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Research Article

Effect of Ascorbic acid and Niacin on Protein, Oil Fatty Acids and Antibacterial Activity of *Lupinus termis* Seeds

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ABSTRACT
The aim of this work is to study the effect of foliar application of various concentrations (50, 100 and 200 ppm) of ascorbic acid and niacin on protein, oil, fatty acids and antibacterial activity of bitter lupine (*Lupinus ter*mis L.) seeds. The results obtained showed that, foliar application of both ascorbic acid and niacin had significant effect on protein and oil content of lupine seeds. Gas liquid chromatography of the oil showed increase in unsaturated fatty acids with all foliar application treatments except 200 ppm nicotinic acid compared with control. Palmitic acid (C18:0) and oleic acid (C18:1) were the most affected saturated and unsaturated fatty acids in response to different vitamins types and levels. The best ratio of linoleic over linolenic (2.18:1) was obtained with using 200 ppm of ascorbic acid. It was noted that, nicotinic acid at all concentrations caused more increases in essential fatty acids (ω-6 and ω -3) than ascorbic acid treatments at the same concentration. The results of this study showed that there was no almost antibacterial activity for *L. termis* oil extract against all the tested strains. Ethanol extracts had the highest inhibition zone in the treatment with foliar application of ascorbic acid, which increased by increasing the treatment concentration to reach its maximum value at 200 ppm. The G-ve bacteria were more resistant to the plant extract than gram-positive bacteria such as *Ps. aeruginosa* exhibited more resistant than *B. subtilis* when they were tested with *L. termis* extract.

Keywords: *Lupinus termis* L., ascorbic acid, niacin, oil, fatty acid, protein, antibacterial activity

INTRODUCTION

Bitter lupine (*Lupinus termis* L.) is considered one of The family Fabaceae (Leguminosae), its seeds are a valuable ancient legume which contains high amount of protein, dietary fiber, oil, minerals and different functional components as well as its adaptation to poor soils and dry climates (Hassan et al., 2012 and Mostafa et al., 2013). In fact, Lupine seeds have been used for human consumption and as a medicinal plant in Egypt (Kattab, 1986 and ARC, 1994). The predominant fatty acid in *Lupinus termis* oil was oleic acid and its concentration was 52.22 ± 2.32%. Bitter lupine seed oil contained 33.81% of total essential fatty acids. Overall the amount of saturated fatty acids was lower than unsaturated fatty acids in bitter lupine seed oil.

Vitamins are compounds that are required in relatively small amounts but that cannot be synthesized in quantities large enough to meet the normal needs of the organism. Vitamins could be considered natural and safety bio-regulator compounds which relatively in low concentrations exerted profound influences upon many physiological processes. Vitamin C is referred to as ascorbic acid. It is one of the most important water soluble antioxidants in plants that have synergistic effects on growth, yield and yield quality of many plant species. These compounds have beneficial effects on catching the free radicals or the active oxygen that produced during photosynthesis and respiration processes (Foyer et al. 1991 and Pastori et al. 2003). Vitamin C provides protection against the harmful side effects of light during photosynthesis, the process by which light energy is used to convert carbon dioxide into plant matter (Noctor et al. 1998). Ascorbic acid (vitamin C) is an important metabolite involved in many cellular processes, including cell division (De Gara et al., 2003), cell wall expansion (Pignocchi and Foyer, 2003), many other important enzymatic and non-enzymatic reactions (Smirnoff, 2000); as well as in regulating plant growth and development, since it plays an important role as plant growth regulator (Athar et al. 2008). Furthermore, the endogenous level of AA has recently been suggested to be important in the regulation of developmental senescence and plant defence against pathogens (Pastori et al., 2003; Barth et al., 2004; Pavet et al., 2005). Moreover, ascorbic acid is very important for the regulation of photosynthesis, flowering and senescence (Barth et al. 2006).

