Journal
J Biol Chem
Environ Sci, 2008,
Vol.3 (2):59-82
www.acepsag.org

EFFECT OF NICOTINAMIDE ON ANTIMICROBIAL ACTIVITY OF LAVENDER ESSENTIAL OIL

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ABSTRACT

Lavendula officinalis plants were grown on puts filed with perlite substrate culture under unheated plastic greenhouse. Two cuts were obtained to investigate the effect of three nicotinamide concentrations (0, 75 and 100 mg/L). Foliar spray and addition in irrigation water were used for nicotinamide application, also two different irrigation scheduling (10, 15 times/ day) were used to perform twelve different treatments. The oil herbage was distilled and oil volume was measured. The essential oil compositions were identified by GLC.

Essential oil samples were tested for their antimicrobial activity against 20 microorganisms including five Gram positive bacteria: *micrococcus roseus, Micrococcus luteus, Staphylococcus aureus, Bacillus subtils, Bacillus cereus,* five Gram negative bacteria: *Escherichia coli, Erwinia carotovora, Serratia marcines, Pseudomonas fluoresceus, Salmonella typhimurium,* six Fungi: *Fusarium solani, Fusarium oxysporum, Rhizoctonia solani, Aspergillus flavus, Aspergillus niger, Penicillium sp.,* four Yeast: *Saccharomyces cerevisiae, Saccharomyces roxii, Candida utilis, Candida albicans.* The antimicrobial potency was determined by measuring the inhibition zone (mm) using whole plat diffusion technique.

Irrigation level of 15 times /day gave the highest oil value (0.31%) in first cut and the lowest oil value (0.21%) in case of the second cut. However, irrigation for 10 times/day is completely sufficient for obtaining significant high oil yield in both first cut (0.63 ml/herb) and second cut (0.51 ml/herb). Both oil percentage and yield

showed less values in the second cut compared with the first one for both irrigation levels. Oil yield showed significant high values, with application of 75 ppm (0.68 ml/herb) compared with those of control (0.50 ml/herb) and 100 ppm (0.48 ml/herb). GC data showed the superiority of 1, 8 cineol which varied from one cut to another according to the rate of irrigation and application of nicotinamide. Linalool was the second major constituent in all treatments, and showed vacillation due to nicotinamide application and the rate of irrigation from one cut to another. Other constituents (Limonene, α, β pinene, Camphor, Linalyl acetate, Terpinene-4-ol, Lavandulyl acetate, and geranayl acetate) exhibited comparatively low values. Chemotypic classification of *Lavendula officinalis* investigated in this study under perlite soilless conditions is 1, 8 Cineol, Linalool type.

Antimicrobial inhibitory effect of most of resultant lavender essential oils increased with increasing their volume from 2 to 5µl. Most strains of fungi, gram positive and negative bacteria showed high sensitivity and inhibited to great extent. Different strains of yeast showed high resistance and survived essential oils tested. Neither irrigation rate frequencies 10 and 15 times/day nor application of nicotinamide had clear and marked effect on the antimicrobial activity of the resultant lavender essential oils. Data clearly indicate the marked inhibitory effect of lavender crude oil samples against several human and plant pathogens and non pathogenic micro organisms.

Keywords: Nicotinamide, *Lavendula officinalis*, essential oil, antimicrobial activity.

INTRODUCTION

Essential oils have been used in perfumeries flavoring, food preservation, traditional medicine, anti-oxidant, anti inflammatory, anthelmintic, anti carcinogenesis, anti-bacterial, anti-fungal and anti-viral agents. Also it raises the immunity of human by increasing the anti body production (Tzakau *et al*., 2001; Kitic *et al*., 2002).Thus essential oils are favored because they are natural, inexpensive when produced in large scale, possess low mammalian toxicity, biodegradable, and can simultaneously fulfill the roles of more than one synthetic alternative (Hay and Waterman, 1993). Also they may offer solution for controlling various environmental problems associated with livestock wastes (Varel *et al*., 2004). Many studies have proved the growth regulatory impact of several vitamins,

especially through foliar spraying with micro concentrations (ppm). However this technique has been used in several studies dealing both medicinal and aromatic plants aiming to increase essential oil concentration and quality. Nicotinamide (vit.B5) may be more softy than the known hormonal regulators which recently are known as carcinogens (Youssef & Iman, 2003). Essential oils distilled from members of Lavandula genus have been used in both cosmetics and therapeutics for centuries with the most commonly used species being *L. angustifolia, L. latifolia, L. stoechas and L. intermedia*. Although there is considerable information about the biological activity of these oils, much of this has not been substantiated by scientific or clinical evidence. Among the claims made for lavender oil are that is it antibacterial, antifungal, carminative (smooth muscle relaxing), sedative, antidepressive and effective for burns and insect bites (Cavanagh & Wilkinson 2002).

The antibacterial effect of Labiatae aromatic plants against bacteria, as recorded in the *in vitro* bioassays, not only explains the scarce presence of the bacteria on their leaves but may have applications in agriculture as a frost-control method for sensitive crops (Karamanoli *et al*., 2000).

