https://dx.doi.org/10.4314/ejppri.v7i3.14

Egypt. J. Plant Prot. Res. Inst. (2024), 7 (3):413-421

وزارة الزراعة واستصلاح الأراضي
Ministry of Agriculture and Land Reclamation
مركز البحوث الزراعية
Agricultural Research Center

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

PLANT PROTECTION RESEARCH INSTITUTE
PPRI

Field application of wild thyme extract *Thymus serpyllum* for controlling land snails *Monacha cartusiana* (Gastropoda: Hygromiidae)

Mohamed, Ahmed Ayyad; Sara, Samir and Ahmed, El Morsy Hussein

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ARTICLE INFO

Article History

Received:30 /7/2024

Accepted:29 /9/2024

Keywords

Land snails, control, *Monacha cartusiana*, *Thymus serpyllum*, volatile oil extract, thymol, GC/MS and ^{1}H-NMR and field application.

.

Abstract

One of the available and the safer medicinal and aromatic plants is *Thymus serpyllum*. Like most natural compounds of plant origin. Thyme oil extract was used in this research paper as a potential alternative in controlling the land snail *Monacha cartusiana* (O. F. Müller) (Gastropoda: Hygromiidae). The volatile oil of *T. serpyllum* was obtained via steam distillation and analyzed using the GC/MS technique; the monoterpenoid alcohol; thymol compound was separated by chromatographic technique, and its structure was elucidated using ^{1}H-NMR, in addition to testing thyme extract in open field conditions, which gave an acceptable percentage population reduction of 66.58 and 51.22 when compared to the recommended pesticide, neomyl, which poses 75.76 and 59.74 for the residual and the mean population reduction percentage, respectively. The results obtained can be largely relied upon in terms of using thyme extracts extensively in the control process of land snails in general, especially since these compounds are safe and environmentally friendly and their results are respectable when applied in field conditions.

Introduction

Molluscs are one of the most diverse creatures on Earth (Guerra-García *et al*., 2021). Terrestrial gastropods, including land snails, are one of the most important pests that cause multiple problems in the agricultural sector, whether crops, vegetables, fruits, or even ornamental plants have not been spared from this pest (Ayyad *et al*., 2023). One of these pests is *Monacha cartusiana* (O. F. Müller) (Gastropoda: Hygromiidae), both a threat and a waste of agricultural economic returns. *M. cartusiana* is one of the pests that have become increasingly harmful in recent times, and many studies and research have been conducted on methods of controlling it (Ali *et al*., 2023a). Natural compounds with botanical sources (Ali and Ayyad, 2022) are used in pest control operations alongside and in parallel with synthetic organic compounds and on all types of pests, whether animal (Ayyad *et al*., 2023) or insect. Since this pest is the focus of attention of many researchers working in the field of pest control, some nanomaterials with green chemistry, along with plant extracts, have been included in research concerned with controlling land snails and *Monacha* in particular (Helmy *et al*., 2022). The molluscicidal ability of three botanical natural oils, compared with neomyl as a reference pesticide against *Monacha* snail, was assessed. The tested oils were applied under laboratory and field conditions. All tested compounds gave a considerable control effect against the *Monacha* snail. The obtained reduction of population after twenty-one days of the applied treatment was 93.28%, 76.76%, 64.16%, and 50.75% for neomyl (Reference pesticide), *S. aromaticum*, *N. sativa*, and *B. alba*, respectively. A bioassay test showed that *S. aromaticum* oil was the most effective and *B. alba* was the least effective. In light of these results, clove oil, black cumin oil, and mustard oil, as botanical natural oils safe for the environment, can be used to control this pest (Abdel-Rahman, 2020).

