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Efficacy of some volatile oils against adults of the white garden snail *Theba pisana* and the brown garden snail *Eobania vermiculata* (Gastropoda: Helicidae) and their effects on carbohydrate hydrolyzing enzyme activity

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Abstract

The objective of this study is to investigate the molluscicide impacts of some volatile oils, such as geranium oil (*Pelargonium graveolens*), French lavender oil (*Lavandula dentate*), and lemon eucalyptus oil (*Corymbia citriodora*), against adults of *Theba pisana* (Müller) and *Eobania vermiculata* (Müller) (Gastropoda: Helicidae) snails at concentrations (7, 14, and 28%). Then, gas chromatography (GC) is a powerful analytical technique utilized to separate, identify, and quantify components in tested volatile oils. Data showed that the maximum effect of mortality percentages was (46.67 and 40.00%) of Lemon eucalyptus oil at the highest concentration after 28 days of *T. pisana* and *E. vermiculata* snails, respectively. Furthermore, the outcomes of biochemical investigations explained that Lemon eucalyptus oil for *T. pisana* snails were reported (-9.69, -9.81 and -8.59%) and (-16.53, -21.37 and -23.53%) of amylase and invertase enzymes, respectively, at the highest concentration, whereas *E. vermiculata* snails were recorded (-9.13, -6.41 and -5.55%) and (-2.10, -2.54 and -2.61%) of the same enzymes, respectively. Finally, data mentioned that the tested oils can be categorized in declining order of their efficiency as follows: *Corymbia citriodora* > *Lavandula dentate* > *Pelargonium graveolens* on *T. pisana* followed by *E. vermiculata*, possibly due to differences in the size of snails.

Introduction

Terrestrial gastropods, including land snails, live in various habitats, ranging from arid to Mediterranean (Schweizer *et al*., 2019). In Egypt, the only class of mollusks that has effectively invaded land and spread widely due to human activity is the gastropod, which primarily occurs in conjunction with the movement of soil and plant matter. Land snail populations have grown in recent years, with numerous species being observed in most Governorates (Abed, 2017, and Khidr *et al*., 2020).

Land snails have been known to infest a wide variety of crops, including field crops, cereals, ornamental plants, oil plant seeds, medicinal fruits (Mango, strawberry, citrus, peach, and palm trees), and vegetables (Carrot, lettuce, and cabbage) (Heiba *et al.*, 2018, and Ibrahim *et al*., 2017) and destroy agricultural products with their bodies, feces, or slime, which lowers their quality and costs money (Ali, 2017).

Regrettably, farmers' careless and chaotic use of pesticides has a negative impact on both human health and the environment. Along with these drawbacks, chemical treatment is a laborious procedure that is not effective enough (Chen *et al.*, 2017). Around the world, mineral oils are seen to be a potential pest control tool against a wide range of pests whereas they are used against the brown garden snail *Eobania vermiculata* (Müller) (Gastropoda: Helicidae) (Farag and Elnagar, 2020) and the white garden snail *Theba pisana* (Müller) (Gastropoda: Helicidae) (Sakla *et al*., 2021).

Also, some plant oils were used against *Monacha cartusiana* (Muller) (Gastropoda: Hygromiidae) snail, (Farag and Sabry, 2017). Nowadays, it is believed that the most practical way to control snails is to use natural molluscicides such as botanical oils (Abd El-Rahman, 2020). Botanical pesticides, which are natural plant derivatives, provide an alternative way to reduce the use of chemical insecticides and could increase the number of environmentally friendly pest control products available (Edyta *et al.*, 2018). Consequently, this study is to investigate the effects of some volatile oils as substitute pesticides against the adults of the white garden snail *T. pisana* and the brown garden snail *E. vermiculata*.

Materials and methods

1. Materials:

1.1. Tested land snail:

Adult land snails, white garden snails (*T. pisana*), and brown garden snails (*E. vermiculata*) were gathered from an orchard in Menia El-Kamh district, Sharkia Governorate, Egypt, that was planted with navel oranges (*Citrus sinensis* L.). The snails were promptly taken to the lab in white cloth bags after being gathered. A glass terrarium filled with damp clay soil that was adjusted to 75% of the water field capacity was used to house healthy and comparable snails. For two weeks prior to treatment, the snails were fed lettuce leaves every day to help them acclimate.