Antifungal activity of essential oils extracted from *Origanum syriacuin*, lavandin *(Lavandula officinalis)* and lemongrass (*Cymbopogon citratus)*, in vitro against *Botrytis cinerea* and *Rhizoctonia solani*. Oil extracted from *Origanum syriacum* inhibited the growth of *B. cinerea* and *R. solani* by 80-100%, whereas oils from *L. officinalis* and *C. citratus* showed less antifungal activity (Shimoni *et al.,* 1993). Antimicrobial activities of *Lavandula officinalis* and *Lavandula angustifolia* using a micro atmospheric technique were more potent against filamentous fungi than the other essential oils exhibit higher activities against a battery of microorganisms (bacteria and fungi) (Gvozdikova *et al.,* 1990; Larrondo *et al.,* 1995; Adam *et al.,* 1998; and Inouye, *et al*., 2003). Biological activities of 105 commercial essential oils against 25 species of bacteria, 20 strains of *Listeria monocytogenes,* and 3 filamentous fungi *(Aspergillus niger, A. ochraceus* and *Fusarium culmorum).* Their antioxidant action was also determined by GC. however, a great variability between the biological action of different samples of individual oils and groups of oils under the same general name, lavender, eucalyptus or chamomile, which was reflected in

differences in chemical composition (Lis-Balchin *et al.*, 1998). There were antifungal activity of lavender essential oil on naturally contaminated corn and maize with moulds of *Alternaria spp*. and *Fusarium verticillioides*. Maize kernels and ears were also inoculated with spores of the aflatoxigenic strain *Aspergillus parasiticus* and with spores of *Aspergillus niger*. Lavender essential oil showed fungistatic effect on maize kernels at concentrations of 0.27 ml/l and 4 µl/g during 30 days against *Aspergillus niger*, and at a concentration 24 µl /g against *Aspergillus parasiticus*. Lavender oil at 125 µl /g also showed a sporicidal effect against *Aspergillus parasiticus*. Kernels treated with various concentrations of lavender essential oils were given as feed to cows, horses and pigs. Only pigs showed a tolerance to lavender essential oil. Mycelial growth and mycotoxin production on maize kernels were inhibited by lavender essential oil (Pepeljnjak *et al.*, 2004).

In this study the nicotinamide effect on the percentage and yield of the resultant Lavender essential oil in addition to their GC profile have drowned the attention as well as the antimicrobial potency of these oils which were investigated against 20 microorganism strains including 5 gram positive, 5 gram negative, 6 fungi (molds) and 4 yeast. Also this study puts stress on the positive variations that may be resulted due to cultivating lavender plant in perlite soilless culture, and the regulatory effect of nicotinamide applications.

MATERIALS AND METHODS

Lavender Plants (*Lavendula officinalis*) were grown on puts filed with perlite substrate culture under unheated plastic house (9 x 60 x 3.25 m). Two cuts were obtained (three replicates of each treatment) to investigate the effect of three nicotinamide concentrations (0, 75 and 100 mg/L), using two different methods of nicotinamide application; foliar spray and addition in irrigation water also two different irrigation scheduling (10, 15 times/day) were used to perform twelve different treatments.

Experimental design and Statistical analysis

The obtained data were statistically analyzed using the general linear model procedure described in SAS User's Guide (SAS, 1998), according to the following model: $Y_{ijkl} = \mu + L_i + C_j + M_k + LCM_{ijk} + E_{ijkl}$, where Y_{ijk} = Observed trait, μ = The overall mean, L_i = The

effect of irrigation (i = 1, 2), C_j = The effect of concentration (j = 1, 2, 3), M_k = The effect of methods (k = 1, 2), LCM_{ijk} = Interactions between i the irrigation, j the concentration and the k methods and E_{ijkl} = Random error. Mean differences were tested by Duncan's Multiple Range test (Duncan, 1955)

Extraction of essential oil

The oil herbage was distilled and oil volume was measured according to (Guenther 1960).

GLC analysis of the essential oil

The essential oil composition was identified by GLC (Gas Liquid Chromatography/Pye Unicampro-GC according to (Radwan 1978). Separation conditions were: Column OV-17 (Methyl Phenyl Silicone, 1.5 X 4mm), 70°C/5min with rate 8°C/min up to 200°C /50 min with Inj. and Dect. temp. 250°C & 300°C respectively.

Antimicrobial activity

The tested micro-organisms source was Microbiology department Faculty of Agriculture Ain Shams University, Cairo, Egypt and they were: five Gram positive bacteria: *micrococcus roseus, Micrococcus luteus, Staphylococcus aureus, Bacillus subtils, Bacillus cereus,* five Gram negative bacteria: *Escherichia coli, Erwinia carotovora, Serratia marcines, Pseudomonas fluoresceus, Salmonella typhimurium,* six Fungi: *Fusarium solani, Fusarium oxysporum, Rhizoctonia solani, Aspergillus flavus, Aspergillus niger, Penicillium sp.,* four Yeast: *Saccharomyces cerevisiae, Saccharomyces roxii, Candida utilis, Candida albicans.*

The following media were used throughout this investigation**:**

Nutrient agar medium

Peptone (5.0 g), Beef extract (3.0g), Agar agar (20.0g), Distilled water up to 1000 ml, pH adjusted to 7.2. This medium was used for propagation and maintenance of Gram positive and negative bacteria. Inoculated plates or tubes were incubated at 30°C ± 2° C for 24 hr (CA I M, 1987).

Universal medium

Peptone (5.0 g), Glucose (10.0g), Yeast extract (3.0g), Malt extract (3.0g), Agar agar (20.0g), Distilled water up to 1000ml, pH adjusted to 5.8. This medium was used for propagation and maintenance of yeast strains. Inoculated plates or tubes were incubated at 30°C ± 2° C for 24 hr (CAIM, 1987).

Malt extract medium

Malt extract (30.0g), Agar agar (20.0g), Distilled water up to 1000ml, pH adjusted to 5.5. This medium was used for propagation and maintenance of fungal strains. Inoculated plates or tubes were incubated at 28°C ± 2°C for 3 to 7days (CAIM, 1987).