Vitamin B_3, generally referred to as niacin, is a water-soluble vitamin. This vitamin can generally be found in two distinctive forms, namely nicotinic acid and nicotinamide. Niacin and its derivative nicotinamide are

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dietary precursors of two coenzymes, namely; nicotinamide adenine dinucleotide (NAD), which can be phosphorylated (NADP) and reduced (NADH and NADPH). Over 400 enzymes require the niacin coenzymes, NAD and NADP, mainly to accept or donate electrons for redox reactions (Penberthy and Kirkland, 2012). NAD is the sole substrate for PARP enzymes involved in DNA repair activity in response to DNA strand breaks; thus, NAD is critical for genome stability (Brody 1999). NADP (NADPH) is utilized in anabolic reactions (biosynthesis), such as synthesis of nucleic acids, fatty acids, and cholesterol (Higdon, 2002). Vitamin B_3 can serve as a wonder drug for correcting lipid metabolic disorders besides it functions as a B group vitamin (Zeb Shah et al., 2013).

There are three types of omega fatty acids: omega-3, omega-6 and omega-9. Omega-3 and omega-6 fatty acids are two types of polyunsaturated fat. They are considered essential fatty acids because the body cannot manufacture them. Omega-9 fatty acids are from a family of monounsaturated fats that also are beneficial when obtained in food. Omega-3 and omega-6 fatty acids are used to create cell membranes and hormones that regulate the hormonal cycles (Okuyama et al., 2007; Johnson et al., 2008).

Recently, the World Health Organization reports that at least 75 - 95% of the world populations of developing countries were chiefly rely on traditional medicines and major part of traditional therapies involves the use of plant extract products or their active constituents (Molly, 2011). Traditional medicine usage is a common practice in developed and developing countries at the primary healthcare (Essawi et al., 2000).

The aim of this work is to study the effect of foliar application of various concentrations (50, 100 and 200 ppm) of ascorbic acid and niacin on protein, oil, fatty acids and antimicrobial activity of bitter lupine seeds.

MATERIALS AND METHODS

In order to consider two water soluble vitamins on Botanical Characteristics, Phytochemical Components and Antimicrobial Activity of bitter Lupine (*Lupinus termis* L.) Seeds, we had done a field experiment in the Experimental Farm, Applied Research Center for Medicinal Plants, National Organization for Drug Control and Research, Egypt. Lupine seeds were obtained from Applied Research Center for Medicinal Plants. The experimental design was a completely randomized block design with three replications for each treatment. The total area of each replicate was $3m^2$. Seeds of lupine were sown in beds on 18th October during winter season of 2010 – 2011. All the recommended cultural practices for lupine production were applied according to the Egyptian Ministry of Agriculture. Foliar application of two water soluble vitamins vitamin C (ascorbic acid) and vitamin B_3 (nicotinic acid) were applied with three concentrations (50, 100 and 200 ppm), as well as untreated plants (control; distilled water). All vitamin concentrations were sprayed twice with freshly prepared solutions at 45 and 60 days from planting. The spraying was done manually using a spraying bottle, on both sides of the leaves evenly. The experimental soil was loamy sand with the following properties: 18.7% coarse sand, 69.5% fine sand, 3.1% silt and 8.7% clay, 0.55 m.mohs/cm, EC, 7.8 pH, 3.2% $CaCO_3$, 10, 5 and 388 ppm of N, P(P_2O_5) and K(K_2O) respectively, and 3, 4.4, 0.98 and 0.25 ppm of Fe, Mn, Zn and Cu, respectively.

Phytochemical components of bitter lupine seeds:

Protein content: The nitrogen content of biter lupine seeds were determined according to Kjeldahl method and then crude protein was calculated by multiplying the nitrogen values by a factor of 6.25 (AOAC, 1994).

Extraction and Determination of Fixed oil: Crude oil of dried seeds of *Lupinus termis* L. was extracted with n-hexane for 10 hr using soxhlet extractor (Adewuyi et al. 2009).

