

Molluscicidal activity of thymol and thyme essential oil against two land snail species, *Succinea putris* and *Eobania vermiculata*

Mona A Ali, Doaa A Abou El Atta

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

Abstract

The control of land mollusks, one of the most damaging agricultural pests in the world, depends on synthetic molluscicides. Thus, the creation of eco-friendly alternatives to traditional molluscicides is necessary. So, this study aimed to determine the molluscicidal activity of the monoterpene compounds, Thymol and Thyme essential oil against adult's *Succinea putris* (*S. putris*) and *Eobania vermiculata* (*E. vermiculata*) land snails. Contact technique gave better results than did poisonous bait technique. In comparison to *E. vermiculata*, the land snail *S. putris* showed greater sensitivity to all compounds tested. The results exhibited that Agrinate was the most effective one followed by Thymol (the active ingredient), and then Thyme essential oil which had the lowest affect against the adult of *S. putris* and *E. vermiculata* with LC₅₀ (593.30, 1570.51 and 224.14 ppm) and (1577.48, 3344.91 and 873.59 ppm), respectively using contact technique. When thymol, thyme oil, and Agrinate, used as poisonous bait, they have LC₅₀ values of (0.77, 2.29, and 0.31%) and (3.44, 6.31, and 1.36%) for *S. putris* and *E. vermiculata*, respectively. Also, Thymol prevents mold formation and has antifungal activity in poisonous bait. The results showed that using Thymol to control harmful land snails would be as effective as using synthetic molluscicides.

Keywords: Thymol, thyme essential oil, land mollusks, *Succinea putris*, *Eobania vermiculata*, Molluscicidal activity

Introduction

In many parts of the world, herbivorous terrestrial mollusks are serious agricultural pests. Fruits, vegetables, field crops, and ornamental plants suffer economic losses as a result. Territory snails severely destroy plants by damaging their roots, leaves, tree trunks, and fruits (Godan, 1983) [1], (Barker, 2002) [2]. The need to manage these pests' populations is increasing. In numerous regions of Egypt, the two land snails, the amber snail *Succinea putris* (Stylmatophora: Succinidae) (*S. putris*) and the brown garden snail *Eobania vermiculata* (Stylmatophora: Helicidae) (*E. vermiculata*), have been noted as pests that attack different plants (Eshra, 2013) [3]. Synthetic molluscicides are difficult to utilize since they are costly and hazardous to people as well as other non-target living creatures, resulting in serious environmental damage (Bakry *et al.*, 2017), (Osman *et al.*, 2019) [4, 5]. So it is necessary to employ alternate, ethical, and safe pesticides. Molluscicides with a plant source are environmentally better than synthetic products since they are easily available, economical, harmless, biodegradable, and cheap (Mossalem *et al.*, 2013), (Osman *et al.*, 2014) [6, 7]. Pest control options created from plant-derived compounds have been developed. In particular, monoterpenoids have lately been employed as molluscicides (Powell and Bowen 1996) [8] and insecticides (Rice and Coats 1994) [9] for pest control. Thyme (*Thymus vulgaris* L.) is present in *Lamiaceae* species' essential oils and comprises a mixture of monoterpene compounds. The main ingredients of this oil are the naturally occurring terpenoid thymol and its phenol isomer, carvacrol (Nickavar *et al.*, 2005) [10], (Amiri, 2012) [11], both of which have a long history of use in industry and traditional medicine (Patil *et al.*, 2021) [12], (Brendha *et al.*, 2022) [13]. Thymol possesses insecticide, bactericide, acaricide, and fungicide properties due to its phenolic formation (Escobar *et al.*, 2020) [14]. Thyme oil has antioxidant, bactericidal, and antimicrobial effects (oferl *et al.*, 2009) [15]. Additionally,

terpenoids, flavonoid glycosides, phenolic acids, and flavonoid aglycones were found in *Thymus* species (Vila *et al.*, 2002) [16].

