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The impact of α-tocopherol and nicotinamide on performance of lupine plant grown under sandy soil conditions

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Abstract

To study the influence of α-tocopherol (α-TOC) and nicotinamide (NAM) on growth and physiological responses of *Lupinus termis* plant, field experiments were conceded at Experimental Station of National Research Centre, Nubaria Region, Behaira Governorate, Egypt. Three levels of α-TOC (1 mM, 2 mM, 3 mM) and NAM (1 mM, 5 mM, 10 mM) were applied as foliar spray. Growth measurements were performed at 75 days after sowing. Seed yield and yield attributes as well as seed quality were also determined at harvest stage. Foliar application with α-TOC and NAM significantly improved lupine plants in sandy soil in terms of growth, photosynthetic pigments, indole-3-acetic acid, seed yield, yield components and seed quality (oil%, carbohydrate%, phenolic compounds (mg/g) and flavonoid compounds (mg/g) and antioxidant activity). NAM treatments were more effective than α-TOC. 10 mM NAM was the most pronounced treatment. It is worthy to mention that α-TOC (2 mM) and NAM (10 mM) caused marked decreases in saturated fatty acids of seed oil accompanied by increases in unsaturated fatty acids, hence improving oil quality. The current research suggested that α-TOC and NAM as a foliar application increased lupine growth, seed yield and seed quality under reclaimed sandy soil.

Keywords: Lupinus termis L, Vitamin E, Vitamin B3, Niacin, Sandy Soil.

1. Introduction

Bitter lupine (*Lupinus termis* L.) as Egyptian leguminous plant is cultivated for soil improvement, human consumption and animal feed. This crop has high nutritional value due to its high protein (35-45%) and oil content (10-15%), its seeds have a nutritional quality similar to that of soybeans and superior to those of many other leguminous plants **[1]**. Thus, a legume strategic objective was planned for extending cultivating lupine in reclaimed desert areas for sustainable production and progressive conservation and development of soil fertility. Improvement and optimizing efficient growth yield and seed quality of lupine plants required application of potential bio-regulator treatment(s) for amelioration of the stressful conditions in sandy arid lands.

one of the existing challenges is to maintain crop productivity, even under the occurrence of adverse conditions in sandy soil. In this sense, we are looking for products or elements with a protective character that can maintain adequate development of the plant. Among the studied elements are vitamins. Vitamins are bio-regulator compounds that control many physiological processes, such as enzyme synthesis, function as co-enzymes **[2]**, regulate metabolism **[3]** and affect plant growth at relatively low concentrations **[4]**.

α-TOC is a lipophilic antioxidant, which mainly protects membranes from oxidative damage **[5]**. α-TOC is generated exclusively in photosynthetic membranes of plant leaves that are concerned in the quenching and scavenging of reactive oxygen species **[6]**. α-TOC is a powerful antioxidant, protecting lipids and cell membranes by scavenging free radicals and inhibiting lipid peroxidation through donating its phenolic hydrogen's to lipid free radicals **[7, 8, 9 &10]**. Under stress condition, one molecule

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of α-TOC can deactivate 120 oxygen molecules **[11]**. In addition, α-TOC can modulate signal transduction pathways **[9, 3, & 12]** and phytohormone levels **[13]**. α-Toc protect metabolic processes, affect many enzyme activities, minimize the damage caused by oxidative processes **[14]**, maintaining membrane stability **[15]**, regulate the transport of electrons in photosystem-II **[16]**, protect photosystem II from photo-inhibition **[17]**, improve membrane permeability to small ions and molecules **[18]** and control certain gene expressions **[19, 20]**.

Nicotinamide (NAM) is a constituent of pyridine dinucleotide coenzymes NADH and NADPH, which are directly, associated with some enzymatic redox reactions in living cells. The nicotinamide concentration may increase in plants after situations that cause oxidative stress and induce defensive metabolism **[21]**. Under stress NAM regulates secondary metabolite accumulation and induces defense metabolism manifestation in plants **[22]**.