Extraction and Determination of Fatty Acids: Fatty acid methyl esters of the oil were prepared by refluxing the sample with mixture of methanol: benzene: sulfuric acid (20:10:1) according to Mendhan et al. (2000). Some water was added to it, again extracted with ether (3 times) and evaporated to get the fatty acid methyl esters. The obtained Fatty acid methyl esters were dissolved in n-hexane and 1 µl of samples was injected and analyzed by GC (gas chromatography). The fatty acids compositions were performed with GC equipped model 6890 Hewlett-Packard (Pittsburgh, PA, USA) with FID detector on a split injector. HP5 capillary column (25 m × 0.12 µm) was used with the injector and detector temperature maintained at 225ºC and 275ºC, respectively. The oven temperature was programmed from 120ºC to 300ºC at 20ºC/min. The carrier gas was nitrogen at a flow rate of one ml/min. One µL of sample was injected by hand and in the split mode (1:20). Fatty acids were identified by comparison of their retention times with those of the reference standards.

Test organisms: Five bacterial strains sensitive to antibiotic (American Type Culture Collection, ATCC), mostly human pathogens, were employed for the screening test. Three G +ve (Staphylococcus aureu ATTC 25923, Staphylococcus epidermidis ATTC 35984 and Bacillus pumilus ATTC 7065) and two G -ve (Pseudomonas aeruginosa ATTC 27853 and Escherichia coli ATTC 25922) were kindly provided from the stock culture of Microbiology Department, National Organization for Drug Control and Research (NODCAR), Cairo, Egypt.

Antimicrobial activity:

Preparation of plant extract: The plant extracts were prepared by immersing 10 gm of dried powdered biter lupine seed in 50 ml of solvents i.e. methanol 80% and ethanol 80% at room temperature for 48h (ZHANG et al., 2008). After filtration, crude extracts were obtained by removing the solvent in rotary evaporator and stored in sterile bottles at 4°C until further use. The extracts were dissolved in the same solvent with which it has been extracted (methanol 80% and ethanol 80%) to a final concentration of 1mg/ml for agar well diffusion method.

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Maintenance of stock culture: Stock cultures of the used microbial strains were maintained on nutrient agar tube slants for bacterial strains at 4°C, sub-cultured monthly throughout this study.

In vitro antimicrobial studies of the selected plants: The antimicrobial activities of all studied treatments were determined by using agar well-diffusion method according to the National Committee for Clinical Laboratory Standard (NCCLS, 1994). 50 ml molten NA medium for bacterial strains which had previously been seeded with 1 ml of the starter inoculums of the tested organisms were poured into a sterile Petri plates (18 cm) and left for solidification at room temperature. 10 mm wells were made in each plate and filled with 100 µl of the treatment concentrated. Plates were left at room temperature for one hour to enable pre diffusion of the substances into the agar, and then incubated at 37 °C for 24 h. Antimicrobial activity was expressed as the diameter of inhibition zone in millimeter. Control holes were filled with distilled water and/or solvents without treatment.

Statistical analysis: Results are expressed as the mean value ± SD of three separate determinations. The data were statistically analyzed using analysis of variance (ANOVA) and least significant difference (Steel and Torrie, 1984). Significant differences between any two means were determined at the $P \leq 0.05$ level.

RESULTS AND DISCUSSIONS

Protein content of *Lupinus termis* seeds: Analysis of variance (ANOVA) showed that foliar application of both ascorbic acid and nicotinic acid had significant effect on protein content % of *Lupinus termis* L. seeds compared with control (Table 1). The results in Table 2 observed that protein content % increased with increasing ascorbic acid, and this increase was gradually positive up to 100 ppm concentration that gave the highest increasing percentage of protein (6.30%) compared with control. On the other hand, there is no significant difference between this treatment (100 ppm of ascorbic acid) and 50 ppm of ascorbic acid.

