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Impact of Waste and Fresh Sunflower and Soybean Oils Mixture on some Land Snail's Eggs and Effects on Aminotransferase Enzymes Activity

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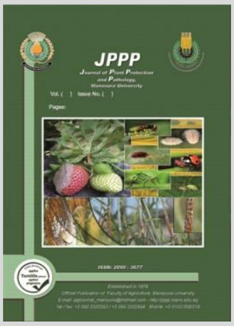

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ABSTRACT

This study aims to assess the molluscicide effect of a mixture of waste and fresh sunflower and soybean oils on eggs of land snails Eobania vermiculata, Theba pisana, and Monacha cartusiana. Then, a potent analytical method for separating, identifying, and quantifying the constituents of an oil mixture is gas chromatography (GC). Results showed that the maximum reduction in hatching percentages was 53.33% and 46.67% for E. vermiculata in a mixture of fresh sunflower and soybean oils (FO) and waste oils (WO), respectively, while the lowest was 63.33% and 60.00% for M. cartusiana, respectively. Furthermore, the outcomes of biochemical investigations demonstrated that the highest concentrations of (FO) for M. cartusiana eggs were reported (61.91 and 147.27%) and (127.34 and 196.25%) of AST and ALT, respectively. In contrast, WO recorded (95.42 and 188.82%) and (154.93 & 221.40%) of AST and ALT, respectively. FO for E. vermiculata eggs was recorded at (76.19 and 178.23%) and (93.64 and 187.84%) of AST and ALT, respectively, whereas WO (85.60 and 200.72%) and (110.57 and 216.41%) of AST and ALT, respectively. Finally, the results revealed that the tested compounds can be categorized in declining order of their efficiency as follows: WO > FO on E. vermiculata, followed by T. pisana and M. cartusiana.

Keywords: Sunflower – Soybean – Egg – Oil – Snail Enzyme.

INTRODUCTION

Land snails such as *M. cartusiana*, *E. vermiculata*, and *T. pisana* eat a variety of plants, including cereals, vegetables, fruit, herbs, and several ornamentals. Also, snails can also spread a variety of plant illnesses, and the mucus trails they leave behind can contaminate cereals, fruits, vegetables, and all plant components Emara *et al.* (2024). Unfortunately, pesticide poisoning has long been considered a serious public health issue (Boedeker *et al.*, 2020). So, attention is increasingly being paid to the utilization of plant extracts such as oils (fresh and expired) as molluscicide to control of land snails. The application of plant oils as a natural strategy to control the destruction effect of land snails is gaining significant attention. Which, an attempt to develop alternatives to traditional pesticides, natural chemicals extracted from plants have attracted considerable interest as potentially beneficial bioactive compounds Farag and Sabry (2017). During the frying process, edible oils like sunflower and soybean undergo various transformations and they go through many changes. These alterations render the oil unsafe for human consumption. They include oxidation, polymerization, and the generation of hazardous compounds. There is movement to utilize these materials as valuable resources, as they represent a severe risk to human health and the environment (Doğan, 2016). Because soybean oil has a high content of polyunsaturated fatty acids, its oxidative stability index is lower (Sayyad *et al.* 2017). One of the key considerations when choosing the best kinds of cooking oils and cooking techniques is the oxidative stability of vegetable oils Yee *et al.* (2018). So, this study aims to evaluate the effect of mixture of sunflower and soybean oils FO and WO against eggs of *M. cartusiana*, *E. vermiculata* and *T. pisana* snails.

MATERIALS AND METHODS

Materials

Assessed land snails: *E. vermiculata* obtained from a sago palm plantation in Banishebl, Zagazig region, Sharkia Governorate, Egypt. *T. pisana* collected from an orchard farmed with navel orange in Menia El-Kamh district, Sharkia Governorate, Egypt. Finally, *M. cartusiana* obtained from Egyptian clover fields in the Sheeba locale, Zagazig district, Sharkia Governorate, Egypt, A glass terrarium filled with damp clay soil set to 75% of the water field capacity utilized to host healthy and comparable individuals. Prior to receiving treatment for acclimatization, the snails fed fresh green cabbage leaves every day for two weeks Ismail *et al.* (2010).