Thus, the current study aims to study the molluscicidal activity of the monoterpene compounds thymol and thyme oil as safe natural molluscicide compounds compared with the recommended molluscicide Agrinate against the land snail species, *Succinea putris* and *Eobania vermiculata*.

Materials and methods

1. Tested Compounds

1.1 Thymol (Active ingredient).

White crystalline monoterpene component with aromatic odour.

Thymol was purchased with a purity of 99.5% from the El-Gomhoria company in Mansoura, Egypt.

1.2 Thyme essential oil.

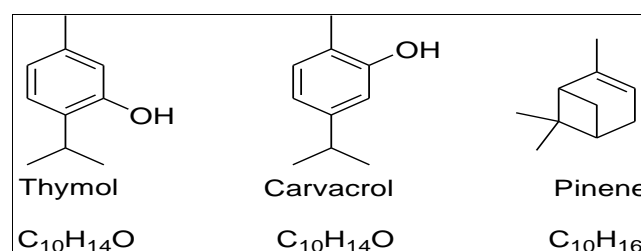
Thyme oil was extracted and obtained through Pure Life Company for Investment and Agricultural Development, Giza, Egypt.

Effective material in Thyme essential oil

Thymol 1.28 - 7.6%

Carvacrol 75 - 81%

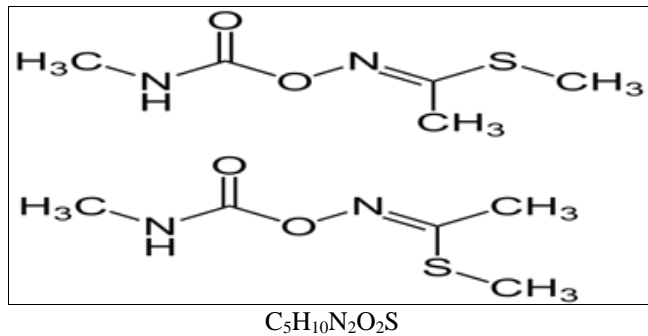
Pinene 11- 22%



Chemical structure of Thymol, Carvacrol and Pinene

1.3 Methomyl

Agrinate 24% SL carbamate recommended molluscicide.
 Chemical name: S-methyl N (methyl carbonyloxy) thioacetamide



Chemical structure of Methomyl

1. Tested snails

In the Mansoura district of the Dakahlia Governorate, adults of the amber snail, *S. putris* and the brown garden snail, *E. vermiculata* both of which are around the same size and age, were taken from contaminated field crops and ornamental plants, respectively. The collected snails were housed in a plastic container with moist soil inside. To acclimatize, fresh lettuce (*Lactuca sativa* L.) leaves were fed to snails every day for 14 days. Only healthy snails were employed in the experiments; dead and sick ones were discarded (Ali *et al.*, 2017) [17].

Molluscicide test

1. Contact technique

Treatments with Thymol, Thyme oil, and Agrinate were made at various concentrations (500, 1000, 2000, and 4000 ppm). On a Petri plate (9 cm in diameter), two mL of each concentration were placed and gently moved in a ring shape (Ascher and Eliyahu, 1981) [18]. Water was evaporated at room temperature, leaving a thin film that contained the tested chemicals. The tested compounds were displayed to

ten adult test snails at various concentrations. The only substance in the control treatment was water. Dead snails were recorded and discarded after 1, 3, 5, and 7 days.

1.2 Poisonous baits technique.

Thymol, Thyme oil, and Agrinate were made at four concentrations (0.5, 1, 2, and 4%). In order to prepare 100 parts of bait, the amounts of each compound were mixed with five parts of sugar cane syrup and completed with wheat bran (El-Okda, 1981) [19]. A bran bait control treatment was made using only bran and sugar cane syrup. Five grammes of each toxic bait were placed on top of sterile, wet sandy loam soil in each box. To keep the snails from escaping, ten adult snails were put in each box and then enclosed completely with muslin clothing netting and fixed with rubber bands. Three replications of the control and each tested substance were performed. The deceased snails were recorded and discarded from the test snails during daily checks. Following treatment, 1, 3, 5, and 7 days later, the mortality percentage was noted.