Increasing nicotinic acid concentration gradually increased protein content % in ascending order up to 200 ppm. On the other hand, there was no significant difference between the treatment of nicotinic acid at 50 ppm and control. It is also cleared that, there was no significant difference between the treatment of ascorbic acid at 50 ppm and the treatment of nicotinic acid at 200 ppm. These results could be explained by the findings obtained by Price (1966) who reported that ascorbic acid increased protein content of wheat grains. Mohamed (2013) found also that foliar application of vitamin C, vitamin B_{12} and folic acid had significant promotive effect on proteins % of wheat Cv. Masr-1 seeds as compared with the control.

Table 1: ANOVA of the effects of ascorbic acid and nicotinic acid on protein % and oil % of biter lupine (*Lupinus termis* L.) seeds.

Source of variation	d.f.	Mean Squares Protein, %	Oil, %
Vitamins	6	1.452*	5.63*
Error	14	2.028	0.078
Total	20	----	----

** means statistically significant at 5% level*

The positive effect of ascorbic treatments on protein content may be due to increasing N concentration in seeds that explained by the finding of Talaat (2003) who showed that the accumulation of nitrate by ascorbic acid foliar application may be due to the positive effect of ascorbic on root growth which consequently increased nitrate absorption. Dolatabadian, and Modarres Sanavy (2008) found that 100 and 200-ppm ascorbic acid prevented of protein degradation in sunflower. In rape seed seedlings, protein content increased by raised ascorbic acid concentration. Also, El-Quesni et al., (2009) indicated that foliar application of ascorbic acid to hibiscus plants significantly increased total carbohydrate %. Dolatabadian, et al. (2010) found that protein percentage of grain corn increased due to 150 ppm ascorbic acid foliar application. Vitamin C protects carbohydrates, fats, proteins and nucleic acids (DNA and RNA) from damage induced by free radicals and other reactive species (Higdon and Victoria, 2012). Ascorbic acid acts as a modulator of plant development through hormone signaling and as coenzyme in reactions by which carbohydrates, fats and proteins are metabolized (Pastori et al. 2003). Moreover, ascorbic acid is very important for the regulation of photosynthesis, flowering and senescence (Barth et al., 2006). Noctor and Foyer (1998) reported that ascorbic acid scavenges reactive oxygen species and prevents protein oxidation and degradation.

Table 2: Protein and oil of biter lupine (*Lupinus termis* L.) seeds affected by ascorbic acid and nicotinic acid.

Foliar application treatment		Protein, %	Oil, %
Control		35.55±1.2	4.25±0.25
Ascorbic acid (Vitamin C)	50 ppm	37.48±2.3	5.00±0.30
	100 ppm	37.79±0.9	4.19±0.08
	200 ppm	36.37±1.8	2.99±0.23
Nicotinic acid (Vitamin B3)	50 ppm	35.76±1.0	4.79±0.41
	100 ppm	36.65±0.8	5.24±0.29
	200 ppm	37.07±1.4	7.46±0.30
LSD		0.47	1.12

Oil content of *Lupinus termis* seeds: Data presented in Table 1 show that oil % in *lupinus termis* L. seeds were

Table 3: Fatty acids profile of biter lupine (*Lupinus termis* L.) oil affected by ascorbic acid and nicotinic acid.