Thymol was used as an oil in the process of controlling snails as a potential pesticide in addition to some known traditional compounds. It had an acceptable effect in reducing the snail population by 55.82% compared to neomyl, which gave 72.61% when applied in the field using toxic baits (Ali, 2017). The activity of monoterpenoid compounds, thymol, agrinate, and thyme essential oil, against different genera of snails, *Eobania Vermiculata* (Müller) *and Succinea Putris* (L.) (Gastropoda: Hygromiidae), was investigated. The results exhibited that agrinate was highly effective, followed by thymol and thyme essential oil, which had the lowest effect with LC_{50} values of (593.30, 1570.51, and 224.14 ppm) and (1577.48, 3344.91, and 873.59 ppm), respectively, using the contact application method. In addition, thymol prevents mold formation and has antifungal properties (Ali *et al*., 2023b).

This research paper is one of many attempts to find an alternative from the available environmentally friendly, effective, and low-cost alternatives to the process of controlling the land snail *M. cartusiana*, which has become an increasing threat to the agricultural sector in general.

Materials and methods

1. Solvents:

Hexane, chloroform, and methanol all the solvents were HPLC-grade purchased from Loba Chemie Company.

2. Processing of thyme (Volatile oil):

The plant was classified and identified in the herbarium of the Faculty of Science - Mansoura University, by Dr. Maha Al-Shami. The volatile oil of leaves and shoots (500 g) was extracted by using steam distillation (Clevenger) and then analyzed by GC/MS to afford the identified 14 compounds.

3. Separation of thymol:

The separated essential oil was dissolved in a reasonable amount of methanol. And was chromatographed over ready silica gel plates using mixtures of hexane/chloroform in a 97:3% ratio to afford crystalized thymol that was subjected to ^{1}H-NMR analysis.

4. Gas Chromatography/Mass Spectrometry (GC/MS):

The volatile fraction was performed by Varian GC interfaced to a Finnegan SSQ 7000 mass selective detector (SMD) with an ICIS V2.0 data system for MS identification of the GC components. The column used was a DB-5 (J&W Scientific, Folsom, CA) cross-linked fused silica capillary column (30 m. long, 0.25 mm. internal diameter) coated with poly dimethyl-siloxane (0.5µm. film thickness). The oven temperature was adjusted from 50˚C for 3 minutes, at isothermal, then heating by 7˚C/min to 250˚C and isothermally for 10 minutes, at 250˚C. Injector temperatures were 250˚C and 150˚C, respectively. The mass spectrometer had a delay of 3 minutes.

To avoid the solvent plead and then scan from m/z 50 to m/z 300. Ionization energy was set at 70 eV (Agricultural Research Center, Dokki, Cairo).

5. ^{1}H-NMR spectra:

The extracted oil was treated with methanol and set on the TLC plates using an elution system 97% of Hexane + 3% Chloroform. The ^{1}H-NMR spectra were recorded on a (Brucker Wpsy) 500 MHz spectrometer, respectively. TMS was used as an internal standard, deuterated chloroform was used as a solvent, and the chemical shift (δ) was expressed in ppm. The coupling constant (J) in Hz and the acronyms (s) for singlet, (d) for doublet, and (m) for multiplet were used to define the type of protons. ^{1}H-NMR spectra were measured at Cairo University, Microanalytical Center.

6. Baits and field application:

In an infested field with a high density of terrestrial gastropods of *M. cartusiana*, the field experiment was carried out. Three replicates of thyme extract and of the pesticide neomyl were prepared in addition to the control replicates. Each replicate consisted of 93 parts bran with 5 parts of sugarcane black honey as an attractant in addition to two parts of the substance under test (Thyme extract), and similarly for the pesticide neomyl and also for the control but without adding any materials. A suitable equal quantity of bait was placed on a piece of plastic in the middle of the clover, and it was moistened every week using a hand sprayer. Readings of live snails per unit area of the bait site were taken before the treatments and after one, three days, one week, and two weeks. The results were analyzed using the Minitab 18 program. The corrected percentage of snail population reduction was calculated using the Henderson and Tilton equation (Shahawy, 2019). The molluscicidal field examinations were carried out in Aga District, Mansoura, Egypt.

