# Synthesis of Maltose Polyester from Waste Sunflower Oil and Evaluation of its Molluscicidal and Biochemical Effects against *Eobania vermiculata* Snail (Muller 1774)

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## ABSTRACT

Improper disposal of waste sunflower cooking oil (WSCO) poses significant environmental challenges and health risks including cancer, heart disease and neurological disorders. Therefore, this study was aimed at using WSCO as a raw material to synthesise maltose polyester (MPE) compound under certain conditions, including 3% Na<sub>2</sub>CO<sub>3</sub> as a catalyst,  $144^{\circ}$ C temperature and 8 h high vacuum. The physicochemical characterizations of the resulting products were performed using gas chromatography (GC), Fourier transform infrared (FT-IR) spectroscopy and nuclear magnetic resonance (H<sup>1</sup>-NMR). Besides these aspects, a comparison of the molluscicidal effects of WSCO and MPE with the recommended methomyl pesticide (RMP) was conducted against *Eobania vermiculata* snail. The results showed that 1% RMP caused 100% mortality in both juveniles and adults after 14 days of treatment. While, 18% concentrations of WSCO and MPE resulted in mortality rates of 20 and 46.67% in *E. vermiculata* adults and 33.33 and 53.33% in juveniles, respectively, after 28 days of treatment. Additionally, biochemical investigations showed a decrease in the activity of the amylase and invertase enzymes in adult *E. vermiculata* snail compared to the control group in all treatments. These findings highlight the potential of using WSCO to produce environmentally-friendly agricultural products for molluscan control, offering a sustainable solution to mitigate the risks associated with WSCO disposal.

Key words: Waste cooking oil, snails, esterification, toxicology, molluscicidal

### INTRODUCTION

Food oils, such as sunflower, soy and palm oil, undergo various chemical and physical reactions at high temperatures. These reactions include oxidation, hydrolysis, cyclization, isomerization, and polymerization (Zribi et al., 2016; Ben Hammouda et al., 2017). As a result, various by-products are formed, including free fatty acids, secondary oxidation products and polar compounds. Some of these by-products are unsuitable for human consumption and hurt health (Guillaume et al., 2018). Waste cooking oils (WCO) accumulate in very large quantities and releasing these oils into the environment can cause environmental problems (Dogan, 2016). Therefore, the use of WCO for safe and beneficial production is one of the promising ways.

There has been a current push to explore the potential of WCO by-products for creating biologically valuable substances. Among these substances are the synthetic sugar esters, which result from the reaction between the hydroxyl group of sugar and fatty acyl groups derived from oleochemicals (fatty acids present in oils and fats; Abdelaziz *et al.*, 2023). These synthetic sucrose esters have been recognized for their antibacterial, antifungal and insecticidal properties (Teng *et al.*, 2021). These compounds represent a relatively recent category of insecticidal compounds against insects like *Rhyzopertha dominica* (Chen, 2016).

Land snails pose a harmful threat in agricultural fields (Elkady *et al.*, 2024). The brown garden snail, *Eobania vermiculata* (Muller 1774), is one of the invasive snails that attack

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many ornamental plants in Egyptian localities and cause harm to the plants (Ibrahim et al., 2022). Many methods have been applied to control invasive snails, including mechanical, physical, chemical and biological strategies. Chemical molluscicides remain the most popular and effective method to control E. vermiculata snail, particularly over large areas (Tandingan De Ley et al., 2020). Such chemicals commonly include metaldehyde, methomyl and thiodicarb (Shetaia, 2015). Methomyl bait is used against E. vermiculata snail effectively (Ismail et al., 2022), but at high concentrations, it has toxic effects on the environment and humans (Dantas et al., 2024).

Therefore, there is a need for using alternative environmentally-friendly compounds for effective molluscan control. At present, attention is being drawn to the use of maltose polyester, the most advanced derivative within polyester group. It has notable characteristics, biodegradable, environmentally-friendly, nontoxic, gentle on the skin, tasteless and odourless. These properties make them highly desirable for wide-ranging applications such as the food industry, cosmetics, agriculture and pharmaceuticals (Dogan, 2016; Barros *et al.*, 2023; Shenana *et al.*, 2023). However, its efficacy against *E. vermiculata* snail has not yet been explored.