Antimicrobial activity

All lavender extracts were tested for their antimicrobial activities against 20 microorganisms including 5 Gram positive, 5 Gram-negative bacteria, 6 fungi and 4 yeast. Antimicrobial activity was determined by measuring the inhibition zones (mm) using agar diffusion method (Sleigh and Timburg, 1981).

RESULTS AND DISCUSSION

Effect of nicotinamide applications on lavender essential oil

It is well known that the economic value of lavender plant is attributed to its essential oil content therefore we have put stress on the positive variations that may be resulted due to cultivating lavender plant in perlite soilless culture, and the regulatory effect of nicotinamide application. Thus the evaluation and compression among all treatments were based on essential oil percentage together with its yield (m. oil/plant fresh weight). Data obtained are listed in Table (1) for the first cut and for the second cut.

The main features could be summarized on the following statements:

Effect of irrigation level

Dealing with the essential oil percentage (Table 1), the highest irrigation level (15 times /day) gave the highest value (0.31%) in first cut and the lowest value (0.21%) in case of the second cut.

However it is clear that, irrigation for 10 times/day is completely sufficient for obtaining significant high oil yield in both first cut (0.63 ml/herb) and second cut (0.51 ml/herb) compared with those produced by 15 times/day which reached only 0.47 and 0.39 ml/herb respectively.

The significant highest oil yield - in spite of its percentage – using 10 times/day in both first and second cuts is attributed to the highest vegetative growth(fresh weight/plant) (data not shown) compared with 15 times/day. On other word, lavender essential oil yield together with its fresh weight increased with decreasing irrigation rate which save money through lowering the case of lavender cultivation in perlite soilless culture. The compression

between first and second cuts herbage reveal that both oil percentage and yield showed less values in the second cut compared with the first one, and this hold true for both irrigation levels, e.g. in case of 10 times/day oil percentage was 0.27% (first cut) and decreased to 0.25% in second cut. Also oil yield values were 0.63 and only 0.51 ml/herb in case of first and second cut respectively.

Table (1): Effect of nicotinamide application on essential oil of lavender herbage (first and second cut)

Irrigation	NA concentration	Method	% Essential oil (ml-100 g FW)		oil Yield / Plant	
			First cut	Second cut	First cut	Second cut
10 times / day	control	Addition	0.27 ± 0.01	0.29 ± 0.007	0.66 ± 0.01	0.53 ± 0.003
		Spray	0.27 ± 0.01	0.29 ± 0.007	0.66 ± 0.01	0.53 ± 0.003
	75 mg/L	Addition	0.26 ± 0.01	0.26 ± 0.007	0.63 ± 0.05	0.47 ± 0.029
		Spray	0.34 ± 0.01	0.23 ± 0.024	0.80 ± 0.05	0.50 ± 0.078
	100 mg/L	Addition	0.27 ± 0.01	0.23 ± 0.015	0.57 ± 0.02	0.45 ± 0.027
		Spray	0.23 ± 0.01	0.24 ± 0.015	0.32 ± 0.01	0.62 ± 0.018
15 times / day	control	Addition	0.31 ± 0.02	0.24 ± 0.023	0.35 ± 0.03	0.35 ± 0.035
		Spray	0.31 ± 0.02	0.24 ± 0.023	0.35 ± 0.03	0.35 ± 0.035
	75 mg/L	Addition	0.27 ± 0.02	0.15 ± 0.012	0.39 ± 0.03	0.31 ± 0.03
		Spray	0.35 ± 0.01	0.20 ± 0.012	0.73 ± 0.03	0.49 ± 0.072
	100 mg/L	Addition	0.27 ± 0.01	0.22 ± 0.021	0.51 ± 0.04	0.53 ± 0.067
		Spray	0.39 ± 0.02	0.23 ± 0.009	0.53 ± 0.03	0.36 ± 0.006
Main effects:						
Effect of irrigation						
10 times / day			0.27^{b}	0.25^{a}	0.63^{a}	0.51^{a}
15 times / day			0.31^{a}	0.21^{b}	0.47^{b}	0.39^{b}
Effect of concentration						
control			0.29^{a}	0.26^{a}	0.50^{b}	0.43^{a}
75 mg / L			0.30^{a}	0.20^{b}	0.68^{a}	0.44^{a}
100 mg / L			0.29^{a}	0.23^{b}	0.48^{b}	0.48^{a}
Effect of Methods						
A			0.27^{b}	0.23^{a}	0.55^{a}	0.43^{a}
S			0.31^{a}	0.23^{a}	0.56^{a}	0.47^{a}
probability						
L			0.0001	0.0003	0.0001	0.0001
C			0.3755	0.0001	0.0001	0.211
M			0.0001	0.5903	0.4436	0.1617
L*C*M			0.0001	0.0542	0.0001	0.0073

Where: A= Addition, S= Spray, L_i = effect of irrigation (i = 1, 2), C_j = effect of concentration (j = 1, 2, 3), M_k = effect of methods (k = 1, 2), LCM_{ijk} = Interactions between i irrigation, j concentration and k methods.

The relationship between irrigation rate and both essential oil concentration (%) and yield may be varied from one aromatic plant to another. However our data agree with those of Mahmoud *et al.*, (1992) on Sweet basil *(ocimum basilicum L.),* Simon *et al.*, (1992) on sweet bail, Saxina *et al.*, (1993) on Japanese mint , and Ram *et al.*, (1995) on *Bergamot mint*, who concluded such inverse relationship between soil moisture and oil content.

On the other hand Koriesh and Atta (1986) on *Pelargonium graveolens*, Horonk Csrake (1987) on anise, coriander and dill, found that no direct relationship between soil moisture content and the yield of the resultant essential oil, whilst frequent irrigation ratio resulted in high significant essential oil yield (Ram *et al.,* 1995) on Japanese geranium plant, Saxena and Singh (1996 and 1998) on Japanese patchouli and Japanese mint respectively.