This research aimed to assess growth, physiological and biochemical responses of *Lupinus termis* (Giza 2) to varying levels of exogenously applied α-TOC and NAM under sandy soil conditions.

2. Materials and Methods

A field experiments were performed at the experimental station of National Research Centre, Nubaria District, El-Behaira Governorate, Egypt, during the two winter growing seasons of 2019/2020 and 2020/2021, respectively to investigate the influence of foliar application with different concentrations of α-TOC and NAM on growth, yield and its components as well as chemical composition of lupine plants. Three concentrations of α-TOC (1 mM, 2 mM, 3 mM) and NAM (1 mM, 5 mM, 10 mM) were applied. Tap water was used as control. Egyptian lupine seeds (*Lupinus termis* L. cultivar Giza 2) were kindly provided by the Legume Research Department, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.

The soil of the experimental site was reclaimed sandy soil where mechanical and chemical analysis is reported in Table (1)

2.1. Experimental Procedure

Lupine seeds were sown in newly sandy soil on November. The studied treatments were arranged in a complete randomized block design with three replicates. Experimental plot area was 10.5 m^2 (five rows, 3.0 m width and 3.5 m length and 60 cm apart). Lupine seeds were sown in hills at both sides of the ridge with 30 cm distances between the hills. Lupine seeds were sown at a rate of 40 kg/fed. After sowing, all plots were immediately irrigated. The plants were thinned after 21 days. Agricultural practices for growing lupine plants were conducted in the usual manner according to Ministry of Agriculture, Egypt. After 30 and 60 days from sowing, plants were sprayed with different concentrations of α-TOC and NAM by means of an atomizer. The control plants were sprayed with tap water.

Table 1: mechanical and chemical analysis

Mechanical analysis				
Sand		**Silt 20-0µ%**	**Clay < 2µ%**	**Soil texture**
Course 2000-200µ%	**Fine 200-20µ %**			
46.80	34.79	14.66	5.29	sandy
Chemical analysis				
pH (1:2.5)	**EC (dsm-1)**	**CaCO3**	**OM %**	
7.40	0.16	5.3	0.08	
Soluble Cations meq/I				
Na^+	**K^+**	**Mg^+**	**Ca^{++}**	
0.56	0.14	0.90	1.0	
Soluble Anions meq/I				
CO_3^{--}	**HCO_3^-**	**Cl^-**	**SO_4^{--}**	
0.0	1.28	0.46	0.80	

2.2. Data recorded

2.2.1. Growth Criteria

Ten uniform plants for each treatment were taken randomly from the middle two rows of each plot during the two growing seasons. The sample was taken after 75 days from sowing (vegetative stage). Growth parameters include shoot length (cm), number of leaves and shoots/plant, stem fresh and dry weight/plant, leaves fresh and dry weight/plant.

2.2.2. Determination of photosynthetic pigments

Photosynthetic pigments (Chlorophylls a & b and carotenoids and total chlorophyll) were extracted from fresh leaf tissue and estimated spectrophotometrically according to the method described by **Sumanta *et al.* [23]**. Samples (0.5 g) were extracted into 10 mL 80% aqueous acetone and incubated in the dark for 24 h. After two centrifugations (10 min, 10,000 rpm, 4 ◦C), absorptions were measured at 663, 647, and 470 nm.

2.2.3. Determination of indole-3-acetic acid

Indole-3-acetic acid content was extracted and estimated from fresh leaves by the method of **Larsen *et al.* [24]**.

2.2.4. Yield and yield attributes

At harvest time (120 days after sowing), ten plants for each treatment were chosen randomly from the middle two rows of each plot, to estimate yield and yield attributes. The following characteristics of plant yield were recorded in the two studied seasons (number of pods per plant, weight of pods (g), number of seeds per plant, weight of seeds per plant (g), 100 seeds weight (g) and straw yield/plant (g)).

2.2.5. Chemical composition of seeds*:*

Seeds from each treatment were collected, cleaned and crushed by using a mortar and grinder and prepared for analysis. The following chemical analyses were carried out to determine the quality of yielded seeds:

2.2.5.1. Oil content

The oil content of the yielded seeds was determined according to the procedure reported by **A.O.A.C. [25]**.

