Biosciences, Biotechnology Research Asia Vol. **5**(1), 65-72 (2008)

Chemical composition, antimicrobial and insecticidal activities of the essential oil of *Lamium maculatum* L. grown in Egypt

ZEINAB I.A. EL- SAYED

Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University Zagazig (Egypt)

(Received: January 08, 2008; Accepted: February 06, 2008)

ABSTRACT

GC and GC/MS analysis of the essential oil isolated by hydro distillation of aerial part of *Lamium maculatum L.* showed the presence of thirty compounds which account for 99.9% of the total composition. Twenty four components representing (93.56%) of the oil were identified. The major components were found to be *β- caryophyllene (14.80%), caryophyllene oxide (13.84%), Z, E- α- franesene (10.11%). dihydroedulan I (9.13%). α- humulene (6.06%), bornyl formate (6.03%)* and *α- bisabolene* (5.34%). The oil showed moderate antimicrobial activity. Insecticidal activity of the essential oil was evaluated against the third larval instar of *Musca domestica* on the basis of mortality percentage. The obtained results declared the use of essential oil of *Lamium maculatum L.* as a natural insecticide.

Key words: *Lamium maculatum L, Lamiaceae*, essential oil composition, GC/MS, antimicrobial, Insecticidal activity, *Musca domestica* L.

INTRODUCTION

Lamium (dead nettle) is a genus of 40-50 species of flowering plants in the family *Lamiac*eae[1]. The genus includes both annual and perennial herbaceous plants; they spread by both seeds and stems rooting as they grow along the ground[1,2]. Some species of this genus are traditionally used as food in some countries and the oil obtained from the seed of some species showed strong antioxidant properties, so its use as a food additive[3]. Also, the hydro-alcoholic extract of another species, showed interesting antioxidant, anti-inflammatory and anti-proliferative properties[4]. Finally, in local folk medicine, all the species are used as remedies in menorrhaigia and intermenstrual bleeding, in the treatment of scrofula, for the regulation of sebaceous secretion, and as blood tonic, antispasmodic and anti-inflammatory agent[5,6].

Lamium maculatum L. "white Nancy" is a perennial, herbaceous straggling or half-trailing plant, effective in hanging basket, slightly hairy, with long-petiolate, cordate-ovate leaves and having white flower, ascending in clusters, Corolla-tube two to three time as long as calyx[1].

Lamium maculatum L. has been used in the Chinese folk medicine for the treatment of trauma, fracture and hypertension[7].

The chemical constituents of *Lamium maculatum* L. has been sparsely investigated. The iridoid composition has been studied by paper chromatography, where a spot was detected which indicated the presence of lamioside[8,9]. *Lamium maculatum* has been screened also for irridoid glucosides, ecdysones, phenylethanoids, flavonoids and betaine[7.10-13].

The essential oils obtained from *Lamium* species are scarcely studied. In 1993 the diethyl ether extract of leaves of *Lamium maculatum* was analyzed[14]. While in 1996 the oil of *Lamium garganicum* ssp. *Laevigatum* was examined[16]. In 2005, the composition of the essential oils and in vivo emission of volatiles of four *Lamium* species from Italy was determined[15].

The use of the conventional chemical insecticidal for best control was responsible for the over- pollution of the environment, toxic residues, carcinogenicity and resistance problems of these insecticides. The plant- derived natural products, however, still have immense potential for the control of insects, if there are reasonably effective and harmless to beneficial non-target organisms and the environment. Essential oil still hold an important place in folklore for use as insecticides and repellents, there is also a long tradition of using aromatic plants as insecticide and repellents around the home and in animal bedding[17,18]. Furthermore, essential oils extracted from some plants belonging to *Lamiaceae* were efficient showed insecticidal effect[19,20].

However, to the best of our knowledge, there are no reports about the composition of the essential oil of *Lamium maculatum* L. and its biological activity. Hereby, the present work was carried out to identify its constituents, as well as to determine its antimicrobial activity and to identify its efficacy as an insecticide against the house fly, *Musca domestica* which constitute one of the greatest problems of human public health. This work on the essential oil of *Lamium maculatum L.* was reported for the first time.

EXPERIMENTAL

Plant material

The fresh aerial part of *Lamium maculatum* L. were collected from plants acclimatized and cultivated in the experimental station of Faculty of Pharmacy, Zagazig University in July 2007. The specimen was kindly identified by Dr. Sayed Farag Khalifa, Prof. of Plant Taxonomy, Phytotaxonomy Dept., Faculty of Science, Ain shams University. A voucher specimen has been deposited in the herbarium of Pharmacognosy Dept., Faculty of Pharmacy, Zagazig University.