Effect of nicotinamide application

Variable differences in lavender essential oils were exerted between first and second cut in relation to nicotinamide applications.

In case of first cut (Table 1) essential oil percentage changed within non-significant range (0.29 to 0.30 %) in case of control, 75 and 100 ppm treatments, whilst oil yield showed significant high values, with application of 75 ppm (0.68 ml/herb) compared with those of control (0.50) and 100 ppm (0.48).

Regarding to the second cut, the significant highest percentage of oil was detected in control treatment (0.26 %) compared with those nicotinamide treatments (0.20 and 0.23 %). On the other hand oil yield exhibited non-significant variations among all treatments (0.43, 0.44 and 0.48 ml/herb).

Essential oils content have been influenced by application of growth regulators, such as nicotinamide on lavender plant (Youssef and Iman, 2003), ascorbic acid and zinc on *Cupressus sempervirens* plant (Farahat *et al.* 2007). Early; El-Keltawi and Croteau (1987) stated that cytokinin stimulate monoterpene biosynthes in Mentha piperita.

Effect of methods of nicotinamide application

As general point of view; method of nicotinamide application seemed to be of slight impact on both lavender oil yield and percentage either in first or second cut (Table 1). However methods of application did not affect oil yield in significant manner in both first cut (0.55 and 0.56 ml/herb) and second cut (0.43 and 0.47 ml/herb).

Dealing with oil percentage; only foliar spray in first cut resulted in significant high percentage (0.31 %) compared with addition method (0.27 %). The comparison between first and second cut, reveal higher values of both oil yield and percentage incase of first cut than in second one.

The comparison among the individual treatments, each apart, reveal the superiority of addition 75 ppm nicotinamide to the nutrient solution (irrigation water) at 10 times/day, which gave the highest oil yield in first cut followed by foliar spray at the same concentration (0.80 ml/herb). On the other hand raising nicotinamide to 100 ppm exerted dramatic decrements in oil yield (0.32 ml/herb) due to foliar spray.

In case of the second cut, most treatments irrigated at 10 times/day exhibited higher oil yield than 15 times/day did. The highest oil yield was obtained with 100 ppm nicotinamide foliar spraying (0.617 ml/herb).

At close, depending upon the forementioned findings one can conclude the ideal conditions for achieving maximal oil yield in lavender plant grown in perlite substrate are:

- In first cut; addition of 75 ppm nicotinamide to the irrigation water at 10 times/ day.
- In second cut; foliar spraying with 100 ppm nicotinamide at the same irrigation level i.e. 10 times/day.

GC profile of lavender essential oil

The effect of nicotinamide application and irrigation rate on the different constituents of lavender crude essential oils are printed in Table (2) for the first cut and Table (3) for the second cut.

The main features that may characterized the essential oil investigated in the present study which extracted from lavender plants grown in perlite soilless culture, are:

1- The superiority of 1, 8 cineol (ether), which varied from one cut to another and according to the rate of irrigation and application of nicotinamide e.g. In control; irrigation at 10 times / day resulted in slight differences between first cut (40.66%) and second cut (39.61%). On the other hand, increasing irrigation rate to 15 times /day, exerted marked increase from 38.34% (first cut) to 48.64% in the second cut , which represented the highest value compared with all other treatments including nicotinamide application. Whilst the lowest

value (16.29%) was attained by increasing irrigation rate (15 times / day) with simultaneous foliar spraying with 100ppm nicotinamide.

Concerning with the effect of nicotinamide application , data show remarkable decrease in 1, 8 Cineol compared with control in both first and second cut, and also both irrigation levels, with one exception foliar spraying with 100 ppm in first cut previously irrigated at 10 times / day (43.65%). However the inverse impact of nicotinamide application on 1, 8 Cineol was clearly observed in case of first cut than in second cut, and also with increasing irrigation rate.

2- Linalool (alcohol), was the second major constituent in all treatments, and showed variable fluctuations due to nicotinamide application and the rate of irrigation also from one cut to another .e.g. in case of 10times/day, its value in control increased from 15.14% to 22.60% in first and second cut respectively. Comparison between 10 and 15 times/ day, showed that its values increased with increasing irrigation rate in both two cuts, e.g. its value increased from 15.14% to 18.55 % (first cut) and from 22.61% to 25.89%(second cut) with increasing the rate of irrigation from 10 to 15 times / day .The highest value was 24.27% in first cut treated with 75 ppm foliar spray, whilst in second cut, its highest value 29.97% recorded when plants irrigated by nutrient solution contained 100 ppm nicotinamide for 10 times / day.

3. Limonene (monoterpene hydrocarbon) exhibited comparatively low values compared with either 1, 8 cineole or linalool. The recorded values of limonene in the first cut were relatively higher than those of the second cut at the same level of irrigation e.g. its values in case of 10 times/ day decreased from 7.78% in the first cut to 4.9% in the second cut . Also, in 15 times/ day, its values were 6.95% in the first cut and decreased to 4.64% in the second cut. However the main conclusion may be ; limonene values showed great differences and varied from one cut to another , whilst it showed little response due to increasing irrigation rate (level) from 10 to 15 times/ day.

4. α, β pinene (monoterpene hydrocarbons) came in the fourth order and showed higher values with nicotinamide application and increasing irrigation rate in the first cut compared with control treatment e.g. In case of irrigation control plants at 10 times/ day, α, β, pinene accounted by 6.78% and increased dramatically by nicotinamide application, which reached their maximal value (14.105%) in case of addition 75 ppm, followed by foliar spray with

the same concentration (13.566%). Also it increased to 11.907% when control plants irrigated frequently at 15 times / day.