Statistical analysis

The LC_{50} values were calculated statistically using the method of probit analysis (Finney, 1971) [20], and the mortality percentage was adjusted by Abbott's method (Abbott, 1925) [21].

Results and discussion

1. Mortality percentages of *S. putris* and *E. vermiculata* snails exposed to Thymol, Thyme oil and Agrinate using contact technique

Thymol and Thyme oil were tested in the current study to test their effects on two land snail species, *S. putris* and *E. vermiculata*. According to the data shown in Fig. 1, using the contact technique, Agrinate caused a high mortality rate for *S. putris* and *E. vermiculata*, followed by thymol (the active ingredient), and then thyme essential oil. However, when the concentration of these compounds increased, their toxicity increased.

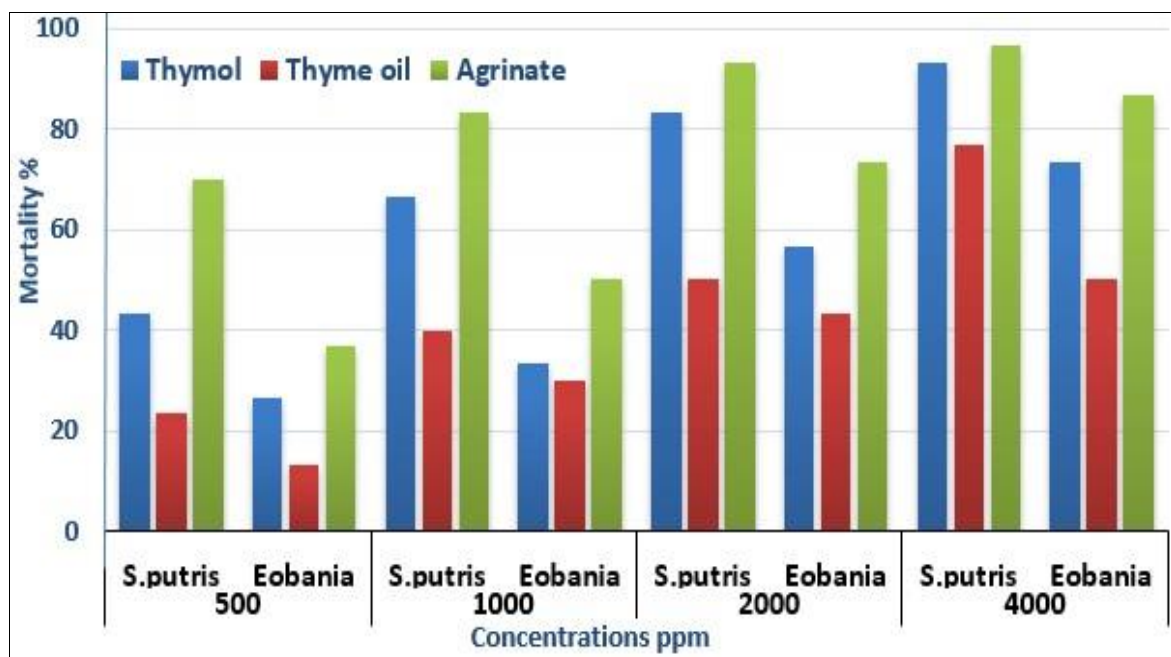


Fig 1: Mortality rates of *S. putris* and *E. vermiculata* snails exposed by contact technique to different concentrations of Thymol, Thyme oil and Agrinate

2. Mortality percentages of *S. putris* and *E. vermiculata* snails exposed to Thymol, Thyme oil and Agrinate using poisonous baits technique:

According to the results shown in Fig. 2, Agrinate caused a high percentage of mortality in two land snail species, *S. putris* and *E. vermiculata*, which was then followed by thymol and finally thyme oil via a poisonous bait technique. When the concentration increased, the toxicity of such compounds also increased. According to Ali (2017) [22], under laboratory conditions, thymol recorded a mortality percentage of 66.66% against *M. cartusiana* adults at 2% poisonous bait. El-Zemity and Radwan (2001) [23] found that several essential oils' main components, such as thymol and

other monoterpenoids including carveol and eugenol, had great molluscicidal activity against the snail species *T. pisana* and *H. aspersa*. Moreover, the results of Mitsue and others, (2022) [24] showed that Terpenecarvacrol at 80 and 100 ppm was 100% lethal to adult aquatic mollusks, *Columella pseudosuccinea*, as were *T. vulgaris* essential oils at 80 ppm. Also, the principal components of the oils used were identified by chromatographic analysis, and the major components of the EO of *T. vulgaris* were carvacrol (23.92%), o-cimeno (22.57%), and thymol (20.23). Agrinate demonstrated high toxicity when total mortality percentages for *E. vermiculata* (86.5%) and *S. putris* (96.5%) were recorded by the leaf dipping technique Ali *et al.* (2017) [17].

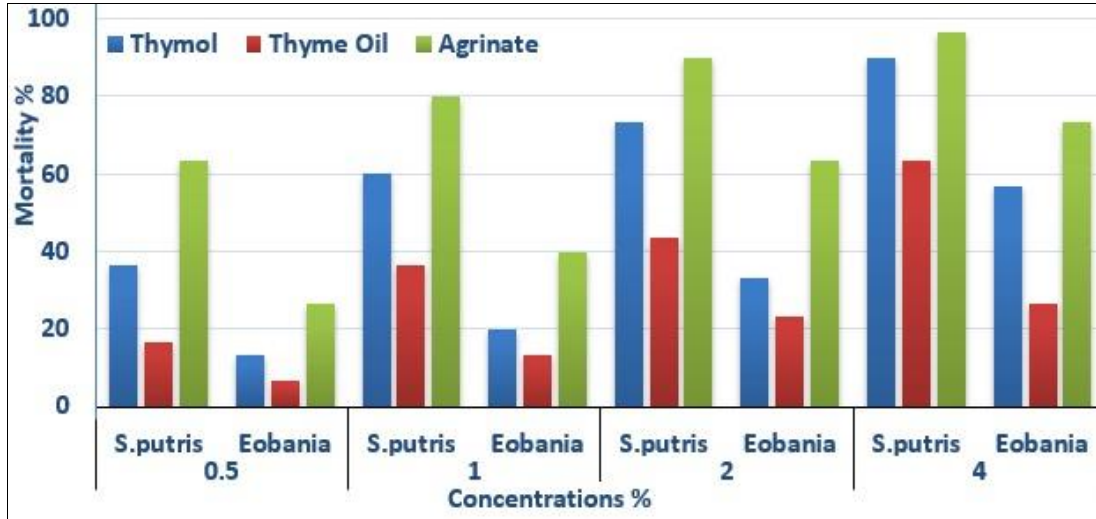


Fig 2: Mortality rates of *S. putris* and *E. vermiculata* snails exposed by poisonous baits technique to different concentrations of Thymol, Thyme oil, and Agrinate

3. Molluscicidal potency of Thymol and Thyme oil against *S. putris*

As shown in Table 1 and Fig. 3 (A and B), Agrinate had greater active toxicity against *S. putris*, followed by thymol and then thyme oil. Using the contact technique, the LC₅₀ and LC₉₀ values for thymol, thyme oil, and Agrinate were (593.30, 1570.51, and 224.14 ppm) and (3303.76, 10921.05, and 1580.55 ppm), respectively. When thymol, thyme oil, and Agrinate, used as bait, they have LC₅₀ values of (0.77, 2.29, and 0.31%) and LC₉₀ values of (4.31, 20.73, and 1.91%), respectively.