Preparation and analysis of essential oil

The oil was obtained from the fresh minced herbs by hydrodistillation for 6 hours in a clevenger type apparatus[21]. The oil was dried over anhydrous sodium sulfate (to give 0.35%v/w) and was kept in a freezer until further analysis. Qualitative and quantitative analysis of the oil were done on 2 µl sample oil solutions "approximately 1% oil in ether" using GC and GC/MS.

The GC analysis of the oil was done on Trace 2000 Gas Chromatogrpahy equipped with a split-splitless injector, attached to an DB-5MS fused silica column (30 m × 0.25 mm; film thickness 0.25 µm), fitted with FID, under the following conditions; helium as carrier gas at 1 ml/min; injector temperature was 250°C; detector temperature 280°C; column temperature programme: 40°C for 2 min., ramp 5°C/1'min. to 250°C. The total run time for GC was 47 min. The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization, all relative response factors being taken as one.

For GC-MS analysis: GC conditions as mentioned above, and the capillary column was directly coupled with a quadrupole mass spectrometer (Finningan MAT SSQ 7000). EI-MS were recorded at 70 ev., Full scan type, Mass Rang 40-400, scan time: 5 sec'.

Identification of the components was performed by aid of the computer liberary search (NIST-Mass lab software package, fissons), comparison of mass spectra with literature data and by comparison of their retention times and mass fragmentation patterns with those of the library database [Wiley (Wiley Int. USA)][15,22,23].

Results of analysis of the essential oil is summarized and presented in (Fig. 1) and table (1). The different chemical classes of the identified compounds are present in table (2).

Screening of antimicrobial activity

Escherichia coli, Bacillus subtilus, *Staphylococcus aureua* and *Candida albicans* were

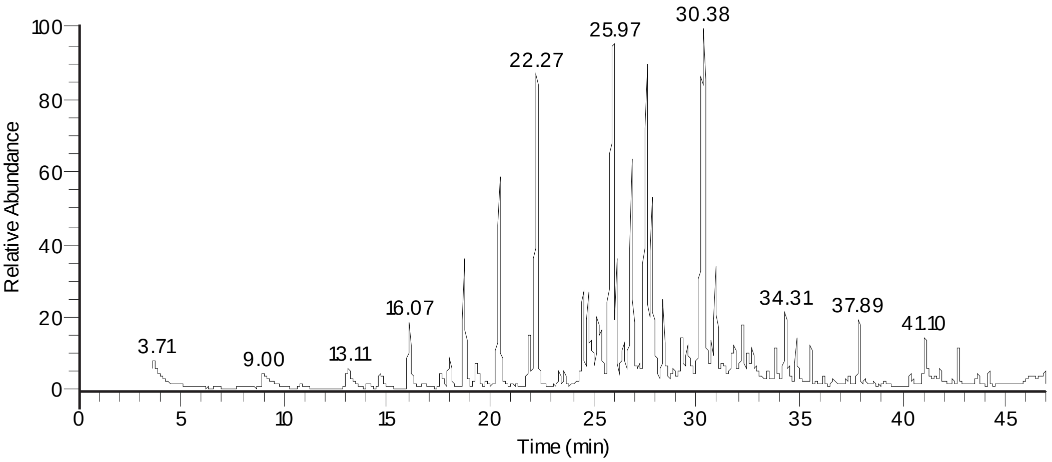

Fig. 1: GC/ MS of *Lamium maculatum* L. essential oil

obtained from Microbiology Department, Faculty of Pharmacy, Zagazig University and subjected to susceptibility testing on Mueller-Hinton agar medium by disc agar diffusion method[24]. The standard antibiotic was Ciprofloxacin while the antifungal was Nystatin 5 and 10 mg/dis respectively. The results were illustrated in Table 3.