Fatty acids composition	Foliar application treatments						
	Control	Ascorbic acid, ppm			Nicotinic acid, ppm		
		50	100	200	50	100	200
Palmitic (C16:0)	7.65	7.22	7.38	7.31	6.89	8.40	7.75
Stearic (C18:0)	0.25	0.27	0.28	0.34	0.36	0.29	0.40
Oleic (C18:1), Omega-9	43.81	44.32	45.15	46.60	47.54	45.67	36.23
Linoleic (C18:2), Omiga-6	21.33	22.82	20.99	20.42	23.71	22.82	22.36
Linolenic (C18:3), Omiga-3	8.68	8.92	8.95	9.36	8.31	8.50	9.24
Eicosanoic (C20:0), Arachidic	5.14	4.74	5.89	5.36	5.14	4.75	5.45
Erucic (C22:1)	5.93	6.19	6.52	6.74	6.58	6.58	6.32
Total identified fatty acids, %	92.79	94.48	95.16	96.12	98.54	97.01	87.76
Saturated fatty acids (SFA) %	13.04	12.22	13.55	13.01	12.39	13.44	13.61
Unsaturated fatty acids (USFA) %	79.75	82.25	81.61	83.11	86.15	83.58	74.15
USFA / SFA	6.12	6.73	6.02	6.39	6.95	6.22	5.45
Essential fatty acids, %	30.01	31.74	29.94	29.78	32.02	31.33	31.60
Linoleic / Linolenic	2.46:1	2.56:1	2.39:1	2.18:1	2.85:1	2.59:1	2.42:1

significantly affected as a result of both applied vitamins (ascorbic acid and nicotinic acid) as foliar application relative to corresponding controls. From the data in Table 2 it appears that spraying lupine plants with ascorbic acid achieved positive increase the oil percent with 50 ppm and then the effect of concentration of ascorbic acid more than 50 ppm was negative on oil percent in lupine seeds compared with control. On the contrary, increasing nicotinic acid concentration increased oil percent in ascending order up to 200 ppm. The highest oil content in plants treated with nicotinic acid was 7.46%, with increment of 75.53% was obtained with the concentration of 200 ppm compared with control. While, in case of ascorbic acid the highest oil content (5.0%) was recorded with increment of 17.88% at 50 ppm than the corresponding values of the control plants. It is also showed that, there are no significant differences between all the treatments namely; ascorbic acid with both the concentration of 50 and 100 ppm, nicotinic acid with both the concentration of 50 and 100 ppm and control.

These results were in agreement with those obtained by Gamal El-Din (2005) on sunflower plants, who reported that ascorbic acid significantly increased oil percentage of seeds. Moreover, Mohamed (2013) showed that foliar application of vitamin C, vitamin B_{12} and folic acid had significant promotive effect on fat % of wheat Cv. Masr-1 seeds as compared with control. Ali (2002) reported also that nicotinic acid or NAD application led to a highly significant increase in oil contents of *Ricinus communis* L. These results also were in harmony with those obtained by Emam at al., (2011) who reported that, the vitamin (vitamin B_9, vitamin C and vitamin B_{12}) treatments did not only stimulate oil production, but also activated the antioxidative properties of flax seeds in terms of increasing the endogenous contents of glutathione, ascorbic acid and total phenols. Dolatabadian, and Modarres Sanavy (2008) report that ascorbic acid decreased lipid peroxidation in sunflower and rape seed. Exogenous ascorbic acid inhibited lipid peroxidation because ascorbic acid is a scavenger of reactive oxygen species (Noctor and Foyer 1998). Furthermore, Pastori et al. (2003) illustrated that ascorbic acid (vitamin C) is one of the most important water soluble antioxidants in plants, acting as a modulator of plant development through hormone signaling and as coenzyme in reactions by which carbohydrates, fats and proteins are metabolized.