Table (1): LC₅₀ and LC₉₀ of Thymol, Thyme oil and Agrinate against *S. putris* land snails when applied in contact and poisonous baits technique

Treatment	Contact (ppm)			Poisonous bait (%)		
	LC ₅₀	LC ₉₀	Slope± S.E.	LC ₅₀	LC ₉₀	Slope± S.E.
Thymol	593.30	3303.76	1.72± 0.42	0.77	4.31	1.72± 0.39
Thyme oil	1570.51	10921.05	1.52± 0.37	2.29	20.73	1.34± 0.37
Agrinate	224.14	1580.55	1.51± 0.49	0.31	1.91	1.62± 0.47

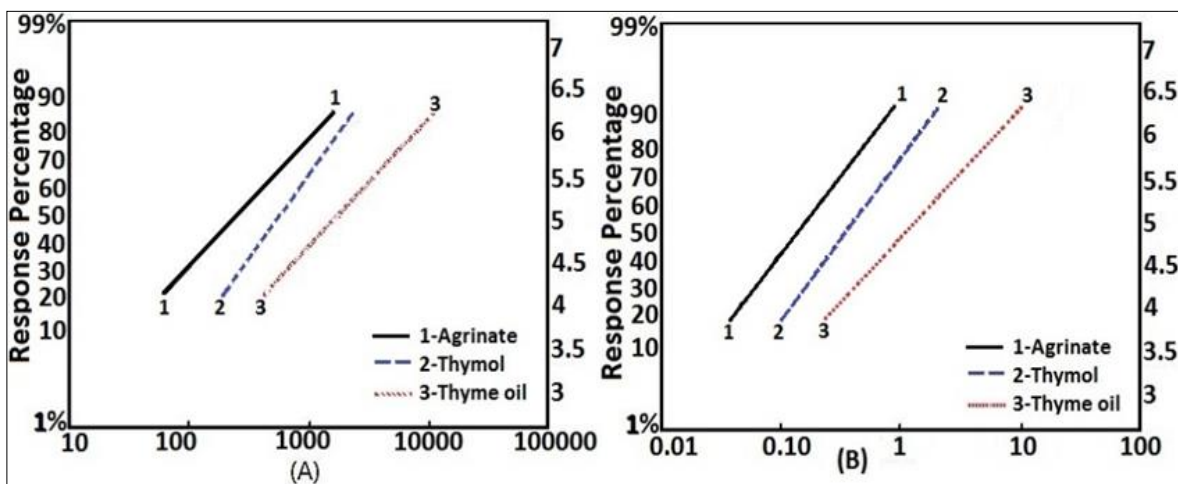


Fig 3: Con/probit regression line of thymol, thyme oil, and Agrinate against *S. putris* using contact (A) and poisonous baits (B) technique.

4. Molluscicidal potency of Thymol and Thyme oil against *E. vermiculata*

As provided in Table (2) and Fig. 4 (A&B) Agrinate was found to be the most active, followed by thymol, while thyme oil was the least active; the LC₅₀ values were (1577.48, 3344.91 and 873.59 ppm), while the LC₉₀ values were (12136.24, 40681.04, and 5247.39 ppm) for thymol, thyme oil, and Agrinate, respectively, using the contact technique. For the bait technique, LC₅₀ values were recorded (3.44, 6.31, and 1.36%) and LC₉₀ values were (26.51, 53.07, and 10.52%) for thymol, thyme oil, and Agrinate, respectively. These results confirmed those of Beltagi *et al.* (2010) [25], who suggested that thymol was the most promising compound, exhibiting molluscicidal potency (LD₅₀ 551.20 µg/snail) against the brown garden snail, *Eobania vermiculata*, when applied topically. The essential oil nanoemulsion's molluscicidal efficacy against *Biomphalaria glabrata* showed an LC₅₀ of 15.04 mg L⁻¹; carvacrol (27.35%) and thymol (24.12%) were the two main components identified in *Thymus vulgaris* L. essential oil (Brendha *et al.*, 2022) [13]. According to Edilene *et al.* (2021) [26], thymol and α-pinene caused concentration-dependent mortality in *B. glabrata* (LC₉₀ 7.11 and 10.34 µg