Evaluated fixed oils: The first was a mixer of fresh sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* L.) oils combined in a 1:1 ratio (FO), purchased from Afco Misr Ataqa company, Suez city, Suez governorate, Egypt. The second was mixture of waste sunflower and soybean oils combined in a 1:1 ratio (WO) obtained from several restaurants in Giza city, Giza Governorate Egypt. After being filtered and dried over anhydrous sodium sulfate, the oil was kept at 5 °C until analysis.

Methods:

Parameters studied: In accordance with PN-ISO 12966-2 (2017), the fatty acid composition recognized, and additional chemical parameters, such as acid and peroxide values and physical properties like specific gravity and smoke point applied to A.O.A.C. (2016), were calculated for both FO and

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WO samples. The Food Technology Research Institute in the Giza Governorate of Egypt conducted the analyses.

Dipping technique: After placed in a Petri dish and cleaned with distilled water, tested land snail's eggs were ready for the subsequent test. Three concentrations 4.5, 9 and 18% from the tested oils were created by mixing 18 ml of tested oils with 81.75 ml of distilled water and 0.25 ml of crude tween 80 (v/v) then diluted to achieve 9 and 4.5%. For a minute, the tested eggs dipped in each concentration. To fill the field to capacity, sieved clay soil (200g) placed in plastic boxes with 3/4 kg of soil and irrigated to reach the soil moisture to 75 % of field capacity. Ten eggs inserted into tiny holes created by a vitreous needle. For every concentration, three replicates were employed, and distilled water and 0.25 ml of tween 80 were used as control. To keep snail hatchlings from escaping, the boxes securely covered with cotton netting that fastened with rubber bands. According to Ismail *et al.* (2010), boxes examined every day, and the incubation period and hatching percentages noted.

Biochemical studies:

Sample preparation: A weight of 0.2 g. of egg – masses weighted after 3 and 7 days after dipped with tested waste oil at (4.5, 9 and 18%) used for biochemical analysis, in addition, to control and kept at 20°C till the investigation time. A Teflon homogenizer used to blend frozen egg samples in purified water. A thorough assessment of research efforts that focus on AST and ALT detection approaches because of their clinical significance is provided by El-Khayat *et al.* (2018), who states that homogenates supernatant is employed merely for the biochemical examination of the activity of AST and ALT enzymes. Among the detection methods are colorimetric.

Statistical analysis: The results' mean, and standard deviation (SD) values were shown. Following one-way analysis of variance (ANOVA), statistical differences were detected using the student's-test ($P < 0.05$) Mishra *et al.* (2019).

RESULTS AND DISCUSSION

Results

Physico-chemical characteristics of sunflower and soybean oils mixture (FO and WO): One of the best indicators of oil damage is the values of acidity and PV (Table 1). For FO and WO, the corresponding oil acidity and peroxide readings were (0.06 and 6.82%) and (5.97 and 18.63 Meq.O_2/Kg), respectively. Whereas Physical characteristics were (0.723 and 0.864), respectively of specific gravity while, smoke points were (220°C/4min and 190°C/5min), respectively.

Table 1. Physico-chemical properties of mixture of soybean and sunflower oils (FO and WO):

Evaluated oils.	Chemical properties		Physical properties	
	Acidity % (as oleic acid)	Peroxide value (Meq.O_2/Kg)	Specific gravity (25°C)	Smoke point
Fresh oil (FO)	0.06	5.97	0.723	220/4min
Waste oil (WO)	6.82	18.63	0.864	190/5min

GC Analyses of mixture of soybean and sunflower oils (FO and WO): According to Table (2)'s analysis of the fatty acid components of FO and WO oils of high percentages of unsaturated fatty acids such as $C_{18:1}$ (23.90 & 22.87%) and $C_{18:2}$ (52.67 & 51.92%) was present in FO and WO, respectively. However, high percentages of saturated fatty acids, such as $C_{16:0}$ (12.28 & 13.49 %) and $C_{18:0}$ (4.04 & 5.97%), provided for fresh and waste, respectively.

Table 2. Fatty acids composition of sunflower and soybean oils mixture (FO and WO).