In the present study, it was aimed at synthesizing maltose polyester (MPE) by using waste sunflower cooking oil (WSCO) and to conduct a comparison of the molluscicidal effects of WSCO, MPE and to recommend methomyl pesticide (RMP) against E. *vermiculata* snail. To this purpose, a dipping bioassay was performed and investigated the impact of WSCO, MPE and RMP on both juvenile and adult mortality and estimated biochemical parameters such as the digestive enzymes amylase and invertase. These findings offer the promise of providing a practical solution to control E. vermiculata snail and making a valuable contribution towards sustainable agriculture.

## **MATERIALS AND METHODS**

Waste sunflower cooking oil was obtained from several restaurants in Cairo city, Cairo Governorate, Egypt. Maltose sugar was obtained from Merck KGaA, 64271 Darmstadt Germany. Bleaching clay was obtained from Arma Food Industries Com. at 10<sup>th</sup> of Ramadan city, Egypt. Molluscicidal methomyl (Copter 90% SP) a carbamate compound (S-methyl N (methyl carbamoyl oxy) thioacetimidate was purchased from Kafer El-Zayat company for pesticides and chemicals in Egypt. All chemical reagents used in this study were provided by Sigma and Al-Gomhoria chemical company of high quality and purity.

The adult and juvenile *E. vermiculata* snails were collected from orchard fields in Belbies district, Sharkiya governorate, Egypt. These were then transported to the laboratory of the Plant Protection Research Institute in white cloth bags. To ensure acclimation, the snails were fed fresh lettuce leaves regularly for two weeks before starting the treatment.

The preparation of MPE was carried out by starting esterification process by mixing methanol with WSCO at molar ratio of 5:1, and 1% H<sub>2</sub>SO<sub>4</sub> at 70°C for 5 h. Following esterification, the mixture underwent warm water washing in a sifting funnel. The bottom product and water were discarded, while the excess methanol was separated (Widayat *et al.*, 2020). The preparation of fatty acid methyl esters (FAME) and isolating/purifying maltose polyester (MPE) was done (Shenana *et al.*, 2023).

The physio-chemical parameters of WSCO and fresh oil were assessed by estimating the peroxide value (PV), refractive index (RI), specific gravity (SG) and free fatty acid (FFA) following the procedures outlined in the A. O. A. C. (2016) standard methods. Further, FFA content was determined by using the PN-ISO 660:2020 method. The total polar compounds (TPC) and the specific extinctions at 232 nm ( $K_{232}$ ) and 270 nm ( $K_{270}$ ) for conjugated dienes and trienes were assessed. Additionally, the fatty acid composition of WSCO, fresh oil and MPE was known by gas chromatography (GC) according to (PN-ISO 12966-2:2017).

The MPE structure was confirmed by using Infra-red absorption spectra (IR) and Protonnuclear magnetic resonance (H<sup>1</sup>-NMR). FTIR spectroscopy was employed to verify the IR absorption spectra, enabling the identification of functional groups present in both the reactants and products. The samples were dissolved and analyzed using a Nicolet iS5 FT-IR spectrometer with an iD5 single-bounce ATR attachment that featured a diamond laminate crystal (Thermo ScientificTM NicoletTM iSTM5 FT-IR). IR spectra were acquired at a resolution of 4.00/cm and 10 scans per spectrum, covering the range from 4000 to 600/cm (Gundlach et al., 2019). While, H<sup>1</sup>-NMR spectra were recorded using Thermo ScientificTM picoSpinTM 45 and 80 NMR spectrometers. This spectroscopic technique was employed to elucidate the organic structure of the molecules. The analysis was conducted under consistent conditions using 45/82 MHz pulsed Fourier transform H<sup>1</sup>-NMR permanent magnet instruments equipped with a capillary cartridge probe. Samples consisting of 2 to 10 mg of maltose esters dissolved in 0.6 to 1 ml of deuterated dimethyl sulfoxide

(DMSO; Gundlach *et al.*, 2019). The toxicity of WSCO, MPE and RMP against E. vermiculata snail was conducted by using dipping technique. The three concentrations (4.5, 9 and 18%) of WSCO and MPE were prepared by mixing 18 ml of each material with 81.75 ml of distilled water and 0.25 ml of Tween 80, then diluted to achieve the desired concentrations. Similarly, concentrations (0.25, 0.50 and 1%) of RMP were prepared by adding 1 g of each material to 99 ml of distilled water, then diluted accordingly. Fresh lettuce leaf discs (5 cm diameter) were dipped for 10 sec in each concentration of the tested compounds. The control replicates were created by submerging fresh lettuce leaves in water. The leaves were allowed to air dry before being fed to snails. Each treatment and control group were replicated three times. Each replicate in a plastic box contained five iuveniles and five adults E. vermiculata snails. covered with muslin cloth and secured with rubber bands. After a 48-h exposure period, the treated leaves were replaced daily with untreated leaves for 28 consecutive days. Dead individuals were checked by using a stainless steel needle and the mortality rates of E. vermiculata snail were determined after exposure at 1, 3, 7, 14, 21 and 28 days.