· mL⁻¹, respectively). Ali, (2017) [22] reported that the most effective one against *M. cartusiana* adults via leaf dipping and poisonous baiting methods was Neomyl, followed by thymol. Also, by using poisonous baits, the LC₅₀ and LC₉₀ were 0.79 and 5.09%, respectively. Thymol and α-pinene inhibit the acetylcholinesterase (AChE) activity of *B. glabrata* at concentrations greater than those utilized in the molluscicide test (Edilene *et al.*, 2021; Jankowska *et al.*, 2017) [26, 27]. Through the suppression of cholinesterase, carbamate molluscicides act as nerve toxins (Young and Wilkins, 1989) [28]. AChE performs an essential function in keep on the physiological processes of mollusks (Kumar *et al.* 2009; Teixeira *et al.* 2012) [29, 30].

Table (2): LC₅₀ and LC₉₀ of Thymol, Thyme oil and Agrinate against *E. vermiculata* land snails when applied in contact and poisonous baits technique

Treatment	Contact (ppm)			Poisonous bait (%)		
	LC ₅₀	LC ₉₀	Slope± S.E.	LC ₅₀	LC ₉₀	Slope± S.E.
Thymol	1577.48	12136.24	1.45± 0.37	3.44	26.51	1.45± 0.39
Thyme oil	3344.91	40681.04	1.18± 0.37	6.31	53.07	1.39± 0.43
Agrinate	873.59	5247.39	1.64± 0.38	1.36	10.52	1.44± 0.37

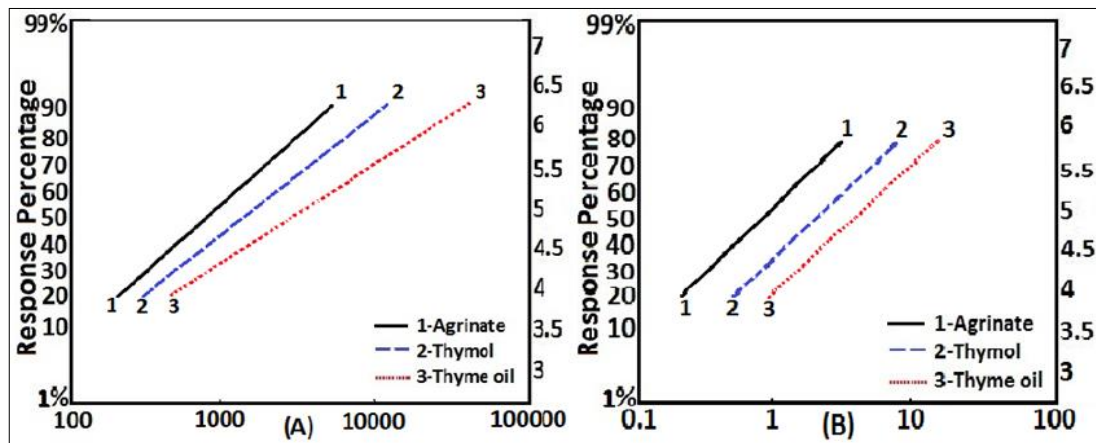


Fig 4: Con/probit regression line of thymol, thyme oil, and Agrinate against *E. vermiculata* using contact (A) and poisonous baits (B) technique

5. Effect of Thymol and Thyme oil on fungal growth on baits

The effect of combining thymol, thyme oil, and Agrinate with wheat bran to form baits is shown in Fig. 5. Photo (A) shows that thymol prevents mold formation and has antifungal activity. Photo (B) shows that the addition of thyme oil to wheat bran changed the status of the bait, resulting in the presence of mold on the bait. Photo (C) indicates that the addition of Agrinate (a recommended molluscicide) did not result in the formation of fungi or mold. Mold can be seen in the control treatment in photo

(D). This result is in agreement with Ferreira *et al.* (2011) [31], who reported that thymol has fungicidal and bactericidal properties. According to Jabeur *et al.*'s (2017) [32] study, *T. vulgaris* essential oil's principal constituents were thymol (76.96%), α-cymene (9.89%), and γ-terpinene (1.92%), which exhibited higher antifungal ability than pure thymol against *M. graminicola*. In the current study, thymol showed a higher antifungal activity than the thyme essential oil used; this may be due to the low percentage of thymol (1.28–7.6%) in the thyme oil components.