1.2. Tested volatile oil:

Geranium oil (*Pelargonium graveolens*), French lavender oil (*Lavandula dentate*), and lemon eucalyptus oil (*Corymbia citriodora*), hydro-distilled volatile oils used in this study, were supplied by the Medicinal and Aromatic Plants Research Department Laboratory, Horticulture Research Institute (HRI), Agricultural Research Center (ARC).

2. Methods:

2.1. Gas chromatography analysis (GC) of volatile oils:

The GC analysis of the volatile oils was carried out utilizing the gas chromatography equipment that met the following requirements: A flame ionization detector is included with the DsChrom 6200 Gas Chromatograph. Column: BPX-5, polysilkphenylenesiloxane 30m × 0.25mm ID × 0.25µm film, 5% phenyl (Equiv.) 1µl is the sample size. The temperature program ramp increases from 70 to 200 ℃ at a rate of 10 ℃ /min. The detector temperature (FID) is set at 280 °C. The carrier gas is nitrogen, and the flow rates are N_2 (30 ml/min), H_2 (30 ml/min), and air (300 ml/min). The main components of the volatile oils were identified by contrasting their retention durations with those of the actual samples injected under the same conditions. Each compound's relative fraction was calculated using the area of the peak that matched it (Farag and Sakla, 2019). Gas chromatography analysis was carried out in

the Medicinal and Aromatic Plants Research Department Laboratory of the Horticulture Research Institute.

2.2. Spraying technique of tested snails:

Geranium, French lavender, and lemon eucalyptus oils were among the essential oils that were bio-tested against *T. pisana* and *E. vermiculata* using three concentrations (7, 14, and 28%) selected from preliminary testing. 28 ml of each item was combined with 71.75 ml of sterile water and 0.25 ml Tween 80 of 28% to create concentrations, which were subsequently diluted to reach 14% and 7% for all tested oils. Tested oils were sprayed on the lettuce leaves utilizing a spraying technique. In plastic boxes with a capacity of 3/4 kg, 15 healthy snails were employed for each of the three replications of each concentration. Cups were covered with muslin cloth and secured with rubber bands to prevent snails from escaping. Following the next two days of exposure, untreated leaves were added every day for a total of 28 days. Snails were tapped with a stainless-steel needle at regular intervals of 24 hrs. following treatment to track their death. As soon as the dead snails were counted, they were removed. Mortality rates were noted following 1, 3, 7, 14, 21, and 28 days of therapy. For the control test, fresh lettuce leaves were put in a water suspension devoid of compounds (Abd El-Rahman, 2020).

2.3. Biochemical studies:

2.3.1. Sample preparation:

After the mature mollusks' shells were removed, the soft tissues of the *T. pisana* and *E. vermiculata* snails were collected to evaluate the activity of the digestive enzymes amylase and invertase. The tissues were homogenized in distilled water at a 1:10 (w/v) ratio after being weighed. After that, the homogenates were centrifuged for 20 minutes at 5°C at 5000 rpm. To evaluate the activity of the amylase and invertase enzymes, the resultant supernatants were immediately examined (Mohammed and Elshewy, 2016).

2.4. Statistical analysis:

The standard deviation (SD) and mean values for the results were displayed. Students't-test ($P < 0.05$) was used to detect statistical changes after a one-way analysis of variance (ANOVA) (Mishra *et al.*, 2019).

Results and discussion

1. Gas chromatography analysis (GC) of the tested volatile oils:

The primary components and percentages of the assessed essential oils are displayed in Table (1) and Figures (1,2, and 3) after a gas chromatography examination. The main components of geranium oil are citronellol (34.49%), geraniol (15.52%), linalool (11.20%), and citronellyl format (6.87%) (Figure 1). However, 1,8-cineole (44.53%) and linalool (24.98%) were the primary components of French lavender oil (Figure 2). Lastly, the primary components of lemon eucalyptus oil were citronellal (59.00%), citronellol (12.42%), and isopulegol (10.97%). (Figure 3), according to earlier studies, citronellol, makes up the largest portion of geranium essential oil. Geraniol was the second main ingredient, according to data from Iancu *et al.* (2017) and Szutt *et al.* (2019). On the other hand, Antar *et al.* (2024) and Martins *et al.* (2019) investigated the constituents of various French lavender oil hybrids. Finally, Mohareb *et al.* (2025) reported that citronellal, citronellol, and isopulegol were identified as the primary constituents of lemon eucalyptus oil.