Fatty acids	Fresh oil (FO)	Waste oil (WO)
Myristic acid $C_{14:0}$	0.13	0.27
Palmitic acid $C_{16:0}$	12.28	13.49
Palmitoleic acid $C_{16:1}$	0.09	0.11
Margaric acid $C_{17:0}$	0.08	0.10
Myristoleic acid $C_{17:1}$	0.04	0.03
Stearic acid $C_{18:0}$	4.04	5.97
Oleic acid $C_{18:1}$	23.90	22.87
Linoleic acid $C_{18:2}$	52.67	51.91
Linolenic acid $C_{18:3}$	5.27	3.15
Arachidic acid $C_{20:0}$	0.32	0.35
Eicosenic acid $C_{20:1}$	0.15	0.11
Behenic acid $C_{22:0}$	0.34	0.43

Impact of sunflower and soybean oils mixture (FO and WO) on eggs of tested land snails: The three concentrations (4.5, 9 and 18%) of FO and WO oils showed no significant difference from the control during the incubation periods and the hatching percentages for all tested land snails except *T. pisana* snail of the hatching percentages according to results by the dipping technique in Table (3). Data showed that the highest impact of hatching percentages were 53.33 and 46.67 % of *E. vermiculata* for FO and WO respectively where the lowest effect of hatching percentages were 63.33 and 60.00 of *M. cartusiana* for the same tested oils, respectively.

Table 3. Effect of sunflower and soybean oils mixture (fresh FO and WO) on eggs of tested land snails.

Oils	Conc.	No. of eggs	M. cartusiana			T. pisana			E. vermiculata		
			No. of Juveniles	Incubation period(days)	Hatching %	No. of Juveniles	Incubation period (days)	Hatching %	No. of Juveniles	Incubation period (days)	Hatching %
FO	4.5	30	27^{a}	16.67	90	26^{ab}	15.33	86.67^{ab}	25^{a}	18.67	83.33
	9	30	24^{ab}	17.33	80	22^{bc}	16.67	73.33^{cd}	21^{bc}	19.33	70.00
	18	30	19^{c}	18.67	63.33	18^{c}	17.33	60^{d}	16^{de}	21.67	53.33
WO	4.5	30	26^{ab}	17.00	86.67	25^{ab}	15.33	83.33^{bc}	23^{ab}	19.33	76.67
	9	30	22^{bc}	18.33	73.33	20^{bc}	17.00	66.67^{cd}	19^{cd}	20.33	63.33
	18	30	18^{c}	19.67	60	17^{c}	17.67	56.67^{d}	14^{e}	22.33	46.67
Control	____	30	28^{a}	16.33	93.33	28^{a}	14.67	93.33^{a}	26^{a}	17.67	86.67
P			0.0005 ***	0.3488^{ns}	0.1762^{ns}	0.0106*	0.5790^{ns}	0.0095**	0.0001 ***	0.9575^{ns}	0.1384^{ns}
$L.S.D._{0.05}$			4.03	3.31	29.67	6.07	3.92	19.96	3.80	10.32	32.43

Changes in (AST and ALT) enzymes activities in eggs of *M. cartusiana* and *E. vermiculata* snails after exposing to sunflower and soybean oils mixture (FO and WO): Table (4) showed that the changes in the activity of AST and ALT enzymes in eggs of *M. cartusiana* and *E. vermiculata* snails treated FO and WO at three concentrations 4.5, 9, 18% compared with control. All treatments caused a gradual augmentation in the activity of both enzymes for 3 days and reached their maximum level after the seven days of treatment whereas, the highest concentration

Table 4. Vitality of AST and ALT enzymes on eggs of *M. cartusiana* and *E. vermiculata* snails after exposing to sunflower and soybean oils mixture (FO and WO).