Fig 5: Photo showing poisonous baits mixed with (A) Thymol, (B) Thyme oil, (C) Agrinate and (D) control.

Conclusion

The results showed that thymol and thyme oil, as natural monoterpene compounds that are safe to use, have the potential to be used in molluscicide activities against *S. putris* and *E. vermiculata* land snails. Thymol has considerable promise as an eco-friendly alternative to synthetic molluscicides due to its effectiveness against land snails. It also acts as an antifungal when used as a poisonous-baits.

References

- Godan D. Pest Slugs and Snails; Springer: Berlin, Germany, 1983, 445.
- Barker GM. Mollusks as Crop Pests; CABI Publishing: Wallingford, UK, 2002, 441.
- Eshra EH. Survey and distribution of terrestrial snails in fruit orchards and ornamental plants at Alexandria and El-Beheira Governorates, Egypt. Alexandria Science Exchange Journal, 2013: 34:242-248.
- Bakry FA, El-Garhy MF, Abd El-Atti M, Atwa MT. Effects of the extracts of Euphorbia pulcherima and *Atriplex nummularia* on the infectivity of *Schistosoma haematobium* to *Bulinus truncatus* snail. ABM. J, 2017:1(2):30-41.
- Osman GY, Abdel-Azeem HH, El Garhy MF, Al Benasy KS. Sublethal toxicity of miltefosine and artemether to molecular aspect of *Biomphalaria alexandrina* snails. Chem. Bio. Phy. Sci. Sec. B, 2019:9(3):405-413.
- Mossalem HS, Abdel-Hamid H, El-Shinnawy NA. Impact of artemether on some histological and histochemical parameters in *Biomphalaria alexandrina*. African J. Pharm. Pharmacol, 2013:267(31):2220-2230.
- Osman GY, Mohamed AH, Sheir SK, Hassab EL-Nabi SE, Allam SA. Molluscicidal activity of mirazid on *Biomphalaria alexandrina* snails: biological and molecular studies. Int. J. Advan. Res., 2014:2(2):977-989.
- Powell A, Bowen LD, The screening of naturally occurring compounds for use as seed treatment for the protection of winter wheat against slug damage. In slug and snail pests in agriculture (ed. By Henderson, I.F.), British Crop Protection Council, BCPC Monograph, 1996:66:231-236.
- Rice PJ, Coats JR. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidea) and Southern corn rootworm (Coleoptera: Chrysomelidae) J. Ecom. Entomol., 1994:87(5):1172-1179.
- Nickavar B, Mojab F, Dolat-Abadi R, Analysis of the essential oils of two *Thymus* species from Iran," Food Chemistry, 2005:90(4):609-611.
- Amiri H. Essential oils composition and antioxidant properties of three *Thymus* species. Evidence-Based Complementary and Alternative Medicine, 2012. Article ID 728065.
- Patil S, Ramu M, Shirahatti R, Shivamallu PS, Amachawadi RG. A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of *Thymus vulgaris* Linn. *Heliyon*, 2021:7(5):e07054.
- Brendha AS, Patrícia MP, Paulo VR, Maria GL, Francisco NS, Larissa KA, et al. Molluscicidal activity against *Biomphalaria glabrata* of the bioactive nanoemulsion of the essential oil of *Thymus vulgaris* Linn (Thyme). Research, Society and Development, 2022:11(15):e427111536784.
- Escobar A, Miriam P, Gustavo R, Guillermo, Arabian J. Chemistry, 2020:13(12):9243-9269
- Oferl MH, Buchbauer G, Jirovetz L. Correlation of antimicrobial activities of various essential oils and their main aromatic volatile constituents. Journal of Essential Oil Research, 2009:21(5):459-463.
- Vila R. Flavonoids and further polyphenols in the genus *Thymus*. in *Thyme: The Genus Thymus*. Medicinal and Aromatic Plants—Industrial Profiles, E. Stahl-Biskup and F. Saez, Eds., 2002, 75, Taylor and Francis, New York, NY, USA, 2002.
- Ali Mona A, Al-Ghnam Heba A, Abou El Atta Doaa A. Efficiency of certain bio-agents as biological control against two land snail species, *Eobania vermiculata* (Müller) and *Succinea putris* (Linnaeus) under laboratory conditions. J. Plant Prot. and Path., Mansoura Univ., 2017:8(6):265-269.
- Ascher, K, Eliyahu M, The residual contact toxicity of bay sir 8514 to *Spodoptera littoralis* larvae. Phytoparasitica, 1981:9(2):133-138.
- El-Okda MM. Response of two land molluscs to certain insecticides. Bull. Ent. Soc. Egypt Econ. Ser., 1981:12:53-57.
- Finney DJ. Probit analysis. Cambridge univ., London, 1971, 333.
- Abbott WS. A method of computing the effectiveness of an insecticide. J. Econ. Entomol, 1925:18(2):265-267.
- Ali Mona A. 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., 2017:8(4):189-194.
- El-Zemity SR, Radwan MA. Molluscicidal and antifeedant activity of some essential oils and their major chemical constituents against *Theba pisana* snails. Arab Univ. J. Agric. Sci. Ain Shams Univ. Cairo, 2001:9(1):483-493.
- Mitsue I, Natânia CS, Ygor HS, Jankerle NB, Mariana DCI, Barbara RA, Vagner TQ, et al. Chemical composition and effect of essential oils of *Thymus vulgaris* and *Origanum vulgare* on adults and ovigerous masses of *Pseudosuccinea columella*. Research Square, 2022:11:1-14
- Beltagi, SM, Al-Shinnawy SM, Elkattan NR, Nady H. Effects of sublethal doses of selected botanical molluscicides on oxygen consumption of the brown garden snail, *Eobania vermiculata*. Eypg. J. Hosp. Med., 2010:40:402-410.
- Edilene CGR, José ACL, Tássio RSA, Daniella PBS, Samara AB, Gleycka CCG, et al. Everton GGS, Paulo RCF, Alexandra MSS, Ricardo LS, Denise FC. Molluscicidal activity of monoterpenes and their effects on inhibition of acetylcholinesterase activity on *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*. Acta Tropica, 2021:223(11):106089.
- Jankowska M, Rogalska J, Wyszowska J, Stankiewicz M. Molecular Targets for components of essential oils in the insect nervous system-A Review. Molecules, 2017:23:34. <https://doi.org/10.3390/molecules23010034>.