Henderson-Tilton's formula

$$\text{Corrected \%} = \left[1- \frac{\text{n in Co before treatment * n inT after treatment}}{\text{n in Co after treatment * n inT before treatment}} \right]$$

Where n =Snail Population , T = Treatment, Co = Control

7. Statistics analysis:

All statistics were applied to One-way ANOVA, represented as mean ± SE. Using Tukey's method, confidences with a 95% simultaneous confidence level were computed; 0.05 or less probability was observed as significant. All statistical analysis was achieved using Cohort Software (Cho *et al.*, 2004).

Results and discussion

1. Separation and identification via GC/MS:

The GC/MS chromatogram (Figure 1) and Table (1) show that 14 compounds have been separated and their structures were elucidated (Figure 2). The molecular formula and the ratio of each compound in the volatile extract are listed and arranged according to their retention time.

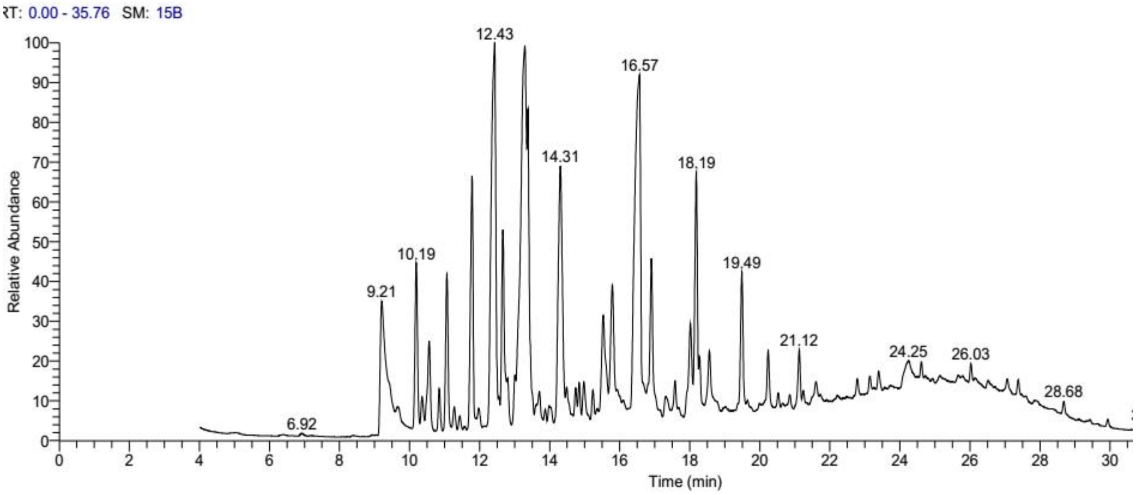

Figure (1): GC/MS analysis of thyme extract.

Table (1): Main components of thyme extract identified by GC/MS.