Fatty acids profile of bitter lupine (*Lupinus termis* L.) oil: Fatty acids stored in plant seeds are usually unbranched compounds with an even number of carbons ranging from 12 to 22 and with 0 to 3 cis double bonds. It is well known that fatty acids composition of oils is an indication for oil quality. The oil quality is usually valued according to the content of essential fatty acids (EFAs) (Johnson et al., 2008). The omega 3, 6, 9 groups of fatty acids all contains essential fatty acids necessary for good health (Emam et al., 2011). GLC of main fatty acids composition of the yielded bitter lupine oil is presented in Table 3. The total percentages of total fatty acids composition were ranged from 98.54% to 87.76% as results of different concentrations of ascorbic acid and nicotinic acid foliar application. Data in Table 3 shows that, unsaturated fatty acids (USFA) increased with all foliar application treatments except spraying lupine plant with 200 ppm nicotinic acid compared with control. The amounts of total unsaturated and essential fatty acids were higher than saturated fatty acids with all treatments. Spraying lupine plants with 50 ppm nicotinic acid resulted in the highest value of USFA being 86.15% with increment of 8.03% compared with control. The highest value of essential fatty acids (32.02%) was recorded with the same treatment (50 ppm nicotinic acid) with increment of 6.70% compared with control. The lowest percentages of saturated fatty acids (SFA); 12.22% was obtained with the concentration of 50 ppm ascorbic acid with reduction percentage of 6.29% compared with control.

Seven fatty acids namely; palmatic, stearic, oleic, linoleic, linolenic, ecosanoic and erucic in lupine oil were identified as affected by foliar application of vitamin C and vitamin B_3 (Table 3). Data in this table refers to that; either exogenous foliar application of vitamin C treatments with all concentration or vitamin B_3 at 50 ppm caused decreases in palmatic acid compared with control. On contrast, stearic acid was increased with increasing ascorbic acid or nicotinic acid concentrations compared

Table 4: Antibacterial activity of *Lupinus termis* seed extracts affected by foliar application of ascorbic acid and nicotinic acid by well diffusion method.

Vitamin foliar application treatments		*Staphylococcus aureus*			*Staphylococcus epidermids*			*Bacillus pumilus*			*Pseudomonas aeruginosa*			*Escherichia coli*		
		Lupines termis seeds extract														
		Oil	Eth	Meth	Oil	Eth	Meth	Oil	Eth	Meth	Oil	Eth	Meth	Oil	Eth	Meth
		Inhibition zone, mm														
Control		0	16	0	0	20	0	0	18	21	0	0	10	0	10	0
Ascorbic acid (vitamin C)	50 ppm	0	17	11	0	19	0	0	18	19	0	0	0	0	11	0
	100 ppm	0	20	0	0	20	0	0	20	19	0	0	0	0	0	0
	200 ppm	0	22	0	0	20	0	0	22	20	0	0	0	0	11	0
Nicotinic acid (vitamin B_3)	50 ppm	0	15	17	0	0	17	0	17	0	0	11	0	0	11	0
	100 ppm	0	14	19	0	0	18	0	19	19	0	10	0	0	0	10
	200 ppm	0	0	17	0	14	16	0	17	16	0	0	0	0	0	0

with control. The lowest content of eicosanoic acid (20:0) was obtained with ascorbic acid at 50 ppm and with nicotinic acid at 100 ppm. In case of erucic acid (C 22:1), all concentrations of vitamin C or vitamin B_3 increase erucic acid content. It is obvious that essential fatty acids (C18:2 & C18:3) were increased with all applied treatments except at 100 and 200 ppm ascorbic acid. It was noted that, nicotinic acid at all levels caused more increases in essential fatty acids than ascorbic acid treatments. Generally, ascorbic acid and nicotinic acid treatments at 50 ppm level caused decreases in saturated fatty acids accompanied by increases in unsaturated fatty acids. It is imperative to mention that, nicotinic acid at levels of 50 and 100 ppm caused more increases in total unsaturated fatty acids than ascorbic acid treatments. On the other hand, the concentration of 50 ppm with both ascorbic acid and nicotinic acid caused more decreases in total saturated fatty acids.