The comparison between first and second cut reveal their higher existence in first cut, which tended to diminish dramatically in the second cut, especially in nicotinamide treatments.

5. GC profile also reveals the presence of five identified compounds which existed in relatively minute proportions (i.e. minor components). These constituents are arranged according to their RT (retention time) as follows:

Camphor (0.912 - 3.282%) in first cut and (1.73 - 4.4 %) in the second cut; Linalyl acetate (0.856 - 5.523%) and (0.14 - 5.31%), Terpinene -4-ol (0.734 - 5.459%) and (0.06 - 0.84%), Lavandulyl acetate (1.461- 4.901%) and (0.02 - 3.12%), finally geranayl acetate (1.593 - 5.12%) and (0.1 - 5.46%) in case of the first and second cut respectively.

Table (2): Volatile oil profile of Lavender herbage (first cut) treated with nicotinamide

Treatment (mg/ l) / Components %	CONT A	I1C1	I1C2	S1C1	S1C2	CONT B	I2C1	I2C2	S2C1	S2C2
a-Pinene	5.469	6.718	5.768	6.612	5.673	6.244	3.974	6.172	6.812	6.358
b-Pinene	1.312	7.387	6.551	6.921	5.809	5.683	3.477	9.2769	7.118	6.499
Limonene	7.783	9.754	9.201	7.155	5.892	6.949	6.556	5.7383	10.798	7.135
1-8 Cineole	40.659	26.529	23.248	34.985	43.646	38.339	19.067	23.735	24.954	36.062
Camphore	0.912	2.203	2.519	1.458	1.215	2.617	2.625	0.9174	3.282	1.352
Linalool	15.141	20.578	18.569	24.273	22.537	18.548	13 561	22.524	18.441	23 206
Linalyl acetate	1.637	4.054	5.523	3.047	0.856	4.347	3.897	2.3494	5.135	3.243
Terpinen-4-ol	2.03	1.237	1.646	0.765	1.694	1.609	3.854	0.8142	5.459	0.964
Lavandulyl acetate	1.461	2.609	3.415	1.794	1.558	3.424	4.901	2.3494	3.346	2.000
Geranyl acetate	1.593	3.408	5.12	2.507	2.066	2.565	5.089	5.5597	2.622	2.954
Total Un identified	20.805	15.088	17.936	10.474	9.051	9.614	32.429	20.564	12.033	10.227

Where: I1C1= irrigation with 75mg/l NA for 10 times/day, I1C2= irrigation with 100mg/l NA for 10 times/day, S1C1= spray with 75mg/l NA for 10, CONT.A= irrigation without NA(with nutrition solution) for 10 times/day, S1C2= spray with 100mg/l NA for 10 times/day, I2C1= irrigation with 75mg/l NA for 15 times/day, I2C2= irrigation with 100mg/l NA for 15 times/day, S2C1= spray with 75mg/l NA for 15 times/day, S2C2= spray with 100mg/l NA for 15 times/day, CONT.B= irrigation without NA(with nutrition solution) for 15 times/day.

Table (3): Volatile oil profile of Lavender herbage (second cut) treated with nicotinamide

Treatment (mg /l) / Components %	CONT A	I1C1	I1C2	S1C1	S1C2	CONT B	I2C1	I2C2	S2C1	S2C2
a-pinene	1.34	1.67	1.74	1.59	1.80	1.000	1.43	1.62	0.76	5.65
b-pinene	4.37	5.31	4.77	4.60	4.60	3.086	5.43	5.42	4.21	3.53
limonene	4.90	6.04	5.30	4.12	3.90	4.642	6.81	6.04	9.35	7.10
1-8 cineole	39.61	39.28	33.45	37.59	37.21	48.462	38.47	38.91	36.51	16.29
camphore	4.40	2.89	3.12	2.92	3.17	2.119	1.73	2.26	2.60	4.38
linalool	22.60	21.36	16.64	29.97	24.52	25.891	23.00	19.31	16.15	15.81
linalyl acetate	3.94	4.05	5.20	2.72	5.31	1.659	4.08	5.20	0.14	3.31
terpinen-4-ol	0.60	0.69	0.84	0.06	0.71	0.058	0.08	0.08	0.13	0.28
Lavandulul acetate	0.83	0.02	1.34	1.24	0.02	0.051	0.02	0 02	3.12	0.40
Geranyl acetate	3.98	2.57	3.67	0.10	0.10	5.460	2.84	2.96	12.13	5.51
Total Un identified	13.33	16.08	23.91	15.01	18.55	7.57	16.11	18.18	14.89	37.48

Where: 11C1= irrigation with 75mg/l NA for 10 times/day, 11C2= irrigation with 100mg/l NA for 10 times/day, S1C1= spray with 75mg/l NA for 10, CONT.A= irrigation without NA(with nutrition solution) for 10 times/day, S1C2= spray with 100mg/l NA for 10 times/day, I2C1= irrigation with 75mg/l NA for 15 times/day, I2C2= irrigation with 100mg/l NA for 15 times/day, S2C1= spray with 75mg/l NA for 15 times/day, S2C2= spray with 100mg/l NA for 15 times/day, CONT.B= irrigation without NA(with nutrition solution) for 15 times/day.

Depending upon the abovementioned finding, which reveal the superiority of 1, 8 cineol followed by linalool (Abdel-Reheem *et al.*, 2006), the chemotypic classification of Lavender plant investigated in the present study under perlite soilless conditions is 1, 8 Cineol, Linalool type, which may be distinguish it from the other lavender types. In this connection Garcia –Vallejo *et al.*, (1990) concluded that *Lavender sp.* have been classified into different chemo types according to the major constituents of each type which are generally more than 10 % in its corresponding oil.