The estimation of the digestive enzymes activity amylase and invertase was estimated by collecting the soft tissues of *E. vermiculata* snail after removing the shells of adult mollusks. The tissues were weighed and homogenized in distilled water at a ratio of 1:10 (w/v). The homogenates were then centrifuged at 5000 rpm for 20 min at 5°C. The resulting supernatants were promptly

analyzed to assess the activity of the amylase and invertase enzymes (Mohammed and Elshewy, 2016).

The results were presented as mean values along with the standard deviation (SD). Statistical variations were determined using one-way analysis of variance (ANOVA) followed by a student's t-test (P < 0.05).

#### **RESULTS AND DISCUSSION**

The quality of WSCO and fresh oil was assessed through various physico-chemical properties, which provided insights into both its nutritional and physical characteristics. These properties included iodine value, peroxide value, saponification value, free fatty acid content and colour appearance, etc. (Bukola et al., 2015). WSCO exhibited higher values for all physico-chemical parameters compared to fresh oil (Table 1). These elevated values suggested higher toxicity and potential health risks associated with WSCO. The acidity value served as a pivotal indicator of the presence of free fatty acids (FFA) in fats and oils, playing a critical role in determining their quality, freshness and suitability for consumption. Typically, lower acidity levels are indicative of superior-quality edible oils, while higher levels may suggest deterioration or rancidity. Meanwhile, the peroxide value served as an indicator of oil quality and stability, offering insights into its susceptibility to oxidation and subsequent rancidity during storage, heating, or oxidation processes (Maliki et al., 2020). Notably, the results aligned with previous findings of Farag and Sabry (2017) demonstrating that waste frying oils generally had higher acidity and peroxide values compared to fresh oils. These observations are consistent with the findings reported by Kaleem et al. (2015), which emphasized the potential health risks associated with oil

**Table 1.** Physico-chemical properties of fresh and waste sunflower oil

Property	Fresh oil	Waste oil
Acidity % (% as oleic acid)	0.06	4.54
PV (Meq.O <sub>2</sub> /kg)	3.5	16
TPM (%)	0.0	10.5
K <sub>232</sub> value (conjugated diene)	1.95	5.2
K <sub>268</sub> value (conjugated triene)	0.93	1.65
Viscosity (mpa.s) at 30°C	35	64.2
Refractive index	1.4775	1.5889
Density (kg/m³)	54.9	64.8

rancidity, including the production of harmful chemicals linked to long-term health consequences such as cancer, heart disease and neurological disorders.

The total fatty acids obtained from fresh oil, WSCO and MPE were identified by GC (Table 2). The results revealed that fresh sunflower oil contained a high concentration of unsaturated fatty acids (86.57%). In comparison, WSCO and MPE had slightly lower levels (83.16 and 82.55%), respectively. The total saturated fatty acid contents for fresh oil, WSCO and MPE were 13.16, 13.18, and 13.23, respectively. The major unsaturated fatty acids in fresh oil, WSCO and MPE were linoleic acid (cis) C<sub>18:2C</sub> (59.53, 55.44 and 55.15%), followed by oleic acid C<sub>18:1</sub> (26.01, 25.92 and 25.70%), respectively. Among the saturated fatty acids, palmitic acid  $C_{16:0}$  had the major percentage (6.65, 6.87 and 6.76) followed by stearic acid  $C_{18:0}$  (5.55, 5.20 and 5.07%), respectively. It is worth noting that linoleic acid (trans)  $C_{18:2T}$  was absent in the fresh oil but it was present in both WSCO and MPE. This observation confirmed their potential for harm, as the presence of linoleic acid is often utilized as an indicator of oil degradation (Farag and Sabry, 2017). Similar perceptions revealed that sugar polyesters feature significant levels of unsaturated fatty acids, where linoleic acid and oleic acid were identified as the primary unsaturated fatty acids, while palmitic acid emerged as the dominant saturated fatty acid, followed by stearic acids (Farag and Sabry, 2017; Shenana et al., 2023).