2.2.5.2. Total carbohydrates

Total carbohydrates were determined colorimetrically by the phenol-sulfuric acid method as described by **Dubois *et al*. [26].** 0.1 g of fine dry powder of plant was added in a sugar tube with 10 ml sulfuric acid (1N H_2SO_4). After boiling and cooling, One ml sugar solution was transferred into a test tube and 1 ml 5% aqueous phenol solution was added followed by 5 ml concentrated sulfuric acid. Absorbance was measured at 490 nm. A standard curve was prepared using a known concentration of D-glucose.

2.2.5.3. Total phenolic compounds content (TPC)

TPC content in lupine seeds was extracted and measured with Folin Ciocalteu reagent as the method described by **Tavarini *et al.* [27].** The methanolic extract (0.5 ml of 1 mg/ml solution) was mixed with 2.5 ml of Folin-Ciocalteu reagent (10%) and 2 ml of sodium carbonate (7.5% w/v). After vortexing for 15 s, the reaction mixture was incubated at room temperature in the dark for 30 min and the absorbance was measured at 765 nm using a UV-1601 Schimadzu UV-Vis spectrophotometer. The TPC was calculated using the regression equation from the calibration curve constructed using gallic acid standards (0 to 300 ppm) treated in the same manner as the extract. The results were expressed as mg gallic acid equivalent per gram of dry weight of the extract (mg GAE/g DW).

2.2.5.4. Total flavonoids content

Total flavonoids content was measured by the aluminium chloride colorimetric assay as described by **Ordoñez *et al.* [28]**. Plant extract was obtained by immersing 100 mg of dried plant sample in 3 mL of methanol. To 1 mL of extract, we added 4 mL double-distilled water, 5% sodium nitrite (0.3 mL), and 10% aluminium chloride (0.3 mL), followed by incubation for 5 min. Sodium hydroxide (2 mL) was added to the reaction mixture. Absorbance was measured at 510 nm. Flavonoids concentration was calculated according to a calibration curve using quercetin as a standard. The results were expressed as mg of quercetin equivalents per gram of dry weight of the extract (mg QE/g DW).

2.2.5.5. DPPH radical scavenging activity

Antioxidant activity of lupine seeds were extracted and determined using the **Brand-Williams *et al.* [29]** technique dependent on the free radical scavenging effect of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reagent.

2.1.1.6. Determination of fatty acids

The fatty acids composition was prepared from an aliquot of total lipids according to **Harborne [30]**. Identification and quantitative determination of fatty acids were performed using Gas Liquid Chromatography (GC). Fatty acids were identified by comparison of their retention times with those of the standards.

Statistical analysis

The data were expressed as means ± standard deviations (SDs) of three replicate values for growth criteria and all the chemical analyses. The obtained data were statistically analyzed using ASSISSTAT Software. The differences between treatment means were compared by LSD at 5% probability level according to **Silva and Azevedo [31]**.

3. Results and Discussion

Vegetative growth parameters

Data presented in Table 2 revealed that all α-TOC and NAM concentrations significantly increased shoot length, number of leaves/plant, number of shoots/plant and fresh and dry weight of stem and leaves compared with untreated plants. It was noted that 2mM α-TOC significantly increased dry weight of stem and leaves relative to control by 2.9 and 1.98 times, respectively. Likewise, 10 mM NAM significantly increased dry weight of stem and leaves relative to control by 2.78 and 2.0 times, respectively. α-TOC may play a role in cell division or cell enlargement **[32]**, DNA replication **[33]** and increments of endogenous phytohormones in plant **[34]**. Furthermore, α-TOC associated with different metabolic processes such as, better water status, uptake of essential nutrients, up-regulation of the oxidative defense system, improved rate of photosynthesis, and synthesis of chlorophyll **[35, 36]** preservation of membrane stability and mitigation of lipid peroxidation **[37]**.