No	Retention time (min.)	Compound Name	Molecular formula	Peak Area %
1	9.2	Thymol	$C_{10}H_{14}O$	3.32
2	10.19	alpha copaene	$C_{15}H_{24}$	3.86
3	10.56	1-methyl-2,4-di(prop-1-en-2-yl)-1-vinylcyclohexane	$C_{15}H_{24}$	2.20
4	10.85	1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-1H-cyclopropa[e]azulene	$C_{15}H_{24}$	0.88
5	12.43	(1E,6E)-8-isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	$C_{15}H_{24}$	14.1
6	12.8	1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(prop-1-en-2-yl) naphthalene	$C_{15}H_{24}$	0.61
7	13.3	1,2,3,4,4a,7,8,8a-octahydro-4-isopropyl-1,6-dimethylnaphthalen-1-ol	$C_{15}H_{26}O$	10.05
8	13.39	3-methyl-2,6-di(prop-1-en-2-yl)-3-vinylcyclohexanol	$C_{15}H_{26}O$	3.75
9	14.31	2,3,4,6,7,8-hexahydro-8-isopropyl-2,5-dimethylnaphthalen-2-ol	$C_{15}H_{26}O$	7.94
10	15.53	1,2,3,4,4a,7,8,8a-octahydro-4-isopropyl-1,1,6-trimethylnaphthalene	$C_{15}H_{26}O$	2.88
11	16.57	6-isopropyl-3-methyl-2-(prop-1-en-2-yl)-3-vinylcyclohexanol	$C_{15}H_{26}O$	15.6
12	18.28	1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-1H-cyclopropa[a]naphthalen-3-yl 3-methylbutanoate	$C_{20}H_{32}O_2$	0.75
13	18.56	hexadecahydro-10,13-dimethyl-2-methylene-17-(6-methylheptan-2-yl)-1H-cyclopenta[a]phenanthren-3-ol	$C_{28}H_{48}O$	1.17
14	20.24	1,2,3,5,6,7,8,8a-octahydro-4,8a-dimethyl-6-(prop-1-en-2-yl) naphthalen-2-ol	$C_{15}H_{24}O$	1.21

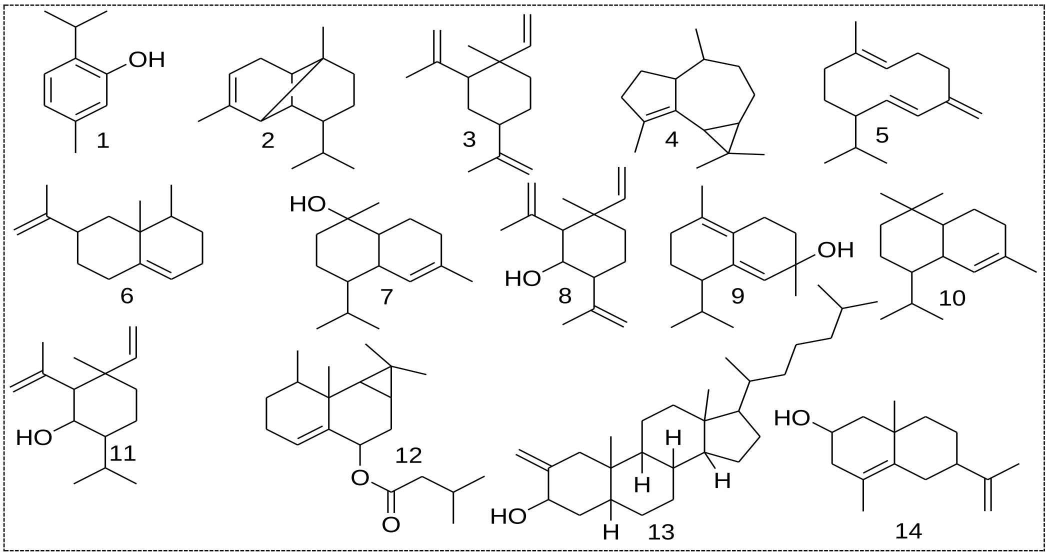

Figure (2): Molecular structures of thyme extracted volatile oil compounds separated by GC/MS.

The natural terpenoid group, whether monoterpenes, sesquiterpenes, diterpenes or triterpenes, is considered one of the effective groups in the field of pest control in general (Kakouri *et al*., 2022). Monoterpenes, which contain ten carbon atoms (C10) in their structural structure, have an important role in many research papers that prove the effectiveness of this group as pesticides for many pests like *Aphis gossypii* Glover (Hemiptera, Aphididae) (Smith *et al*., 2018).

2. Thymol ^{1}H-NMR:

Thymol was isolated as a faint yellow liquid material after steam distillation processing. The ^{1}H-NMR spectrum (Figure 3), (Table 2) was characteristic of the thymol monoterpene compound (Reyhani *et al*., 2022). Two methyl groups at δ 1.39 ppm (6H, s, Me-9,10), δ 2.41 ppm (3H, s, Me-4) in addition to δ 3.33 ppm (H, s, H-8) and a broad singlet signal at δ 4.72 ppm for the hydroxy group; in complete agreement with the proposed structure.