Screening of insecticidal activity of essential oil against *Musca domestica L.*

Rearing technique used for *Musca domestica*[25,26]

The mass rearing in the laboratory was achieved by collecting a batch of outdoor larvae from their natural breeding sites in manure and were reared in the laboratory at 28 ± 2^{5}C, 65 ± 5% R.H. and 12h. illumination. Studies were carried out under laboratory conditions of the Plant Protection Department, Faculty of Agriculture, Zagazig University at 28 ± 2^{5}C and 65 ± 5% R.H. To evaluate insecticidal activity of essential oil of *L. maculatum* L. on the third larval instar *Musca domestic* the following technique was used:

Technique for insecticidal effect of essential oil

Formulation of 5% essential oil in acetone were prepared. The formulated essential oil was applied topically on dorsal surface of the thorax of 3rd instar larvae of *Musca domestica* L. (ten larvae) with different doses; 15,20, 25 and 30 μ liter/ larva of essential oil (5%) by using Automatic micro pipet. After application, larvae were put in small plastic cups, 7cm in diameter, and covered with standard larval rearing medium. This experiment was replicated three times. The larvae of control groups were also treated with 25 μ liter/larva of acetone and also replicated three times.Larval mortality percentage of the treated and control larvae were calculated and recorded after 12 and 24h.of treatments. Treated and control larvae were also subjected periodically for moulting and developing until pupation. All data were analyzed by ANOVA. Insecticidal results is shown in table 4.

RESULTS AND DISCUSSION

The volatile oil of *Lamium Maculatim L* "White Nancy" was prepared by hydrodistillation[21] of fresh aerial part; to yield (0.35% v/w). The oil was viscous, orange- yellow in colour, lighter than water and exhibited characteristic agreeable aromatic odour.

GC/ MS analysis of the oil led to identification of the majority of the components, which were listed in table 1, along with their quantitative data and their retention time. The identification of the compounds was based on comparison of their mass spectra with those of wiley

Table 1: The results of GC/ MS analysis of the essential oil of *Lamium maculatum* L.

S.No.	Name	R_t	M^+	B. P	Major peaks	Area%
1.	Cis-3-Hexen- 1-ol	9.01	100	67	82, 55, 69, 41	1.03
2.	1-Octen- 3-Ol	13.12	128	57	43, 72, 85, 99	0.88
3.	Fenchone	16.07	152	81	69, 109, 137, 53	2.11
4.	Camphor	18.03	152	95	81, 55, 69, 108	0.93
5.	Chrysanthenol <cis>	18.77	152	81	41, 121, 93, 67, 107	3.53
6.	Borneol	19.33	154	95	55, 41, 110, 139, 121	0.97
7.	Bornyl formate.	20.47	182	95	121, 136, 93, 53, 41	6.03
8.	Bornyl acetate	21.90	196	95	43, 136, 121, 154, 55, 67	1.47
9.	Dihydroedulan I	22.26	194	179	43, 69, 107, 90, 98	9.13
10.	a- copaene	24.51	204	161	119, 105, 91, 77, 55	2.16
11.	b- Bourbonene	24.78	204	81	123, 161, 80, 105, 91	2.35
12.	4- MethyL-5- isopropyliden-8-oxo- Nona-1, 3- diene	25.20	192	119	43, 134, 91, 121, 77, 159	1.31
13.	Unidentified	25.34	204	91	43, 159, 119, 133, 97	1.11
14.	b- caryophyllene	25.96	204	91	133, 105, 79, 69, 161, 189	14.80
15.	Unidentified	26.50	-	119	43, 69, 194, 93, 136, 151	0.89
16.	a- Humulene	26.89	204	93	121, 80, 147, 79	6.06
17.	Z, E-a- Franesene	27.59	204	93	119, 107, 55, 69, 79, 161	10.11
18.	a- Bisabolene	27.86	204	93	107, 79, 55, 121, 133, 161	5.34
19.	d- Cadinene	28.38	204	161	119, 105, 134, 91, 189	1.84
20.	Unidentified	29.30	220	41	79, 106, 93, 55, 121, 149	1.03
21.	Germacerene-D-4- OL.	29.59	222	81	43,69, 93, 107, 123, 161	1.05
22.	Caryophyllene oxide	30.37	220	41	79, 91, 67, 55, 107	13.84
23.	Humulene epoxide II	30.96	220	43	109, 67, 96, 138, 123	2.52
24.	Epi-a- Cadinol.	31.83	222	43	161, 136, 79, 93, 204, 121	1.03
25.	b- Eudesmol	32.23	222	59	149, 79, 121, 164, 204, 189	1.41
26.	Unidentified	33.84	-	93	133, 79, 69, 55, 147, 189	0.81
27.	a- Bisabolol oxide A	34.31	238	143	43, 125, 93, 67, 178	2.11
28.	Unidentified	34.88	-	41	91, 119, 133, 161, 187	1.04
29.	Unidentified	37.89	270	131	190, 160, 91, 55, 229	1.56
30.	Phytol	41.10	296	71	43, 55, 123, 81, 137	1.54

R_t, Retention time; M^+, molecular ion peak; B.P, base peak.