These results were in harmony with those obtained by Emam et al., (2011) on flax (*Linum usitatissimum L.*) seeds who found that the used vitamins (vitamin B_9, vitamin C and vitamin B_{12}) treatments markedly decreased the saturated fatty acids; palmitic acid level (C 16:0). This was also in accordance with the results obtained by Youssef and Iman (2003) who indicated that oil composition (fatty acids) responded greatly to foliar spray of the vitamins nicotineamide, ascorbic and thiamin at different rates of application. In addition, Aml et al., (2011) observed that exogenous application of ascorbic acid on two sunflowers (*Helianthus annuus L.*) hybrids increased monounsaturated fatty acids (oleic acid), polyunsaturated fatty acids (linoleic acid) and saturated fatty acids (stearic acid).

Data presented in Table 3 also illustrated that there are three types of omega fatty acids namely; omega-3, omega-6 and omega-9 were identified. Regarding omega-9 (oleic acid), all applied treatments caused marked increases in omega-9 except at 200 ppm nicotinic acid treatment compared with control. The highest value of omega-9 (47.54%) was obtained with 50 ppm nicotinic acid foliar application with increment of 8.51% compared with control. All concentrations of nicotinic acid increased omega-6 (linoleic fatty acid), while this fatty acid increased with the concentration of vitamin C at 50 ppm only. Both 100 and 200 ppm concentrations impacted negatively on omega-6 fatty acid compared with control. Nicotinic acid at 50 ppm achieved the highest omega-6 value (23.71%) with increment of 11.16% compared with control. Omega-3 (Linolenic acid) gradually increased with increasing the concentration of vitamin C up to 200 ppm. This supports the result obtained by Emam et al., (2011) who reported that vitamin treatments stimulated linolenic acid (18:3, □-3) production in flax seeds. Vitamin B_3 at 200 ppm gave the positive result for omega-3 while spraying lupine plant with 50 or 100 ppm gave the negative effect on omega-3 compared with control. Both ascorbic acid at 200 ppm and nicotinic acid at 200 ppm gave high values compared to other treatments with increment of 7.83 and 6.45% respectively compared to control. It should be noted that, the effect of ascorbic acid was more than that of nicotinic acid on omega-3 content.

The results further indicate that the best ratio of linoleic over linolenic (2.18:1) was obtained with using 200 ppm of ascorbic acid. Level of linoleic acid relative to linolenic acid may increase the probabilities of a number of diseases (Hibbeln et al., 2006). However, it has been suggested by Hu (2001) that linoleic to linolenic acid ratio of 10: 1 or less results in reduction of cardiovascular diseases. Although plants can synthesize both the basic omega−6 and omega−3 structures, animals lack this capacity and must obtain them from dietary sources. Deficiency of the omega−6 fatty acid linoleic acid leads to poor growth, fatty liver, skin lesions, and reproductive failure (Connor et al., 1992). Omega−3 Fatty acid deficiency causes reduced vision, abnormal electroretinogram results, and, perhaps, impaired cognition and behavior (Neuringer et al., 1984). Thus, diets rich in corn, safflower, sunflower, and peanut oils, all of which are high in linoleic acid and low in α-linolenic acid, can lead to omega−3 fatty acid deficiency. Thus, a high ratio of omega−6 to omega−3 fatty acids in the diet accentuates omega−3 fatty acid deficiency (Connor, 1999). The increase in linolenic acid with vitamin treatments might be attributed to the acceleration of the biosynthetic pathway of linolenic acid (Joshi et al. 1998).

It should be noted that, the positive effect of niacin is here described by transformation into co-enzymes (NAD and NADP) also these vitamins are involved in fatty acid

synthesis and oxidation reactions (Fidanza and Audisio, 1982). In plants, the major site of fatty acid biosynthesis is within the plastid; this is in contrast to animals, which produce fatty acids in the cytosol. Reducing power required for fatty acid biosynthesis is provided by NADPH formed during photosynthesis in chloroplasts or via the oxidative pentose phosphate pathway in plastids of nongreen tissues (Ulf-Ingo, 2001). In addition to, fatty acids synthesis, a carbon supply and ATP also requires large amounts of reducing power (co-enzymes), in the form of NADPH and NADH (Slabas and Fawcett, 1992). In this respect, it is noted that NADP functions as a hydrogen donor in reductive biosynthesis (anabolism), such as in fatty acid and steroid synthesis while NAD functions as an electron carrier for intracellular respiration as well as a co-factor for enzymes involved in the oxidation (catabolism) of fats, proteins, carbohydrates and alcohol to produce energy (Brody, 1999 and Cervantes-Laurean et al., 1999).