2. Impact of some volatile oils against adults of tested land snails:

Results presented in Tables (2 and 3) reported that mortality percentages were (13.33 and 6.67%), (20.00 and 13.33%) and (33.33 and 20.00%) of geranium oil (13.33 and 13.33%), (26.67 and 20.00%) and (40.00 and 33.33%) of French lavender oil, (20.00 and 13.33%), (33.33 and 26.67%) and (46.67 and 40.00%) of lemon eucalyptus oil at

concentrations (7,14 and 28 %) after 28 days of *T. pisana* and *E. vermiculata* snails, respectively, compared control. Additionally, the data demonstrated a strong correlation between the quantities in the tested snails examined and the passage of time. These data are consistent with advanced studies showing that the volatile oil of *Eryngium triquetrum* has molluscicidal activity against the snail, *Biomphalaria glabrata* (Say) (Gastropoda: Planorbidae) (De Carvalho Augusto *et al*., 2020). Tiwari (2023) reported the molluscicidal impact of *Syzygium aromaticum* volatile oil against *Lymnaea acuminata* Lamarck (Gastropoda: Lymnaeidae) snail. Farag *et al.* (2024) studied the synthesis of maltose polyester from waste sunflower oil as a molluscicidal against *E. vermiculata* snails.

Changes in amylase and invertase enzymes in adults of *T. pisana* and *E. vermiculata* snails after exposure to some volatile oils: The amylase and invertase enzymes activity variations in adults of *T. pisana* and *E. vermiculata* snails.

Table (1): Gas chromatography analysis for some volatile oils used in the current study.

Geranium oil			French lavender oil			Lemon eucalyptus oil		
No.	**Component**	**%**	**No.**	**Component**	**%**	**No.**	**Component**	**%**
1	α-pinene	0.83	1	α-pinene	4.43	1	α-pinene	1.77
2	Myrcene	0.29	2	Camphene	1.53	2	Sabinene	2.54
3	β-pinene	2.15	3	Sabinene	4.36	3	β-pinene	1.35
4	P-Cymene	1.13	4	P-Cymene	1.31	4	P-Cymene	1.35
5	Limonene	1.27	5	Limonene	1.98	5	1,8-Cineole	1.49
6	α-terpineol	0.73	6	1,8-Cineole	44.53	6	Citronellal	59.00
7	Isomenthone	5.54	7	Linalool oxide	1.06	7	Isopulegol	10.97
8	Linalool	11.20	8	Linalool	24.98	8	Borneol	2.31
9	Citronellyl formate	6.87	9	Camphor	1.84	9	Menthol	2.45
10	Geranyl formate	7.09	10	Lavandulol	1.57	10	Terpineol	0.76
11	Citronellol	34.49	11	Borneol	4.34	11	Citronellol	12.42
12	Citronellal	0.81	12	γ-Terpineol	4.28	12	Citronellyl acetate	0.78
13	Geraniol	15.52	13	Hexenyl butanoate	0.53	13	Caryophyllene oxide	2.82
14	Citronellyl butyrate	0.88	14	Linalyl acetate	0.87		Total	100
15	Geranyl butyrate	0.39	15	Lavandulyl acetate	0.05			
16	Eugenol	6.32	16	Thymol	1.43			
17	β-Caryophyllene	1.16	17	Eugenol	0.86			
18	Cis-rose oxide	3.35	18	β-farncene	0.21			
	Total	100		Total	100			

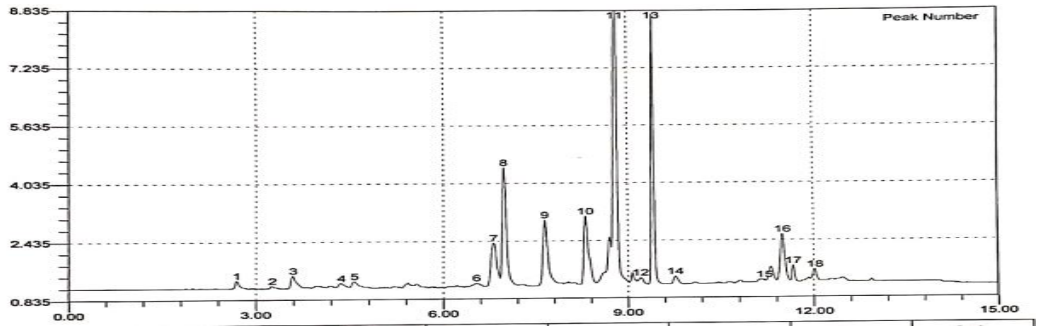


Figure (1): Gas chromatography chromatogram for geranium oil used in the current study.