NAM can induce plant metabolic processes through increasing the content and activity levels of endogenous promoters as indole-3-acetic acid **[38]**. The rise in IAA, as illustrated in Table 2, matches with the rise in growth rate, as described in Table (2). This increase may due to the role of endogenous hormone in stimulating cell division and/or the cell enlargement **[39, 40]** and consequently accelerates growth and yield of crops.

Table 2: Effect of foliar spray by different α-TOC and NAM concentrations on vegetative growth parameters of lupine plants.

Treatments	Conc (mM)	Shoot length (cm)	Number of shoots /plant	Number of leaves /plant	Stem fresh weight/plant (g)	Leaves fresh weight/plant (g)	Stem dry weight/plant (g)	Leaves dry weight/ plant (g)
Control		36.33^{c} ±0.58	3.00^{c} ±0.58	20.67^{d} ±1.53	5.31^{c} ±0.38	2.54^{d} ±0.19	0.36^{c} ±0.05	0.75^{d} ±0.03
α-TOC	1	38.00^{bc} ±0.00	3.67^{abc} ±0.00	21.67^{cd} ±1.53	9.53^{b} ±0.20	6.19^{c} ±0.71	0.88^{b} ±0.34	1.36^{c} ±0.50
	2	40.67^{a} ±2.08	4.33^{a} ±0.58	23.33^{bc} ±1.53	10.53^{ab} ±0.23	7.38^{a} ±0.42	1.05^{ab} ±0.03	1.50^{a} ±0.06
	3	39.67^{ab} ±1.53	4.00^{ab} ±0.58	23.33^{bc} ±0.58	10.21^{ab} ±0.27	7.08^{ab} ±0.17	1.01^{ab} ±0.04	1.45^{ab} ±0.02
NAM	1	36.67^{c} ±0.58	3.33^{bc} ±0.50	22.33^{cd} ±1.53	10.09^{ab} ±0.22	6.77^{b} ±0.61	0.96^{ab} ±0.03	1.44^{b} ±0.09
	5	39.33^{ab} ±0.58	3.33^{bc} ±0.58	25.00^{b} ±1.00	10.54^{ab} ±0.07	6.75^{b} ±0.55	0.96^{ab} ±0.01	1.50^{a} ±0.07
	10	39.67^{ab} ±2.08	3.67^{abc} ±0.00	28.67^{a} ±0.58	10.63^{a} ±0.23	7.07^{ab} ±0.52	1.01^{a} ±0.03	1.51^{b} ±0.08

Photosynthetic pigments and IAA

Table 3 clearly showed that all treatments significantly increased IAA. The highest significant increases in IAA were recorded for 2mM and 3mM of α-TOC. 2mM α-TOC significantly increased IAA by 1.82 times compared to control.

From Table 3, all applied treatments caused marked increases in photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total chlorophylls). The most effective treatments on photosynthetic pigments were 10 mM of NAM followed by 2mM of α-TOC compared to the corresponding untreated control plants.

Regarding the effect of vitamins application on IAA content**,** El-Hariri *et al.* **[41]** mentioned that α-TOC application activated the biosynthesis of the endogenous hormones (cytokine, GA3 and IAA) contents in flax. According to Sadak *et al.* **[42]**, seed priming with NAM increased significantly indole acetic acid (IAA) in *Pisum sativum* L. plants.

From Table 3, all vitamin treatments increased photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total chlorophylls). This may be due to role of α-TOC in preventing lipid peroxidation, protecting chloroplast membranes from photo-oxidation and assisting the transport of electrons in photosystem-II **[43]**. α-TOC helps also to maintain thylakoid membrane structure and function during plant development **[16, 19]** and prevents peroxidation of lipids and polyunsaturated fatty acids **[44]**. Additionally, α-TOC plays a crucial part in the xanthophyll cycle that occurs in chloroplasts, maintaining the chlorophyll concentrations **[13]**. Lalarukh *et al.* **[45]** stated that foliar spray of 200

mg/L α-TOC increased significantly photosynthetic pigments of sunflower plants.

