *N. damascene*, sunflower, soybean, anise, coriander and sweet fennel were significantly increased ($p \leq 0.05$) with FF^{5,18,24,30,31}. The NPK and FF resulted in an increase in TL content³⁹. Espinosa *et al.*²⁰ indicated that NPK×FF plays an important role in various metabolism processes such as TL and FA synthesis. The FF resulted in the highest accumulations of TL of anise, coriander, sweet fennel and *N. sativa*^{5,18}. Similar FA were found in *N. sativa* by previous studies^{40,41}, they revealed the presence of nine different fatty acids were identified in *N. sativa*, linoleic, oleic or linolenic acid were detected as the major fatty acids. The UFA was the major one, the remaining fraction as SFA formed the minor class, SFA were caprylic, capric, lauric, myristic, stearic and arachidic acid while UFA were oleic, linoleic and linolenic acid. This study discovers that the cultivation of *N. sativa* plants with NPK×FF are very important especially in arid and semi arid zones for production the lipids and FA that can be beneficial for producers and farmers in reclaimed areas of Egypt. This study recommended the use of the combination of N (200 kg N ha⁻¹), P (75 kg P₂O₅ ha⁻¹), K (75 K₂O ha⁻¹) with FF (Agronal at 1.5 mL L⁻¹).

CONCLUSION

It can be concluded that NPK resulted in a positive effect of TL and FA composition of *N. sativa* seeds. TL and main FA compositions (oleic, linoleic and linolenic acid) were increased under NPK×FF. Greatest values of main FA were resulted from N₃P₃K₃×FF treatments.

SIGNIFICANCE STATEMENTS

- This study discovers that the cultivation of *N. sativa* plants with NPK×FF is very important
- The increases in TL were highly significant for NPK treatments
- FF caused highly significant increases in TL (%) or yield compared with the treatments of without FF
- The interactions (FF×NPK) resulted in different changes in FA isolated from *N. sativa* seeds

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