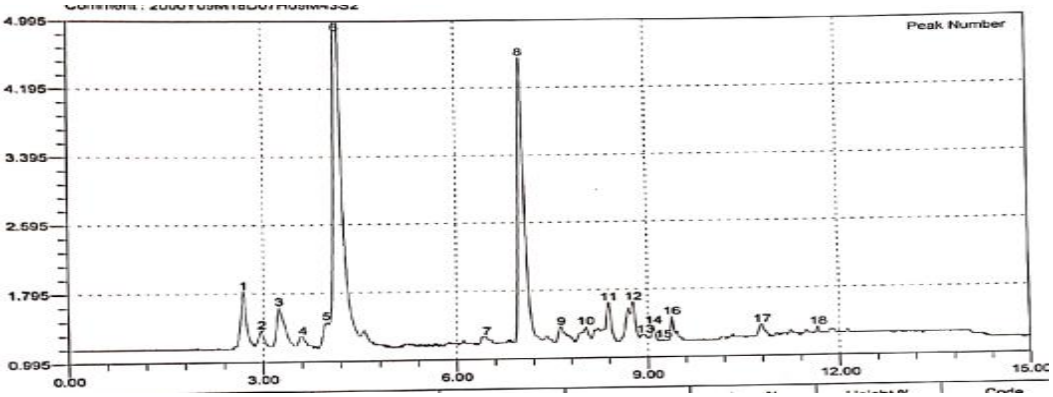


Figure (2): Gas chromatography chromatogram for French lavender oil used in the current study.

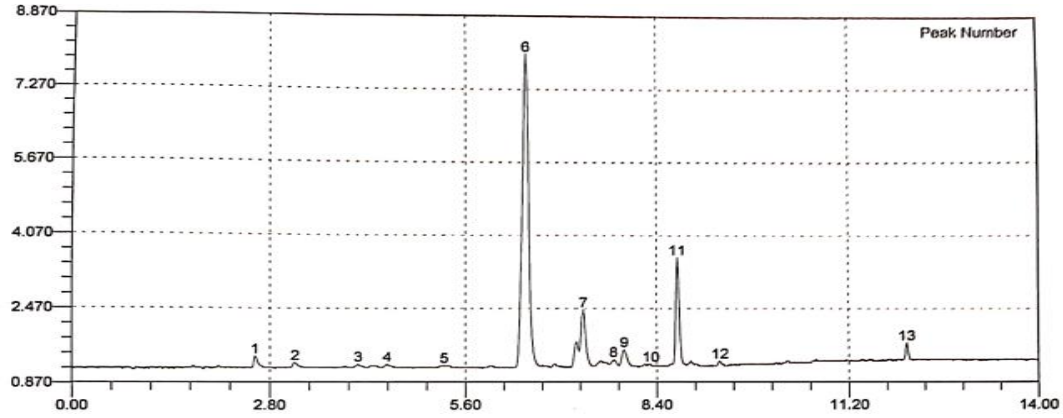


Figure (3): Gas chromatography chromatogram for Lemon eucalyptus oil used in the current study.

Table (2): Impact of some volatile oils against adults of the white garden snail, *Theba pisana*.