Table 2: Essential oil composition with constituent categories

Constituent category	Relative area percentage
Long chain hydrocarbons	1.91
Oxygen containing monoterpenes	24.17
Sesquiterpenes hydrocarbons	45.97
Oxygen containing sesquiterpenes	23.8
Others	4.14

Table 3: The results of antimicrobial screening

Ess. oil and St. antib.	Zone of inhibition in mm *Escherichia coli* G -ve	*Bacillus subtilis* G+ve	*Staphylococcus aureus G +ve*	*Candida albicans*
Ess. oil of *L. maculatum.*	-	13	9	-
Ciprofloxacin	15	24	20	-
Nystatine	-	-	-	24

Evaluation inhibition (7-8 mm) resistant, (9-11 mm) moderate sensitive, >12 mm sensitive

Table 4: Effect of essential oil of *Lamium maculatum* L. on 3rd larval instar of *Musca domestica* treated with topical application method and then reared on standard rearing medium

Extract	Dose, μ liter	% Larval Mortality After 12h.	After 24h	Mean	%Pupation
5% Essential oil of *L.maculatum* in acetone	Control*	0	0	0	100
	15	7	20	1	66.67
	20	14	60	3	20
	25	20	80	4	13.33
	30	24	93.33	4.66	6.66
	L.S.D. 5%		1.24**		1.15**

*acetone was used as control

and library data base and those described by Adams[22], as well as on comparison with literature values[(5,23].

From these results it could be concluded that: Twenty four compounds were identified which representing 93.56% of the total oil sample. The major components were found to be; β- caryophyllene (14.80%), caryophyllene oxide (13.84%), Z,E- α- franesene (10.11%), dihydroedulan I (9.13%), α- humulene (6.06%), bornyl formate (6.03%) and α- bisabolene (5.34%). Grouping the constituents into categories of compounds indicated that; the oxygenated compounds constituted (47.97%) table 2, mainly attributed to monoterpenes (24.17%) and sesquiterpenes (23.80%), oxygenated monoterpenes contain dihydroedulan I (9.13%) as the major constituent. While Caryophyllene oxide (13.84%) was the major oxygenated sesquiterpene.

Furthermore, sesquiterpenes hydrocarbons components participate in the high percentage (45.97%), β-caryophyllene (14.80%) was the major component in the volatile oil of the investigated species. Moreover, some minor components were also detected of which long chain hydrocarbons (1.91%), in addition to phytol (1.54%), that was diterpenoid in nature. Essential oil of *lamium macalatum* L. showing a characteristic agreeable aromatic odour due to the presence of oxygenated components (47.97%), as well as b- caryophyllene (14.80%) and dihydroedulan I (9.13%). Since, β- caryophyllene was reported to have a nice and flowery fragrance odour[27], while dihydroedulan I has a characteristic elderberry odour[28].

Concerning the biological study Table 3 the oil exhibited significant level of activity against *Bacillus subtitles,* moderate activity against *Staphylococcus aureus,* where *Escherichia coli* and

Candida albicans were completely resistant against the essential oil of *Lamium maculatum.* This activity is confirmed with its high concentration of oxygenated terpenoids (47.97%) and caryophyllene oxide (13.84%). The oil rich in oxygenated terpenoids was reported to show antimicrobial activity against *Staphylococcus epidermitis*[29], while caryophyllene oxide was active against *Staphylococcus aureus*[30], used as preservative in food, drugs and cosmetics[31], as well as has been tested as an antifungal against dermatophytes[31].

The presence of oxygenated terpenoids (47.97%) and caryophyllene oxide (13.84%) in the essential oil of *Lamium maculatum* could explain the use of the plant for the treatment of trauma and fracture[7].

The insecticidal activity of the essential oil of *lamium maculatum* was evaluated against the third larval instar of *M. domestica* L. on the bases of larval mortality percentages. It was found out that it showed a promising insecticidal activity. Data in Table 4, showed that the essential oil increased the percent of larval mortality at different doses 15, 20, 25 and 30 µ liter/ larva to reach a very high values (93.33%) at 30µ liter/larva compared with control (0%) after 24 h. The response of the larvae to the oil appears to be concentration-dependent. Statistical analysis assures that there were highly significant differences between the four tested doses and control.