 Table 2. Fatty acid composition of sunflower oil (fresh and waste) and MPE

Fatty acids	Fresh	Waste	MPE
	sunflower	sunflower	
Myristic acid C <sub>14:0</sub>	0.08	0.07	0.07
Palmitic acid C <sub>16:0</sub>	6.65	6.87	6.76
Palmitoleic acid C <sub>16:1</sub>	0.09	0.10	0.10
Margaric acid C <sub>17:0</sub>	0.09	0.09	0.09
Myristoleic acid C <sub>17.1</sub>	0.04	0.04	0.04
Stearic acid C <sub>18:0</sub>	5.55	5.20	5.07
Oleic acid $C_{18:1}$	26.01	25.92	25.7
Linoleic acid (trans) C <sub>18-2</sub>		0.46	0.46
Linoleic acid (cis) C <sub>18-2C</sub>	59.53	55.44	55.15
Linolenic acid C <sub>18.3</sub>	0.72	0.84	0.88
Arachidic acid $C_{20.0}$	0.36	0.42	0.38
Eicosenic acid $C_{20.1}^{20.0}$	0.18	0.22	0.22
Behenic acid $C_{22:0}$	0.43	0.53	0.43
Total SFA	13.16	13.18	13.23
Total USFA	86.57	83.16	82.55

The IR spectra of maltose sugar showed that the methyl and methylene groups of fatty acid chains were seen to vibrate in a stretched state and to be stretched asymmetrically in the range of 2860-3000/cm. The hydroxyl groups' absorption band was located at 3335/ cm (Fig. 1a). In contrast, MPE IR spectra showed absence of the hydroxyl group's absorption band at 3335/cm, while the ester carbonyl absorption band appeared at 1740/cm. These findings revealed that maltose sugar was esterified throughout, including all of its hydroxyl groups (Fig. 1b). These results align with prior study indicating that the sucrose palmitate spectrum exhibited a peak at 1734/ cm due to the stretching vibrations  $(v_{c-0})$  of the ester (Vassilev et al., 2021).

The H1-NMR spectra of maltose sugar and MPE detected signals of fatty acid chains methylene group protons at 1.201-1.226 ppm, methyl group protons on the omega end of fatty acid chains at 0.799-0.826 ppm, besides methylene group protons connected to a double-bonded carbon at 1.933-1.945 ppm. Maltose sugar H1-NMR spectra exposed hydroxyl group protons signals at 4.2-4.7 ppm (Fig. 2a), whereas these signals were not found in the MPE spectrum (Fig. 2b). These findings demonstrated that fatty acids were esterified to sugar molecules and there were no hydroxyl groups in the molecule of MPE. The obtained results were similar to those of Abdelaziz et al. (2023) who found that sucrose stearate's methyl groups had their protons chemically displaced as usual at 0.87-0.99 ppm. The (-CH2) methylene groups produced proton signals in the (1.17-2.02) ppm range. The range of 3.34-5.40 ppm was where glucose protons of glucopyranose were found. The mortality rates of juvenile and adult E. vermiculata snails exposed to WSCO, MPE and RMP were evaluated at concentrations of (4.5, 9 and 18%) WSCO and MPE, as well as (0.25, 0.50 and 1%) RMP for durations of 1, 3, 7, 14, 21 and 28 days. The results demonstrated a clear relationship between dosage and time, with mortality increasing in tandem with higher concentrations and prolonged exposure compared to the control group (Table 3). At 1% RMP, mortality rates reached 100% for both juveniles and adults. Conversely, the 18% concentration of MPE resulted in mortality rates of 53.33% for juveniles and 46.67% for adults after 28 days. Meanwhile, the 18% concentration of WSCO led to mortality rates



Fig. 2. (a)  $H^1$ -NMR spectrum of maltose sugar and (b)  $H^1$ -NMR spectrum of MPE.