Oils	Conc.	1	3	7	14	21	28
Geranium oil	7%	0.00^{b}	0.00^{e}	6.67^{ef}	6.67^{fg}	13.33^{e}	13.33^{e}
	14%	0.00^{b}	6.67^{de}	13.33^{de}	13.33^{ef}	20.00^{de}	20.00^{de}
	28%	0.00^{b}	13.33^{cd}	26.67^{bc}	33.33^{bc}	33.33^{bc}	33.33^{bc}
French lavender oil	7%	0.00^{b}	6.67^{de}	13.33^{de}	13.33^{ef}	13.33^{e}	13.33^{e}
	14%	0.00^{b}	13.33^{cd}	20.00^{cd}	26.67^{cd}	26.67^{cd}	26.67^{cd}
	28%	0.00^{b}	26.67^{ab}	33.33^{ab}	40.00^{ab}	40.00^{ab}	40.00^{ab}
Lemon eucalyptus oil	7%	0.00^{b}	13.33^{cd}	20.00^{cd}	20.00^{de}	20.00^{de}	20.00^{de}
	14%	0.00^{b}	20.00^{bc}	26.67^{bc}	33.33^{bc}	33.33^{bc}	33.33^{bc}
	28%	6.67^{a}	33.33^{a}	40.00^{a}	46.67^{a}	46.67^{a}	46.67^{a}
Control		0.00^{b}	0.00^{e}	0.00^{f}	0.00^{g}	0.00^{f}	0.00^{f}
P		0.0195^{*}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}
L.S.D.$_{0.05}$		3.59	10.15	10.75	10.76	10.77	10.77

Table (3): Impact of some volatile oils against adults of the brown garden snail *Eobania vermiculata*.

Oils	Conc.	1	3	7	14	21	28
Geranium oil	7%	0.00^{b}	0.00^{d}	0.00^{d}	6.67^{de}	6.67^{ef}	6.67^{ef}
	14%	0.00^{b}	6.67^{cd}	13.33^{bc}	13.33^{cd}	13.33^{de}	13.33^{de}
	28%	0.00^{b}	13.33^{bc}	20.00^{ab}	20.00^{bc}	20.00^{cd}	20.00^{cd}
French lavender oil	7%	0.00^{b}	0.00^{d}	6.67^{cd}	13.33^{cd}	13.33^{de}	13.33^{de}
	14%	0.00^{b}	6.67^{cd}	13.33^{bc}	20.00^{bc}	20.00^{cd}	20.00^{cd}
	28%	0.00^{b}	20.00^{ab}	20.00^{ab}	33.33^{a}	33.33^{ab}	33.33^{ab}
Lemon eucalyptus oil	7%	0.00^{b}	6.67^{cd}	13.33^{bc}	13.33^{cd}	13.33^{de}	13.33^{de}
	14%	0.00^{b}	20.00^{ab}	20.00^{ab}	26.67^{ab}	26.67^{bc}	26.67^{bc}
	28%	6.67^{a}	26.67^{a}	26.67^{a}	33.33^{a}	40.00^{a}	40.00^{a}
Control		0.00^{b}	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{f}	0.00^{f}
P		0.0195^{*}	0.0001^{***}	0.0002^{***}	0.0001^{***}	0.0001^{***}	0.0001^{***}
L.S.D.$_{0.05}$		3.59	9.50	10.16	10.76	10.77	10.77

Treated with some volatile oils at three doses (7, 14, and 28%) compared to the control displayed in Tables (4 and 5). The highest concentration of French lavender oil for *T. pisana* snails was found to be (-9.19, -8.83, and -7.92%) and (-2.40, -10.86, and -12.87%) of amylase and invertase enzymes, respectively, while the highest concentration of lemon eucalyptus oil was (-9.69, -9.81, and -8.59%) and (-16.53, -21.37, and -23.53%) of amylase and invertase enzymes, respectively. All treatments resulted in a gradual increase in the activity of the amylase enzyme starting on day one and reaching its maximum level after seven days of treatment. On the other hand, the invertase enzyme's activity gradually decreased after each treatment. Conversely, French lavender oil for *E. vermiculata* snails was found to have amylase and invertase levels of (-3.97, -3.74, and -2.28%) and (-2.70, -2.58 and, -2.65%) at the highest concentration, respectively, while lemon eucalyptus oil for the same snails was found to have (-9.13, -6.41, and -5.55%) and (-2.10, -2.54, and -2.61%), respectively. Finally, because of their different sizes, *T. pisana* snails had greater measured enzyme levels than *E. vermiculata* snails. The findings are in line with the information gathered by Zeng *et al.* (2019), who noted that enzyme bioassays are diagnostic instruments used to evaluate physiological alterations or harm in organisms exposed to contaminants. In many insect species, amylase and invertase are vital digestive enzymes that are necessary for growth, development, and digestion. Mohammed and Elshewy (2016) demonstrated how some plant extracts affected certain biochemical alterations in *E. vermiculata* snails. Farag *et al.* (2024) mentioned the impact of the synthesis of maltose polyester from waste sunflower oil on amylase and invertase enzymes of *E. vermiculata* snail. The study examined the potential for lemon eucalyptus oil to raise *T. pisana* and *E. vermiculata* snail death rates in comparison to the control; all treatments showed that the tested snails' activity for the enzyme amylase increased while that of the enzyme invertase decreased.