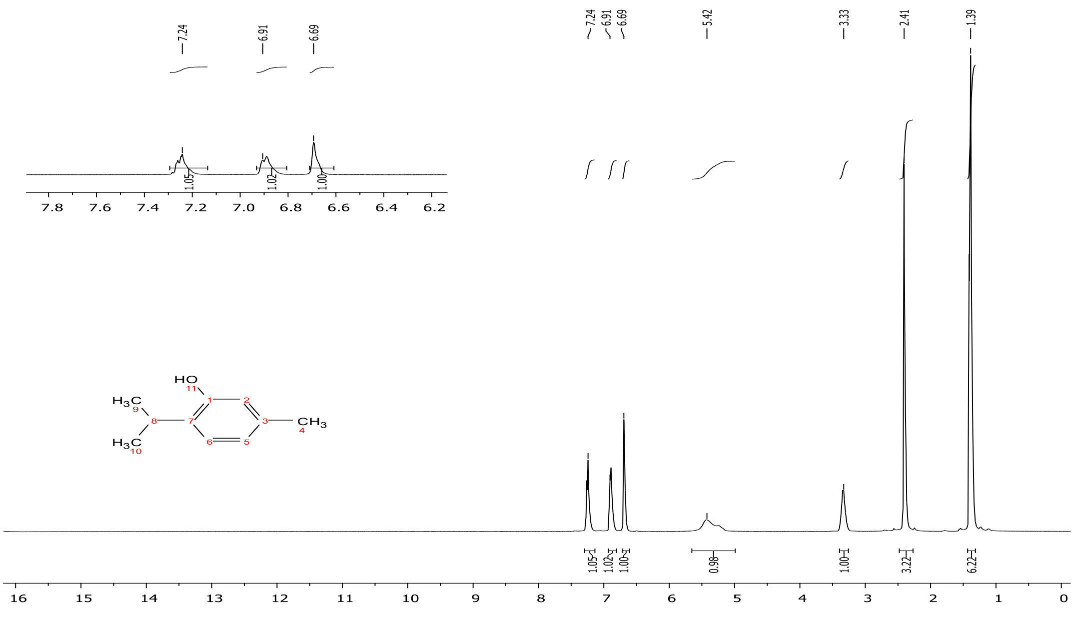

Figure (3): The ^{1}H-NMR Spectrum of the isolated thymol.

Table (2): ^{1}H-NMR of thymol.

H-atom	δ value, ppm	Multiplicity (J, Hz)
2-CH_3	1.39	6H, d (J= 8.5)
CH_3	2.41	3H, s
H	3.33	1H, multiplet
H	6.69	1H, s
H	6.91	1H, d (J=8)
H	7.24	1H, d (J=8)
-OH	5.42	broad, s

3. Field application via thyme extract poisonous bait:

Field assessment experimentation is an applicable actual technique for testing accuracy and the capacity for any potential toxicant, including natural extracts, to be well considered in open field conditions. Data in Table (3) revealed that neomyl was the most operative substance for reducing the population density of *M. cartusiana*, followed by thyme extract. Data revealed that, after the first three days of treatment, the percentages of snail reduction were 43.73 and 35.87 for neomyl and thyme extract, respectively. The residual effects of these compounds were 75.76 and 66.58 reductions, consequently with averages of 59.74% and 51.22% reductions for neomyl and thyme extract, respectively.

The aspect of the open-field application of any materials under investigation takes great importance over ordinary laboratory assessments, especially in the field of pest control (Helmy *et al*., 2022). Many land snails were recorded as damaging invasive pests: *M. cartusiana*, *E. vermiculata*, and *S. putris*. With the gradual population increasing from winter to reach the highest density in the spring season, the control process through field application tests is the true choice to eliminate these different invasive gastropods (Bayoumi *et al*., 2023).