NAM helps in increasing photosynthetic pigments through its role in protecting chloroplast and its membrane from oxidative damage **[46, 47]**.The stimulating influence of vitamins on the biosynthesis of chlorophyll may be attributed to its role in stimulation of enzymes that regulate photosynthetic carbon reduction **[46]**.

Table 3: Effect of α-TOC and NAM concentrations on IAA (µg/g fresh weight) and photosynthetic pigments (mg/100g fresh weight) of lupine plant.

Treatments	Conc. (mM)	IAA	Chlorophyll a	Chlorophyll b	Carotenoids	Total chlorophylls
Control		25.79f±0.17	0.581^{d}±0.09	0.194^{c}±0.04	0.077^{d}±0.01	0.831^{d}±0.14
α-TOC	1	40.94^{d}±0.59	0.649^{cd}±0.01	0.253^{bc}±0.00	0.082^{d}±0.01	0.971^{cd}±0.02
	2	46.92^{a}±0.14	1.349^{a}±0.11	0.415^{a}±0.03	0.160^{ab}±0.02	1.927^{a}±0.13
	3	43.74^{b}±0.12	1.173ab±0.34	0.350^{ab}±0.10	0.138^{bc}±0.03	1.631^{ab}±0.29
NAM	1	38.25^{e}±0.40	0.78^{cd}±0.28	0.274^{bc}±0.09	0.107^{cd}±0.04	1.135^{cd}±0.08
	5	41.74^{c}±0.22	0.951^{bc}±0.09	0.305^{b}±0.06	0.136^{bc}±0.01	1.346^{bc}±0.16
	10	41.80^{c}±0.55	1.427^{a}±0.01	0.415^{a}±0.00	0.185^{a}±0.00	1.973^{a}±0.01

Seed yield and yield components

Table 4 demonstrated that all applied treatments enhanced seed yield and its components significantly expressed as number of pods/plant, weight of pods /plant, number of seeds/plant, weight of seeds/plant, 100 seeds weight, and straw yield. Concentration of α-TOC at 1mM showed non-significant increases in yield parameters. While, 2mM of α-TOC was the most pronounced treatment. Regarding to nicotinamide effect 10 mM of NAM was the most effective one.

The increase in seed yield and yield components (Table 4) is probably referred to the role of α-TOC in improving nutrient uptake from the soil, increasing the photosynthetic rate, enhancing protein synthesis, delaying senescence, increasing the plant growth, and consequently seed yield **[48]**. In addition, Sadak and Dawood **[49]** mentioned that under saline conditions, α-TOC at 0.46 and 0.93 mM caused marked increases in yield and yield attributes of three flax cultivars.

Sadak **[42]** and Dawood *et al.* **[50]** indicated that NAM had a positive impact on growth parameters, amounts of IAA, photosynthetic pigments, seed yield, and yield components as well as biochemical constituents of pea and faba bean seeds.

Table 4: Effect of α-TOC and NAM concentrations on seed yield and yield components of lupine plant.

Treatments	Conc. (mM)	Number of pods /plant	Weight of pods/plant (g)	Number of seeds/plant	Seed yield/plant (g)	100 seeds weight (g)	Straw weight/plant (g)
Control		12.74^{f}±0.01	15.73^{e}±0.21	36.13^{e}±0.20	9.77^{f}±0.02	26.32^{g}±0.25	3.06^{c}±0.2
α-TOC	1	14.77^{e}±0.09	18.71^{d}±0.71	37.89^{d}±0.38	9.97^{f}±0.00	27.03^{f}±0.20	4.19^{b}±0.08
	2	16.10^{a}±0.01	25.21^{b}±0.13	43.67^{b}±0.05	13.96^{c}±0.17	31.97^{c}±0.43	9.41^{a}±0.1
	3	14.89d±0.02	16.62^{e}±0.35	40.83^{c}±0.26	11.37^{e}±0.08	27.85^{e}±0.01	8.66^{a}±0.3
NAM	1	14.78^{e}±0.06	22.80^{c}±0.65	41.02^{c}±0.27	12.901^{d}±0.24	31.46^{d}±0.38	3.92b^{c}±0.3
	5	15.49^{c}±0.09	22.10^{c}±0.63	43.82^{b}±0.77	15.29^{b}±0.13	34.89^{b}±0.32	3.99b^{c}±0.5
	10	15.61^{b}±0.04	28.55^{a}±1.28	47.39^{a}±0.33	17.79^{a}±0.13	37.54^{a}±0.01	4.29^{b}±0.1