Antimicrobial activity of lavender crude essential oil

All essential oil samples were tested for their antimicrobial activity against 20 microorganisms including gram positive (5 strains) gram negative (5 strains), fungi (6 strains) and yeast (4 strains). The antimicrobial potency was determined by measuring the inhibition zone (mm) using whole plat diffusion technique.

The data obtained are listed in Tables (4, 5, 6 and 7) in general; three main aspects could be concluded:

Firstly: the inhibitory effect of most of the resultant lavender essential oils increased with increasing their volume from 2 to 5µl. in spit of the type of the tested microorganism.

Secondly the different types of organisms showed variable responses towards lavender essential oils. Most strains of fungi, gram positive and negative bacteria showed high sensitivity and inhibited to great extent. On the other hand the different strains of yeast showed high resistance and survived essential oils tested. However the slight inhibitory effects were attained at the highest concentration (5µl).

Thirdly: neither irrigation rate frequencies 10 and 15 times/day nor application of nicotinamide had clear and marked effect on the antimicrobial activity of the resultant lavender essential oils.

More details could be discussed as follows:

Lavender essential oil samples had no inhibitory effect against both *B .subtilis* (g^{+ve}) and *Candida ablicans* (yeast) as shown in tables 12 and 14 respectively. However this hold true for oils extracted from control and nicotinamide treated samples.

Dealing with antifungal activity: data in Table (4) reveal the marked inhibitory effect of most oils especially at 5µl against the all tested molds strains.

ASP. flavus showed the relatively highest sensitivity (29mm) in case of control A (5µl). One must put stress on this fungus because of its capability for producing AFB compounds, which are well known as carcinogenic agents (initialed carcinogens). Thus further studies on AFB producing fungi are needed before recommendation of essential oils spraying in closed and welled areas to prevent the reproduction of these human pathogens. On the other hand *Rhizoctonia solani* and *F. oxysponum* exhibited the relatively higher resistance, as their inhibition zone did not exceed than 19 and 20mm respectively. The other remained types i.e. *Asp. niger*, *pencillium sp.* and *F. solani* showed moderate resistance as their maximal inhibition zone were 22, 22 and 24 mm respectively.

Table (4): Antifungal activity of *Lavendula officinalis* crude volatile oil

treatment	crude volatile oil (µl)	Diameter of inhibition zone (mm)					
		F.solani	*F. oxysporium*	*penicillium sp*	*Rhizoctonia solani*	*Asp.flavus*	*Asp. niger*
CONT.A	2	2	0	2	0	0	0
	3	11	18	17	14	12	4
	5	19	17	19	16	29	16
I1C1	2	0	0	0	0	0	0
	3	14	18	14	9	12	19
	5	19	19	18	12	16	12
I1C2	2	4	2	0	0	3	2
	3	9	12	15	9	9	9
	5	13	12	15	9	12	11
S1C1	2	5	3	0	0	0	0
	3	12	14	12	12	7	9
	5	24	16	14	12	7	9
S1C2	2	5	0	0	2	4	6
	3	12	19	12	14	12	12
	5	13	18	19	19	22	22
CONT.B	2	0	5	6	8	14	12
	3	14	14	12	12	12	14
	5	12	16	14	19	13	16
I2C1	2	0	0	12	0	6	0
	3	6	6	20	8	12	9
	5	12	12	22	12	18	14
I2C2	2	6	2	0	0	2	0
	3	12	7	10	10	12	5
	5	22	13	12	12	16	12
S2C1	2	2	2	2	4	2	0
	3	12	14	14	8	5	6
	5	14	16	18	8	15	18
S2C2	2	0	0	0	12	14	18
	5	16	20	12	9	18	12

Where: I1C1= irrigation with 75mg/l NA for 10 times/day, I1C2= irrigation with 100mg/l NA for 10 times/day, S1C1= spray with 75mg/l NA for 10, CONT.A= irrigation without NA(with nutrition solution) for 10 times/day, S1C2= spray with 100mg/l NA for 10 times/day, I2C1= irrigation with 75mg/l NA for 15 times/day, I2C2= irrigation with 100mg/l NA for 15 times/day, S2C1= spray with 75mg/l NA for 15 times/day, S2C2= spray with 100mg/l NA for 15 times/day, CONT.B= irrigation without NA(with nutrition solution) for 15 times/day.

Table (5): Antimicrobial activity of *Lavendula officinalis* crude volatile oil on Gram positive bacteria.

treatment	crude volatile oil (µl)	Diameter of inhibition zone (mm)				
		M.roseus	*M luteus*	*Staph.aureus*	*B.subtilis*	*B. cereus*
CONT.A	2	0	2	0	0	8
	3	4	6	12	0	12
	5	8	8	20	0	20
I1C1	2	0	0	4	0	2
	3	10	4	12	0	8
	5	24	20	18	0	12
I1C2	2	0	2	6	0	6
	3	8	14	14	0	10
	5	14	16	24	0	22
S1C1	2	2	0	0	0	10
	3	10	4	20	0	14
	5	21	12	21	0	22
S1C2	2	2	0	4	0	2
	3	16	4	12	0	14
	5	20	6	16	0	22
CONT.B	2	0	0	0	0	6
	3	12	14	8	0	12
	5	2	16	12	0	20
I2C1	2	0	0	4	0	6
	3	10	12	12	0	12
	5	12	14	20	0	14
I2C2	2	4	0	0	0	4
	3	20	16	10	0	16
	5	21	18	16	0	18
S2C1	2	0	4	0	0	6
	3	20	10	8	0	12
	5	25	11	12	0	24
S2C2	2	0	0	6	0	6
	5	18	20	24	0	20

Where: I1C1= irrigation with 75mg/l NA for 10 times/day, I1C2= irrigation with 100mg/l NA for 10 times/day, S1C1= spray with 75mg/l NA for 10, CONT.A= irrigation without NA(with nutrition solution) for 10 times/day, S1C2= spray with 100mg/l NA for 10 times/day, I2C1= irrigation with 75mg/l NA for 15 times/day, I2C2= irrigation with 100mg/l NA for 15 times/day, S2C1= spray with 75mg/l NA for 15 times/day, S2C2= spray with 100mg/l NA for 15 times/day, CONT.B= irrigation without NA(with nutrition solution) for 15 times/day.