Perhaps the coming days will bring a lot of research that aims to make natural compounds a basic reference and a valid alternative to synthetic compounds and traditional pesticides, reducing the severity of these compounds on the environment on the one hand and the economic side in terms of the relatively high cost on the other hand.

The present proposed study draws attention to potential molluscicides using renewable plant extracts. The extraction was well characterized, deploying mixed techniques like GC/MS and ^{1}H-NMR. The process of controlling land snails in the field is carried out through the toxic bait technique, through which there is no interference of the potential pesticide with the soil, and thus no pollution of irrigation water occurs. In addition, the baits are presented in plastic sheets and are not sprayed, so there is no air pollution. Practical results under field conditions show significant reductions in the snail population at 51.22% compared to the neomyl at 59.74% (The APC-recommended molluscicide). Our eco-friendly extraction strategy offers a greener approach for the simplistic and smooth production of thyme extract as a promising acceptable alternative to the traditional synthetic but costly pesticides, thus paving an approach for the control of *M. cartusiana* and good signs attempts that should be considered in developing strategies for other plant extracts.

Table (3): Mean values ± SE and diminution% for *Monacha cartusiana* snails after the addition of thyme extract and neomyl under field conditions.

Treatment	No. Pre-treatment	Mean (±SE) No. of alive adult gastropods and reduction% after 1, 3, 7, and 14 days										
		1 day		3 days		Initial kil%	7 days		14 days		Residual effect%	Mean of reduction%
	Mean± SE	Mean± SE	Reduction %	Mean± SE	Reduction %		Mean± SE	Reduction%	Mean± SE	Reduction%		
Thyme volatile extract	**44.33± 3.38^{a}**	**34.00± 4.04^{a}**	**23.30**	**23.00± 3.51^{b}**	**48.44**	**35.87**	**19.00±2.52^{b}**	**57.41**	**12.33± 2.96^{b}**	**75.76**	**66.58**	**51.22**
Neomyl	**46.67± 6.89^{a}**	**28.00± 8.02^{a}**	**40.00**	**24.67± 6.89^{b}**	**47.47**	**43.73**	**16.00±3.2^{b}**	**65.93**	**7.33± 3.71^{b}**	**85.59**	**75.76**	**59.74**
Control	**52.00± 5.57^{a}**	**52.00± 3.46^{a}**	—	**52.33± 1.33^{a}**	—	—	**52.33±4.37^{a}**	—	**59.67± 1.20^{a}**	—	—	—
p-value	**0.62**	**0.05**		**0.006**			**0.001**		**0.00**			

***Analysis of variance for means *Monacha* population using one-way ANOVA.**
***Grouping by "Tukey" and 95% confidence.**
***Values of the same column that do not share the same letter (a, b) are significantly different.**
*** —means no result.**

Acknowledgment

I am very appreciative of Dr. Ahmed Abdel-mon'em, Plant Protection Research Institute, Agricultural Research Center for his fruitful discussion, valuable help, and nice conversations.

References

Ali, M. A. (2017): Comparison among the toxicity of thymol and certain pesticides on adults survival and egg hatchability of the glassy clover snail *Monacha cartusiana* (Müller). J. Plant Prot. and Path., Mansoura Univ., 8(4): 189-194.

Ali, M. A.; Abou El Atta, D. A. and Ayyad, M. A. (2023a): Propolis, bee venom and *Beauveria bassiana* toxicity with field application; Controlling the terrestrial gastropod Monacha cartusiana. J. Entomol. Zool. Stud., 11: 43-46. https:// doi.org/10.22271/j. ento. 2023.v11. i1a.9147.