Seed Quality

The obtained data in Table 5 proved that all α-TOC and NAM treatments caused marked increase in oil content of the yielded seeds. The effect of nicotinamide was more pronounced that α-TOC. The highest significant increase in oil content was recorded by 5 mM and 10 mM of NAM followed by 2mM of α-TOC, since 10 mM of NAM significantly increased oil content by 1.42 times relative to control.

All applied treatments significantly increased total carbohydrates content of lupine seeds. 2mM of α-TOC and 10 mM of NAM significantly increased total carbohydrate by 1.05 times compared to control.

Foliar application of α-TOC and NAM significantly enhanced phenolic compounds content, flavonoids, and DPPH of lupine seeds over the untreated plants. The effect of NAM treatments was more pronounced in increasing phenolic compounds

content than α-TOC treatments. Regarding flavonoid, and DPPH, the most pronounced treatments were 2mM of α-TOC and 10 mM of NAM, since they increased flavonoids by 1.65 times and 1.62 times, respectively relative to control and DPPH by 1.35 times and 1.38 times respectively relative to control.

Concerning the promoting effect of α-TOC on the nutritive value (Table 5), Maeda and Della Penna **[18]** indicated that α-TOC plays crucial role in maintaining the photo-assimilate substances, improves the synthesis of sugar in plants by protecting chloroplasts in the plastid membrane and affects sugar transport from the source (leaves) to the sink (seeds) **[51, 52]**. According to Alnusairi **[12]**, secondary metabolites like phenols and flavonoids protect plants under stress and contribute to antioxidant property considerably. El Hariri *et al.* **[41]** verified that an increase in total phenolic compounds content by α-TOC application, which play a role in the regulation of plant metabolic processes, act as a substrate for many antioxidant enzymes **[53]**. These phenolic compounds protect cells from potential oxidative damage and increase stability of cell membranes **[14]**. α-TOC declined lipid peroxidation, increased membrane stability via raising the level of non-enzymatic antioxidants **[54]**. Subedi *et al*. **[55]** observed that the increase in both phenolic compounds and flavonoids content, resulting from connection between polyphenol content and antioxidant activity. Accumulation of total phenolic compounds and flavonoids content prevents oxidative stress by elimination of ROS, thus protecting the structural and functional integrity of key macromolecules **[56]**.

Effect of NAM on chemical composition of lupine seeds was reported in Table 5. NAM application generally stimulated the accumulation of carbohydrates by increasing endogenous levels of certain phytohormones or by acting as activators of carbohydrates synthesis **[57, 50]**. NAM induced and regulated the expression of secondary metabolic accumulation and/or defense metabolism in plants **[22, 58]**. This increase may be due to the role of phenolic in regulating plant metabolic processes and consequently overall plant growth **[59]**. Additionally, secondary metabolites have antioxidative properties **[60]**, which are also demonstrated by their ability to scavenge DPPH radicals.

Table 5: Effect of α-TOC and NAM concentrations on chemical composition of lupine seeds.

Treatments	Conc. (mM)	Oil (%)	Total carbohydrates (%)	Phenolic compounds content (mg/g)	Total flavonoids (mg/100g)	DPPH (%)
Control		9.54^{d}±0.09	41.34^{c}±0.32	50.89^{e}±0.43	35.87^{d}±0.25	42.34^{e}±0.19
α-TOC	1	11.89^{c}±0.64	42.95^{b}±0.30	63.94^{d}±1.42	46.70^{c}±2.96	51.48^{d}±0.03
	2	12.84^{b}±0.00	43.57^{a}±0.05	71.92^{b}±0.43	59.15^{a}±0.62	57.25^{b}±0.60
	3	12.65^{b}±0.42	42.74^{b}±0.12	69.78^{c}±0.40	56.24^{a}±0.70	54.25^{c}±0.40
NAM	1	12.49b^{c}±0.55	42.94^{b}±0.32	70.94b^{c}±0.59	46.10^{c}±0.25	53.92^{c}±1.57
	5	13.07^{ab}±0.04	42.90^{b}±0.00	78.94^{a}±2.29	51.80^{b}±0.55	57.17^{b}±0.40
	10	13.58^{a}±0.04	43.52^{a}±0.05	79.80^{a}±0.45	58.25^{a}±0.40	58.85^{a}±0.05