Table (6): Antimicrobial activity of *Lavendula officinalis* crude volatile oil on Gram -negative bacteria.

treatment	crude volatile oil (µl)	Diameter of inhibition zone (mm)				
		E. coli	*Er. carotovora*	*Serr. marciens*	*Ps. fuorescens*	*S.tyhimurium*
CONT.A	2	2	0	0	0	2
	3	3	14	22	0	8
	5	5	12	14	13	20
I1C1	2	0	0	0	0	2
	3	12	6	9	0	10
	5	26	20	12	12	25
I1C2	2	0	2	6	0	10
	3	8	7	12	12	12
	5	14	16	26	14	24
S1C1	2	0	0	4	6	0
	3	6	8	12	13	12
	5	12	18	25	22	25
S1C2	2	2	0	2	6	3
	3	3	22	10	12	12
	5	5	24	22	24	24
CONT.B	2	12	0	0	0	0
	3	18	12	8	6	12
	5	18	14	20	20	22
I2C1	2	3	2	0	0	9
	3	12	16	9	3	12
	5	15	15	12	5	20
I2C2	2	4	4	0	0	12
	3	20	16	12	14	14
	5	21	20	15	16	16
S2C1	2	6	2	0	0	6
	3	22	14	12	10	12
	5	24	12	22	20	20
S2C2	2	0	0	12	0	9
	5	18	21	24	12	20

Where: I1C1= irrigation with 75mg/l NA for 10 times/day, I1C2= irrigation with 100mg/l NA for 10 times/day, S1C1= spray with 75mg/l NA for 10, CONT.A= irrigation without NA(with nutrition solution) for 10 times/day, S1C2= spray with 100mg/l NA for 10 times/day, I2C1= irrigation with 75mg/l NA for 15 times/day, I2C2= irrigation with 100mg/l NA for 15 times/day, S2C1= spray with 75mg/l NA for 15 times/day, S2C2= spray with 100mg/l NA for 15 times/day, CONT.B= irrigation without NA(with nutrition solution) for 15 times/day.

Regarding to G^{+VE} (Table 5) and G^{-ve} (Table 6), data reveal their highly sensitivity towards the most different oil samples, which ranged between 20-26mm and 24-26mm respectively. All the different strains of yeast survived and showed the highest resistance towards lavender essential oil samples (Table 7). Fortunately the tested yeast strains are non pathogens.

At close, the forementioned data clearly indicate the marked inhibitory effect of lavender crude oil samples against several human and plant pathogens and non pathogenic micro organisms. The relatively highest inhibition was attained on fungi followed by both G^{+ve} and G^{-ve} bacteria. On the other hand these oil samples were less active against yeast. However our data are in good conconance with those reported by Shimoni *et al.,* 1993, Vokou *et al.,* 1993, Larrando *et al.,* 1995, Adam *et al.,* 1998, Karamanoli *et al.,* 2000, Gavanagh and Wilkimson 2002, Tolkunova 2002, Pepeljnjak *et al.,* 2004, and Abdel-Reheem *et al.,* 2006. In this connection Inouye *et al.,* (2003) found that, bark, thyme and lavender oils showed high antimicrobial activity against three fungi and two bacteria. Lavender oil showed relatively high toxicity by solution contact than by gaseous contact. Also, Soylu *et al.,* (2006) found that several essential oils including lavender, thyme have inhibitory effect on Q-infestantsin a close dependent manner.

Considering with the mode of antibacterial action Burt (2004) explained that essential oils comprise large number of components and, in turn their mode of action involves several targets in bacterial cell. The hydrophobicity of essential oils enables them to partition in the lipids of cell membrane and mitochondria rending them permeable and leading to leakage of cell contents i.e. exert bactericidal or at least bacteriostatic properties.

The marked inhibitory effect of lavender crude essential oil tested in the present study may be mainly due to the presence of relatively high concentration of 1, 8 cineole and linalool (Abdel-Reheem *et al.*, 2006). The biological activity of 1, 8 cineole has been proved as fungicidal (Hammar *et al.,* 2003) and as in vitro antibacterial potent (Azuma *et al.,* 2003).