Table (4): Vitality of amylase and invertase enzymes on adults of *T. pisana* snail after exposing to some volatile oils.

Tested volatile oils	Conc.		Amylase enzyme			Invertase enzyme		
			One day	Three days	One week	One day	Three days	One week
French lavender oil	7%	SA	70.12^{b}	70.24^{b}	72.63^{a}	93.48$^{a b}$	91.52^{b}	90.89^{b}
		RA%	-4.01	-4.38	-0.48	-0.26	-0.93	-2.54
	14%	SA	68.58^{d}	69.39^{c}	70.44^{c}	93.21^{b}	90.76^{c}	90.68^{b}
		RA%	-6.12	-5.54	-3.48	-0.54	-1.75	-2.77
	28%	SA	66.34^{f}	66.97^{e}	67.20^{e}	91.47^{c}	82.35^{e}	81.26^{d}
		RA%	-9.19	-8.83	-7.92	-2.40	-10.86	-12.87
Lemon eucalyptus oil	7%	SA	69.82^{c}	70.13^{b}	71.04^{b}	89.31^{d}	85.44^{d}	84.76^{c}
		RA%	-4.42	-4.53	-2.66	-4.71	-7.51	-9.11
	14%	SA	67.98^{e}	68.35^{d}	69.58^{d}	87.49^{e}	81.68^{f}	80.57^{e}
		RA%	-6.94	-6.96	-4.66	-6.65	-11.58	-13.61
	28%	SA	65.97^{g}	66.25^{f}	66.71^{f}	78.22^{f}	72.64^{g}	71.32^{f}
		RA%	-9.69	-9.81	-8.59	-16.53	-21.37	-23.53
Control	___	SA	73.05^{a}	73.46^{a}	72.98^{a}	93.72^{a}	92.38^{a}	93.26^{a}
P			0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***
L.S.D.$_{0.05}$			0.21	0.27	0.35	0.31	0.39	0.30

***SA = Specific activity as (µg /ml) *RA% = (Relative activity %) = [(Treatment – Control) / Control] × 100**

Table (5): Vitality of amylase and invertase enzymes on adults of *E. vermiculata* snail after exposing to some volatile oils.

Tested volatile oils	Conc.		Amylase enzyme			Invertase enzyme		
			One day	Three days	One week	One day	Three days	One week
French lavender oil	7%	SA	98.07^{b}	98.44^{c}	98.13$^{a b}$	125.11^{b}	124.86^{b}	122.48^{d}
		RA%	-1.58	-1.42	-0.08	-1.01	-0.72	-2.09
	14%	SA	97.56^{c}	98.16^{d}	97.05^{c}	124.91^{b}	123.37^{c}	122.82^{c}
		RA%	-2.09	-1.70	-1.18	-1.17	-1.91	-1.81
	28%	SA	95.68^{d}	96.13^{e}	95.97^{d}	122.98^{d}	122.53^{d}	121.78^{e}
		RA%	-3.97	-3.74	-2.28	-2.70	-2.58	-2.65
Lemon eucalyptus oil	7%	SA	97.38^{c}	99.14^{b}	98.01^{b}	124.87^{b}	123.42^{c}	123.12^{b}
		RA%	-2.27	-0.72	-0.20	-1.20	-1.87	-1.57
	14%	SA	91.82^{e}	94.17^{f}	93.57^{e}	123.65^{c}	123.14^{c}	122.61$^{c d}$
		RA%	-7.85	-5.72	-4.78	-2.17	-2.09	-1.98
	28%	SA	90.55^{f}	93.46^{g}	92.76^{f}	123.74^{c}	122.58^{d}	121.82^{e}
		RA%	-9.13	-6.41	-5.55	-2.10	-2.54	-2.61
Control	___	SA	99.64^{a}	99.86^{a}	98.21^{a}	126.39^{a}	125.77^{a}	125.09^{a}
P			0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***
L.S.D.$_{0.05}$			0.37	0.23	0.20	0.26	0.32	0.25

***SA = Specific activity as (µg /ml) *RA% = (Relative activity %) = [(Treatment – Control) / Control] × 100**

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