Fatty acids composition

Good results were achieved at 2 mM of α-TOC and at 10 mM of NAM, so that these treatments will undergo fatty acid fractionation.

The results of gas chromatographic analysis of the methyl esters of fatty acids of lupine oil are shown in Table 6. Data showed that both 2mM of α-TOC and 10 mM of NAM caused marked decreases in saturated fatty acids (Palmitic acid (C16:0), Stearic acid (C18:0) , Arachidic acid (20.0), Behenic acid (C22:0), Lignoceric acid (C24:0) and Eicosapentanoic acid (C20:5) accompanied by increases in unsaturated fatty acids (Oleic acid (C18:1) , Linoleic acid (C18:2) and Linolenic acid (18:3).

Function of α-TOC is the protection of polyunsaturated fatty acids from lipid peroxidation, stabilization of membranes and regulation of different signal transduction pathways **[61, 62, 18, 63]**. Tocopherols operate as a terminator in chain reaction for elimination of polyunsaturated fatty acids **[15]** by scavenging and quenching of oxygen **[64]**. α-TOC disrupts chain of lipid oxidation by reduction of radical intermediates **[65]**. One molecule of the α-, β-, γ-, δ- TOC can prevent oxidation of 220, 120, 100, 30 and 20 molecules of polyunsaturated fatty acids, respectively **[66]**. Ayad *et al.* **[67]** indicated that essential oil percent and yield of *Pelargonium graveolens L.* was increased significantly by α-TOC treatments. These increases may be due to a pronounced enhancement of α-TOC on the synthesis and accumulation of oil. El Lethy *et al.* **[68]** founded that oil yield of flax plant was significantly affected by foliar application of α-TOC.

Reducing saturated fatty acids and improving unsaturated fatty acids could be a successful step in improving the quality of seeds by vitamin treatments. Increasing seed oil's nutritional value and economic importance is due to increase in unsaturated fatty acids in response to vitamins treatments.

Table 6: Effect of α-TOC and NAM on fatty acids composition of lupine oil.

Treatments	Fatty acids										
	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Behenic	Lignoceric + Eicosapentanoic	TSA	UN	UN/TS
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C24:0+C20:5			
Control	6.35	2.42	23.35	49.32	7.62	1.72	6.90	0.83	18.22	80.29	4.40
α-TOC (2mM)	5.24	2.35	25.15	51.25	8.85	1.54	4.82	0.51	14.46	85.25	5.89
NAM (10mM)	6.15	2.35	25.05	52.52	8.35	1.52	3.85	0.62	14.49	85.92	5.87

4. Conclusion

It can be decided that α-TOC and NAM can be easily used as foliar application for *Lupinus termis* plants in the field. Application of α-TOC and NAM at different concentrations enhanced photosynthetic pigments, IAA, Growth criteria and consequently seed yield, yield components and seed quality. Application of 10 mM of NAM was the most prominent treatment. Using α-TOC and NAM compounds opens up a new opportunity for increasing lupine yield and improving quality and provides an easily applicable solution to ensure sustainable agriculture in sandy soil.

5. Declaration:

The authors declare the work is not published anywhere else.

6. Ethics approval and consent to participate

There is no need as clinical trials are not involved in study.

7. Consent for publication:

The authors gave their consent for publication.

8. Availability of data and material:

The data is available but not attached with manuscript**.**

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The authors declare that there is no conflict of interests regarding the publication of this article.

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