Ali, M. A. and Ayyad, M. A. (2022): Toxicological, histological and field application of *Ricinus communis* methanol extract controlling the black rat *Rattus Rattus* (Rodentia: Muridae) compared to bromodialone.Egypt. J. Plant Prot. Res. Inst. , 5 (4): 341–352.

Ali, M. A.; Doaa, A. and Abou, E. A. (2023b): Molluscicidal activity of thymol and thyme essential oil against two land snail species, *Succinea putris* and *Eobania vermiculata*. International Journal of Agriculture and Plant Science, 5(1):61-66.

Abdel-Rahman, A. H.E. (2020): Usage of some botanical oils to control the land snail Monacha sp.(Gastropoda: Helicidae). Egypt. J. Plant Prot. Res. Inst., 3 (4): 1241-1254.

Ayyad, M. A.; Ali, M. A. ; Helmy, E. T. and Soliman, U. A. (2023): Novel triazole derivatives as potential rodenticides against the Norway rat, R. norvegicus: histology, biochemical alternations, and field application. J. Chemical Papers, **77**(10): 5947-5959. https:// doi.org/10.1007/s11696-023-02912-2.

Bayoumi, S.; Omar, N. ; Mohanna, A. ; Ismail, S.; Abed, M. ; El Sayed, A.; El-Akhrasy, F. and Issa, M. (2023): Survey and population dynamics of land snails at Sharkia Governorate, Egypt. Brazilian Journal of Biology, 84: e271247. Doi: 10.1590/1519-6984.271247.

Cho, E.; Smith-Warner, S. A.; Spiegelman, D. ; Beeson, W. L. ; van den Brandt, P. A. ; Colditz, G. A. ; Folsom, A. R. ; Fraser, G. E. ; Freudenheim, J. L. and Giovannucci, E. (2004): Dairy foods, calcium, and colorectal cancer: A pooled analysis of 10 cohort studies._Journal of the National Cancer Institute, **96** (3): 1015-1022. Doi: 10.1093/jnci/djh185.

Guerra-García, J. M.; Martínez-Pita,I. ; García-García, F. J. and Moreira, J. (2021): Diversity, community structure and habitat use of molluscs in marinas from the Iberian Peninsula and Northern Africa. Ocean Coastal Management, 212: 105795. https://doi.org/10.1016/j.ocecoaman.2021.105795

Helmy, E. T.; Ali, M. A. ; Ayyad, M. A. ; Mohamedbakr, H.; Varma, R. S. and Pan, J. H. (2022): Molluscicidal and biochemical effects of greensynthesized F-doped ZnO nanoparticles against land snail *Monacha cartusiana* under laboratory and field conditions. J. Environmental Pollution, 308: 119691. Doi: 10.1016/j.envpol.2022.119691.

Kakouri, E.; Daferera, D. ; Kanakis, C.; Revelou, P. K. ; Kaparakou, E. H.;Dervisoglou, S.; Perdikis, D. and Tarantilis, P. A. (2022): Origanum majorana essential oil—A Review of its chemical profile and pesticide activity. J Life, **12**(12): 1982. Doi: 10.3390/life12121982.

Reyhani, Y. I.; Taghizadeh, M. ; Saberi, S. F. and Faegheh, S. F. (2022): An evaluation of qH NMR: A complementary approach to GC-FID for quantification of Thymol and trans-Anethole in essential oils and supplements. Journal of Pharmaceutical Biomedical Analysis, 220: 114992. https://doi.org/10.1016/j.jpba.2022.114992

Shahawy, W. A. (2019): Field trials on land gastropods infesting some ornamental plants at Kafr El-Sheikh Governorate. Journal of Plant Protection Pathology, 10 (1): 7-11. DOI: 10.21608/jppp.2019.40553

Smith, G. H.; Roberts, J. M. and Pope, T. W. (2018): Terpene based biopesticides as potential alternatives to synthetic insecticides for control of aphid pests on protected ornamentals. J. Crop. Protection,_110: 125-130. https://doi.org/10.1016/j.cropro.2018.04.011.