Table (7): Antimicrobial activity of *Lavendula officinalis* crude volatile oil on yeasts

treatment	crude volatile oil (µl)	Diameter of inhibition zone (mm)			
		Sacch.cerevisiae	*Sacch.rouxii*	*candida albicans*	*Candida utilis*
CONT.A	2	0	0	0	0
	3	0	0	2	0
	5	10	14	4	0
I1C1	2	0	0	0	0
	3	0	0	4	0
	5	8	2	15	0
I1C2	2	0	0	0	0
	3	0	0	3	0
	5	7	4	12	0
S1C1	2	0	0	0	0
	3	0	0	0	0
	5	2	9	9	0
S1C2	2	0	0	0	0
	3	0	0	0	0
	5	9	11	2	0
CONT.B	2	0	0	0	0
	3	0	0	3	0
	5	4	9	12	0
I2C1	2	0	0	2	0
	3	0	0	10	3
	5	2	6	12	6
I2C2	2	0	0	0	0
	3	0	0	10	0
	5	6	4	9	0
S2C1	2	0	0	0	0
	3	0	0	0	0
	5	9	7	9	0
S2C2	2	0	0	0	0
	5	6	5	9	0

Where: 11C1= irrigation with 75mg/l NA for 10 times/day, I1C2= irrigation with 100mg/l NA for 10 times/day, S1C1= spray with 75mg/l NA for 10, CONT.A= irrigation without NA(with nutrition solution) for 10 times/day, S1C2= spray with 100mg/l NA for 10 times/day, I2C1= irrigation with 75mg/l NA for 15 times/day, I2C2= irrigation with 100mg/l NA for 15 times/day, S2C1= spray with 75mg/l NA for 15 times/day, S2C2= spray with 100mg/l NA for 15 times/day, CONT.B= irrigation without NA(with nutrition solution) for 15 times/day.

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تأثير النيكوتين أميد علي النشاط المضاد للميكروبات لزيت اللافندر العطري

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أستُخدم في هذه الدراسة شتلات نبات اللافندر حيث تم زراعتها في أصص مُلِئَت ببيئة البرليت وذلك تحت ظروف الصوبة الزراعية البلاستيكية. عُومِلت النباتات (بعد تقسيمها إلي أثنَتَي عشرةَ مجموعة كل مجموعة بها ثلاثة مكررات) بواسطة ثلاثة تركيزات من النيكوتين أميد (0 و 75 و 100 مجم/ لتر) عن طريق الرش أو الأضافة إلي ماء الري وذلك بمعدلين رش أو ري مختلفين (10 و 15 مرة في اليوم) مما أدي للحصول علي 12 معاملة مختلفة. تم أخذ حشتين من الأعشاب النامية تحت هذه الظروف وتم أستخلاص الزيوت العطرية منها. تم تقدير النسب المئوية للزيوت المستخلصة و حساب أنتاجية الزيت لكل عشب. أيضاً تم إجراء تحليل كروماتوجرافي لمكونات الزيوت المستخلصة بواسطة تقنية كروماتوجرافيا الغاز بأستخدام جهاز ال GC .

تم دراسة نشاط هذه الزيوت ضد عشرون كائن دقيق مُمرض أو غير مُمرض للأنسان أو النبات تضمنت :

- خمس أنواع بكتيريا موجبة الجرام (*micrococcus roseus, Micrococcus luteus, Staphylococcus aureus, Bacillus subtils, Bacillus cereus*).
- خمس أنواع بكتيريا سالبة الجرام (*Escherichia coli, Erwinia carotovora, Serratia marcines, Pseudomonas fluoresceus, Salmonella typhimurium*).
- ستة أنواع فطريات (*Fusarium solani, Fusarium oxysporum, Rhizoctonia solani, Aspergillus flavus, Aspergillus niger, Penicillium sp.,*).
- أربعة أنواع من الخمائر (*Saccharomyces cerevisiae, Saccharomyces roxii, Candida utilis, Candida albicans*).

وكانت أهم النتائج المتحصل عليها كما يلي:

- أدي الري بمعدل 15 مرة في اليوم إلي الحصول علي أعلي نسبة زيت من الحشة الأولي (0.31 %) و أقل نسبة زيت من الحشة الثانية (0.21 %).
- كان الري بمعدل 10 مرات في اليوم كافياً للحصول علي أعلي أنتاجية زيت في كلاً من الحشة الأولي (0.63 مل/ عشب) و الحشة الثانية (0.51 مل/ عشب).
- أعطت الحشة الثانية أقل نسبة مئوية للزيوت المستخلصة وكذلك أقل إنتاجية زيت مقارنتاً بالحشة الأولي وذلك عند مستويات الري أو الرش المستخدمة (10 و 15 مرة /يوم).

- كانت أعلي إنتاجية للزيت العطري في النباتات المعاملة بالنيكوتين أميد بتركيز 75 جزء في المليون (0.68 مل /عشب) مقارنتاً بالتركيزات 0 و 100 جزء في المليون.
- أظهرت نتائج تحليل ال GC أن مركب 1,8 سينيول هو المكون السائد للزيوت الناتجة من كل المعاملات ولكن أختلفت نسبته من حشة لأخري و تبعاً لمعدل وطريقة الري وكذلك تبعاً لتركيز النيكوتين أميد المستخدم.
- اللينالول هو ثاني أكبر المكونات للزيوت العطرية في كل المعاملات وأختلفت أيضاً نسبته من حشة لأخري و تبعاً لمعدل وطريقة الري وكذلك تبعاً لتركيز النيكوتين أميد المستخدم.
- كانت قيم باقي مكونات الزيوت العطرية (Limonene, α, β pinene, Camphor, Terpinene -4-ol, Lavandulyl acetate, Linalyl acetate, و geranayl acetate) قليلة نسبياً.
- زيادة أحجام الزيوت العطرية المستخلصة من 2 إلي 5 ميكروليتر أدت إلي زيادة تثبيط نمو معظم الميكروبات المستخدمه في هذه الدراسة ومنها الفطريات والبكتريا الموجبة والسالبة للجرام.
- أظهرت سلالات الخمائر المستخدمه مقاومه شديدة تجاه الزيوت المستخلصة حيث نمت بصورة طبيعية.
- أوضحت نتائج هذه الدراسة أن لزيت اللافندر الخام تأثير مثبط واضح ضد عدة ميكروبات ممرضة وغير ممرضة لكلاً من الأنسان أو النبات.

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