Antibacterial activity: Antimicrobial screening tests using extracts and oil obtained from *Lupinus termis* (Table 4) indicated that there was no almost antibacterial activity for *L. termis* oil extract against all the tested strains. On the other hand, the antibacterial activity of the ethanol and methanol extracts against G +ve bacterial strains (*Staphylococcus aureu*, *Staphylococcus epidermidis* and *Bacillus pumilus*) is clearly greater than that in G –ve strains (*Pseudomonas aeruginosa* ATTC 27853 and *Escherichia coli* ATTC 25922). As can be seen, ethanol extracts had the highest inhibition zone (22 mm) in the treatment with foliar application ascorbic acid, which increased by increasing the treatment concentration to reach its maximum value at 200 ppm. The present results showed different levels of activity with the two solvents and the oil extract of *Lupinus termis*, revealed that each extract showed a distinctive permutation of target organisms suggests that different bioactive phytochemicals are present in the plant species (Ananil et al., 2000). In accordance with Esawi and Srour (2000) and Keskin et al. (2010) who confirmed that plants differ significantly in their activity against tested microorganisms using different solvents. Devi et al. (2009) reported that the solvents used for the preparation of extracts have a key role in the expression of activity. This observation can be rationalized in terms of the polarity of the compounds being extracted by each solvent (Parekh et al., 2005). Furthermore, explanation by Barbour et al. (2004) that this difference could be due to the nature and level of the antimicrobial agents present in the extracts and their mode of action on the different microorganisms.

In classifying the antibacterial activity as G +ve and G -ve, it would generally be expected that a much greater number of plants would be active against G +ve than G -ve bacteria (McCutcheon et al., 1992), however, the results of this study demonstrated that the gram-negative bacteria were more resistant to the plant extract than gram-positive bacteria such as *Ps. aeruginosa* exhibited more resistant than B. subtilis when they were tested with *Lupinus termis* extract. Because lipopolysaccharide (LPS) layer of gram- negative bacteria in outer membrane have a high hydrophobicity which acts as a strong permeability barrier against hydrophobic molecules (Smith-Palmer, et al., 1998). Hydrophobic molecules can pass through cell wall of gram-positive bacteria easier than the gram-negative bacteria because cell wall of the gram- positive bacteria contained only peptidoglycan (Nikaido and Vaare, 1985; Lambert et al., 2001). These results agree with those reported by (Lan-ciotti, et al., 2004) who confirmed that the antimicrobial effects of essential oil constituents are dependent on their hydrophobicity. On the other hand, Quarenghi et al. (2000) found that Anthemis cotula had activity against both G +ve and G-ve microorganisms. Further report by Esawi and Srour (2000), demonstrated the eight plant extracts tested were active against both G +ve and G -ve bacteria.

CONCLUSION

It could be concluded that foliar application of ascorbic acid and nicotinic acid could play an enhancement role on many metabolic and physiological processes of lupine (*Lupines termis* L.) that reflected in increasing seed quality. This seed quality represented in increasing of protein content, oil content, omega fatty acid group and microbiological activity of seeds. The extract of *L. termis* treated with ascorbic acid and nicotinic acid showed antibacterial activity against G +ve bacterial strains (*Staphylococcus aureu*, *Staphylococcus epidermidis* and *Bacillus pumilus*). This acitivity was clearly greater than that in G –ve strains (*Pseudomonas aeruginosa* ATTC 27853 and *Escherichia coli* ATTC 25922).

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