In contrast, percentages of pupation were decreased gradually recording 100, 66.67, 20, 13.33 and 6.66% at control, 15, 20, 25 and 30 µ liter/larva respectively. Statistical analysis of the data revealed that this decrease in percentage of pupation was highly significant for the four tested doses of essential oil compared with untreated. Concerning insect growth inhibitory activity, the percentage of the larvae that reached pupation decreased drastically with almost all doses. None of the control group was affected. An untreated control batch was replicated to ensure that the solvent treatment continued to have no significant effect on background mortality rate.

The obtained results indicated that the essential oil of *Lamium maculatum L.* as toxicant has a promising effect against the house fly. *M. domestica.* The literature offers no data on chemical composition and the potency of the studied essential oil against hous fly. M. domestica or any other insect.

It was reported that the insecticidal activity of volatile oils against the house fly *M. domestica* was due to the presence of high percentage of sesquiterpenes[32-34]. Accordingly, the insecticidal activity of essential oil of *Lamium maculatum L.* is probably deduced to its high percentage of sesquiterpenes content (45.97%) and *β- caryophyllene* (14.80%), which is known to possess local anaesthetic in vivo and vitro[(35)].

The obtained results have provided evidence to the potential use of *Lamium maculatum* L. essential oil as insecticide.

Recently, essential oils derived from plants have received much interest as potential bioactive agents against house fly *M. domestica*[32,33,36,37].

These results suggest that further investigations into the use of essential oil as insecticidal are warranted, specially in view of the public dislike of synthetic insecticides and resistance and toxicity problems associated with their use.

In conclusion, this study indicated that *Lamium maculatum* L. could be successively introduced, acclimatized and cultivated in Egypt, can be used as antimicrobial against gram +ve microorganisms as well as clearly illustrated the efficacy of the investigated plant, which encourages the development of alternative active ingredients as effective *M. domestica* larvicides which constitute one of the greatest problems of human public health.

Bioassay and identity of the responsible compounds of insecticidal activity and their possible mechanism of action is in progress.

ACKNOWLEDGMENTS

The author is indebted to Prof. Dr. Mahmoud Khalil, Plant Production Department, Efficiency Productive Institute, Zagazig University for introducing plant material from Holland, Europe and helping us in acclimatization and cultivation of the plant in the experimental station of faculty of pharmacy, Zagazig University, special thanks also go to Dr. Hemmet Kamal Abd Ellatif Prof. of Microbiology, Faculty of Pharmacy, Zagazig University for her help in performing antimicrobial activity. Thanks extended to Plant Protection Department, Faculty of Agriculture, Zagazig University for providing us with insect.