Oils	Conc. (%)		*M. cartusiana*				*E. vermiculata*			
			AST μg/ml		ALT μg/ml		AST μg/ml		ALT μg/ml	
			3days	7days	3days	7days	3days	7days	3days	7days
Fresh FO	4.5	SA	8.42ef	13.68d	12.63e	17.29e	10.83d	21.44d	13.05d	22.40e
		RA%	13.32	73.82	57.68	116.40	19.93	120.12	27.69	126.95
	9	SA	10.86cd	17.29c	16.07cd	21.83c	13.89c	25.73b	16.65c	26.92c
		RA%	46.16	119.7	100.62	173.22	53.82	164.17	62.92	172.75
	18	SA	12.03bc	19.46b	18.21b	23.67b	15.91ab	27.10b	19.79b	28.41bc
		RA%	61.91	147.27	127.34	196.25	76.19	178.23	93.64	187.84
Waste WO	4.5	SA	9.62de	17.42c	15.56d	19.34d	11.82d	23.98c	15.23c	24.67d
		RA%	29.48	121.35	94.26	142.05	30.90	146.20	49.02	149.95
	9	SA	12.73b	19.81b	17.47bc	23.15bc	14.51bc	27.03b	18.54b	29.25b
		RA%	71.33	151.72	118.10	189.74	60.69	177.52	81.41	196.35
	18	SA	14.52a	22.73a	20.42a	25.68a	16.76a	29.29a	21.52a	31.23a
		RA%	95.42	188.82	154.93	221.40	85.60	200.72	110.57	216.41
Control	____	SA	7.43f	7.87e	8.01f	7.99f	9.03e	9.74e	10.22e	9.87f
P			0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***	0.0001 ***
L.S.D.0.05			1.58	1.62	1.61	1.63	1.59	1.62	1.61	1.62

SA = Specific activity as (μg /ml) RA% = (Relative activity %) = [(Treatment – Control) / Control] × 100 of FO for *M. cartusiana* snail eggs were recorded (61.91 & 147.27%) and (127.34 & 196.25%) of AST and ALT, respectively whereas WO was recorded (95.42 & 188.82%) and (154.93 & 221.40%) of AST and ALT, respectively. On the other hand, FO for *E. vermiculata* snail eggs recorded (76.19 & 178.23%) and (93.64 & 187.84%) of AST and ALT, respectively while WO recorded (85.60 & 200.72%) and (110.57 & 216.41%) of AST and ALT, respectively.

Discussion

Initially the current study was conducted on sunflower and soybean oils mixture (FO and WO) for their ovicidal activity against on eggs of land snails such as *Eobania vermiculata*, *Theba pisana* and *Monacha cartusiana*. These oils are utilized as nutrition for humans; they are thought to be less dangerous to people than the majority of conventional pesticides. The chemical characteristics of WO oil were the values of acidity (0.06 and 6.82%) and PV (5.97 and 18.63 Meq.O_2/Kg), respectively. WO had a higher percentage of acidity and a higher peroxide value than FO. Physical characteristics included specific gravities of 0.723 and 0.864 and smoke points of 220°C/4min and 190°C/5min, respectively. The data matched those obtained via Kozłowska and Gruczyńska (2018), Abd El-Haleem (2021) and Jaski *et. al.* (2022). The primary components of the tested oils were identified by the GC analysis. Regarding analysis of the fatty acid components of FO and WO oils of unsaturated fatty acids whereas, the highest percentages were $C_{18:1}$ (23.90 & 22.87%) and $C_{18:2}$ (52.67 & 51.92%) of FO and WO, respectively. However, high percentages of saturated fatty acids, such as $C_{16:0}$ (12.28 & 13.49 %) and $C_{18:0}$ (4.04 & 5.97%), provided for fresh and waste, respectively. Similar data reported via Farag and Sabry (2017) reported that fatty acids composition of sunflower and soybean oils. They showed that $C_{18:2}$ acid content is frequently utilized as an indicator of the degree of oil degradation, since the polyunsaturated linoleoyls chain is highly susceptible to oxidation. However, the viscosity of waste cooking oils differed from that of fresh vegetable oils Jabr *et al.* (2022). There was no significant difference between the control and the three concentrations (4.5, 9 and 18%) of fresh FO and waste WO oils during the incubation periods and hatching percentages for all examined land snails with the exception of *T. pisana* snail. The maximum hatching percentages of *E. vermiculata* for FO and WO were 53.33 and 46.67%, respectively, whereas the lowest hatching percentages for *M. cartusiana* for the same tested oils were 63.33 and 60.00%. According to Farag and Sabry (2017) mentioned effect of soybean and sunflower oils on many criterions of *M. cartusiana* eggs. On the other hand, the highest snail mortality rates were seen with a concentration (20%) of usable Kz and expired Kz oils against *Eobania vermiculata* snail Farag and Elnagar (2020). In contrast, Elyamani, *et al.* (2025) reported that utilized some oils such as citronella, lemon grass volatile oils and the mixture of fresh and used sunflower and soybean fixed oils as nutritional additives for silkworm *Bombyx mori*. Finally, the highest concentrations of FO for *M. cartusiana* snail eggs were recorded at 61.91 & 147.27% and 127.34 & 196.25% of AST and ALT, respectively, while the highest concentrations of WO were recorded at 95.42 & 188.82% and 154.93 & 221.40% of AST and ALT, respectively. All treatments resulted in a gradual increase in the activity of both enzymes starting on day three and reaching its maximum level after seven days of treatment. The results are consistent with data from biochemical studies conducted by earlier researchers, which demonstrated the impact of some oils on the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of *Eobania vermiculata* snails Farag and Elnagar (2020) and *M. cartusiana* eggs Abd El-Haleem (2021). Also, Sabry (2004) showed that the toxicity of oil might be attributed to unsaturated and saturated long chain fatty acids namely linolenic, linoleic and oleic acids which may interfere with other physiological sites. Finally, the increase of ALT and AST enzymes activity may be referred to the diffusion of this enzyme from its intracellular sites due to damage caused via the insecticide on the subcellular level Amer *et al.* (1994).