28. Young AG, Wilkins RM. A new technique for accessing the contact toxicity of molluscicides to slugs. *J. Mollu. Stu.*,1989;53:533-536.
29. Kumar P, Singh VK, Singh DK. Kinetics of enzyme inhibition by active molluscicidal agents ferulic acid, umbelliferone, eugenol and limonene in the nervous tissue of snail *Lymnaea acuminata*. *Phytother. Res.*,2009;23:172-177. <https://doi.org/10.1002/ptr.2578>.
30. Teixeira T, Rosa JS, Rainha N, Baptista J, Rodrigues A. Assessment of molluscicidal activity of essential oils from five Azorean plants against *Radix peregra* (Müller, 1774). *Chemosphere*,2012;87:1-6. <https://doi.org/10.1016/j.chemosphere.2011.11.027>.
31. Ferreira P, Geraldo LG, Sthefane D, D'ávila C, Elisabeth C, Bessal dA, The influence of thymol+DMSO on survival, growth and reproduction of *Bradybaena similaris* (Mollusca: Bradybaenidae). *ZOOL*,2011;28(2):145-150.
32. Jabeur MB, Somai JL, Hamada W. Thyme essential oil as an alternative mechanism: biofungicide-causing sensitivity of *Mycosphaerella graminicola*. *J. App. Microb.*,2017;122:932- 939.