REFERENCES

1. Bailey, L.H., Manual of cultivated plants. Macmillan, New York, 854 (1977).
2. Http://en. wikipedia. org/ wiki/ lamium. Accessed on 27th Aug; (2007).
3. Picuric-Jovanovic, K.M., Budincevic, M and Vrbaski, Z. Acta of the symposium "vitamine und Zusatzstoffe" Jena, 24- 25 September (1997).
4. Trouillas et al., *Food chemistry*, **80**: 399-407 (2003).
5. Mazza, F. Itinerari alla scoperta delle erbe officinali del monte amiata, Abbadia S. Salvatore, siena, Italy: Stampa (2000).
6. Bremness, L. Bremness, the complete book of Herbs, dorling Kindersley London (1995).
7. Shuya, C., Xingguo, C. and Zhide, H. *Biomed. Chromatogr*. **17**: 477 (2003).
8. Adema, F. *Acta Bot. Neerl*. **17**: 423 (1968).
9. Kooiman, P. *Acta Bot. Neerl*. **21**: 417 (1971).
10. Alipieva, K., Kokubun, T., Taskova, R. *et al. Biochemical systematic and Ecology*, **35**(1): 17- 22 (2007)
11. Alipieva, Kalina I., Taskova, Rilka M., Jensen, Soren R. *et al., Biochemical systematics and Ecology*, **34**(1): 88- 91; (2006).
12. Savchenko. T, Black ford. M, Sarker S.D and Dinan. L. *"Biochem. Sysl. Ecol.* **28**: 891 (2001).
13. Yuan, Z.X., Patel, A.V., Blunden. G. *et al., "Phytochmistry"* **21**(12): 4351-2l (1992).
14. Abuzeina, A. E. S., Handjieva, N. P. S., Evstatieva, L. *"Dokl-Bulg. Akad. Nauk."*, **46**(6): 37-39 (1993).
15. Guido Flamini, Pier Luigi Cioni and Ivano Morelli *"Food chemistry"* **91**(1): 63- 68 (2005).
16. Roussis, V., Chinou, I., Perdetzoglou, D., and Loukis, A.E. *"Journal of essential oil research"* **8**: 291-293 (1996).
17. Priestle, C.M., Burgess, I.F., Williamson, E.M. *"Fitoterapia"* 77-303 (2006).
18. Williamson, E.M., Priestley, G.M., Burgess, I.F. *"Fitoterapia"* **78**: 521-527 (2007).
19. Catherine Regnault. Roger, Hamraoui, A., Holeman, M., Theron, E. and Pinel. R. *"Journal of chemical Ecology"* **19**(6): 1233- 1244 (1993).
20. Silva, E.J., Döria, G.A., Maya, R.T., Nunes, R.S., Carvulho, G.A., Blank, F.F., Alves, P.B., Marcol, R.M., Cavalcanti, S.C. *"Journal Bioresour Technol"*; Jul. 25 (2007).
21. Egyptian pharmacopeia "Eng- Ext", 3rd ed., Cairo University, Cairo, **429**: 472,733 (1984).
22. Adams, R.P. *"Identification of essential oil components by gas chromatography- mass spectroscopy",* Allured, Carol Stream (1995).
23. Massada, Y. *"Analysis of essential oils by gas chromatography and mass spectrometry"*, wiley, New York (1967).
24. Ericsson, H.M. and Sherris, J.C. *Antibiotic sensitivity testing: reports of an international collaborative study. Acta. Pathol. Microbial. Scand.,* **217**: 1-90 (1971).
25. Busvine, A., laboratory technique for measuring the suscptibility of house flies and blow flies to insecticides. *Lab. Prot.*, **11**: 464-468 (1962).
26. Kelany, I.M., El-deeb, M.A. and El-fishawi, A.A. The effect of aqueous neem, *Azadirachta ind*ica A. Juss, seed kernel extract on the house fly, *Musca domestica* L. *Egypt. J. Biol. P. Cont.,* **1**(1): 121-128 (1991).
27. Ohloff, G. Recent developments in the field of naturally- occurring aroma components. In W. Herz, H. Grisebach, and G.W. Kirby, eds., Progress in the chemistry of Organic Natural products, vol. 35. springer- verlag, Vienna, 1- 589 (1978).
28. Kaack, K., Christensen, L.P., Hughes, M. and Eder, R. *"European food research and technology"* **221**(3-4): 244- 254 (2005).

29. Bülent Köse, Y., Iscan, G., Dermici, B., *et al. “Fitoterapia”,* **78**: 253-254 (2007).
30. Ulubelen, A., Topcu, G., Eris, C., Sonmez, U. *et al., “Phytochemistry”*, **36**(4): 971-974 (1994).
31. Depo yang, Laura Michel, Jean- Pierre Chaumont and Joëlle Millet- Clerc. “*Mycopathologia*”, **148**(2): 79-82 (2000).
32. Shoukry, I.F.I. Proc. Fir. Conf. On the role of science in the development of Egyptian society and environment. 21- 23Oct. 1996, Zagazig University, Benha branch, Egypt 1- 21 (1996).
33. Maher, M. El- Domiaty, Azza, M. EL., Mahmoud. M.A. and Eman, M.R. *“J. Egypt. Acad. Soc. Environ. Develop.,* (A-Entomology) **3**(2): 21- 40 (2003).
34. Perti, G., Lemberkovies, E., Foldvari, M. *et al.,* “*Flavors and fragrances a world perspective*”, 439- 446 (1988).
35. Ghelardini, C., Galeotti, N., Dicesare Mannelli, L. *et al., “Farmaco”* May- July, **56**(5-7): 387-9 (2001).
36. Abdel- Halim, A.S., Morsy, T.A. “*J. Egypt Soc. Parasitol*,; Aug, **35**(2): 631-6 (2005).
37. Abdel- Hady, N.M., Abdel- Halim, A.S., Al- Ghadban A.M. *“J. Egypt Soc. Parasitol”*; Aug, **35**(2): 687- 98 (2005).