CONCLUSION

The paper discussed the possibility that waste oil (WO) would decrease the hatching percentage of eggs of M. cartusiana, T. pisana and E. vermiculata snails compared to the control. The findings showed that the evaluated substances might be arranged as follows in decreasing order of effectiveness: On E. vermiculata, WO > FO, followed by M. cartusiana and T. pisana.

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تأثير خليط زيتى عباد الشمس وفول الصويا الطازج والمستعمل على بيض بعض القواقع الأرضية وتأثيراتهما على نشاط الأنزيمين الناقلين لمجموعة الأمين

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الملخص

تهدف هذه الدراسة إلى تقييم التأثير المُبيدى للرخويات لمزيج من زيتي عباد الشمس وفول الصويا الطازج والمستعمل على بيض القواقع الأرضية (*Eobania vermiculata* و *Theba pisana* و *Monacha cartusiana*) وكذلك استخدام تقنية الكروماتوجرافي الغازي (GC) كطريقة تحليلية فعّالة لفصل مكونات خليط الزيوت وتحديدها وتحديد كميتها, حيث أشارت النتائج إلى أن أعلى نسبة فقس كانت ٥٣,٣٣ و٤٦,٦٧% لقوقع *E. vermiculata* لمزيج زيتي عباد الشمس وفول الصويا الطازج (FO) والمستعمل (WO) على التوالي، بينما كانت أقل نسبة فقس ٦٣,٣٣ و٦٠,٠٠% لقوقع *M. cartusiana* على التوالي. علاوة على ذلك، أظهرت المؤشرات البيوكيميائية أن أعلى تركيزات لزيتFO لبيض *M. cartusiana* سجلت (٦١,٩١ و ١٤٧,٢٧%) و (١٢٧,٣٤ و١٩٦,٢٥%) من AST و ALT على التوالي، بينما سجل WO (٩٥,٤٢ و ١٨٨,٨٢%) و(١٥٤,٩٣ و٢٢١,٤٠%) من AST و ALT، على التوالي, وكذلك سجل FO لبيض *E. vermiculata* (٧٦,١٩ و١٧٨,٢٣%) و (٩٣,٦٤ و ١٨٧,٨٤%) من AST و ALT، على التوالي، بينما سجلت WO (٨٥,٦٠ و٢٠٠,٦٠%) و (١١٠,٥٧ و ٢١٦,٤١%) من AST و ALT، على التوالي. وأخيرا، أظهرت النتائج أن المركبات المختبرة يمكن تصنيفها حسب الترتيب التنازلي لكفاءتها على النحو التالي: FO < WO على *E. vermiculata*، يليه *T. pisana* و *M. cartusiana*.

الكلمات الدالة : عباد الشمس -فول الصويا – البيض -الزيت -انزيمات القواقع