Tested	Concentration						Mortality p	ercentages					
		1 da	y	3 day	ş	7 da	ys	14 da	ys	21 day	ş	28 da	s/s
		Juveniles	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles	Adults
MSO	4.5	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00€	0.004	0.00°	0.00€	0.00 <sup>f</sup>	0.00 <sup>f</sup>	6.67 <sup>sh</sup>	0.00€	$13.33^{\circ}$	6.67 <sup>tg</sup>
	6	0.00 <sup>d</sup>	$0.00^{d}$	0.00 <sup>€</sup>	0.00 <sup>d</sup>	0.00 <sup>€</sup>	0.00°	6.67ef	0.00 <sup>f</sup>	$13.33^{le}$	$6.67^{de}$	$26.67^{d}$	$13.33^{ef}$
	18	0.00 <sup>d</sup>	$0.00^{d}$	0.00 <sup>e</sup>	$0.00^{d}$	$6.67^{e}$	0.00°	$13.33^{de}$	$6.67^{ef}$	$26.67^{de}$	$13.33^{d}$	$33.33^{d}$	$20.00^{de}$
MPE	4.5	0.00 <sup>d</sup>	$0.00^{d}$	0.00 <sup>e</sup>	$0.00^{d}$	$6.67^{e}$	0.00°	$13.33^{de}$	$6.67^{ef}$	$20.00^{ef}$	$13.33^{d}$	$26.67^{d}$	$13.33^{ef}$
	6	0.00 <sup>d</sup>	$0.00^{d}$	$6.67^{de}$	$0.00^{d}$	$13.33^{de}$	$6.67^{de}$	$20.00^d$	$13.33^{de}$	$33.33^{d}$	$26.67^{\circ}$	$33.33^{d}$	$26.67^{d}$
	18	6.67 <sup>cd</sup>	$0.00^{d}$	$13.33^{d}$	$6.67^{d}$	$26.67^{\mathrm{cd}}$	$13.33^{d}$	33.33°	$20.00^{d}$	46.67°	33.33°	$53.33^{\circ}$	$46.67^{\circ}$
RMP	0.25	$13.33^{\circ}$	$6.67^{\circ}$	$26.67^{\circ}$	$20.00^{\circ}$	$40.00^{\circ}$	33.33°	$53.33^{b}$	$46.67^{\circ}$	$73.33^{b}$	$66.67^{\rm b}$	$86.67^{\rm b}$	73.33 <sup>b</sup>
	0.50	$26.67^{\mathrm{b}}$	$20.00^{\mathrm{b}}$	$53.33^{\rm b}$	$33.33^{\mathrm{b}}$	$73.33^{\rm b}$	$53.33^{b}$	$93.33^{a}$	$86.67^{\rm b}$	$100^{a}$	$100^{a}$	$100^{a}$	$100^{a}$
	1	53.33ª	$46.67^{a}$	$86.67^{a}$	60.00ª	$100^{a}$	$86.67^{a}$	$100^{a}$	$100^{a}$	$100^{a}$	$100^{a}$	$100^{a}$	$100^{a}$
Control		$0.00^{d}$	$0.00^{d}$	0.00€	$0.00^{d}$	0.00 <sup>€</sup>	0.00°	$0.00^{f}$	$0.00^{f}$	$0.00^{h}$	0.00°	$0.00^{f}$	0.00 <sup>g</sup>
Р		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
$L.S.D_{0.05}$		7.18	6.22	8.03	7.17	13.43	8.03	9.5	8.79	9.49	8.79	9.5	9.51

percentages can be ranked in descending order of efficacy as follows: RMP> MPE > WSCO. Similar studies showed that sucrose esters possessed antibacterial, antifungal and insecticidal properties (Kroumova *et al.*, 2016; Teng *et al.*, 2021). These results are strengthened by the finding of Chen (2016) who revealed that sucrose esters had insecticidal activity against *Rhyzopertha dominica*. In the same context, sucrose esters exhibited protective effect against cape gooseberry (*Physalis peruviana* L.; Ocampo *et al.*, 2024). Enzyme bioassay can provide diagnostic means to assess change or injury caused to organisms due to exposure to pollutents. The

of 33.33% for juveniles and 20.00% for adults

after the same duration. The mortality

means to assess change or injury caused to organisms due to exposure to pollutants. The amylase and invertase enzymes occurred in the digestive tract of several insects and they played important roles in insect digestion, growth and development (Zeng *et al.*, 2019). Data showed that both WSO and MPE at a concentration of 18% exhibited pronounced decreases in amylase activity with values (-57.35, -45.89, -27.34%) and (-59.43, -49.35, -32.48%) over the period (1, 3 and 7 days), respectively, compared to the control group (Fig. 3). Additionally, RMP at 1% concentration displayed the most substantial reduction in amylase activity with values (-71.63, -53.15 and -38.76%).

Similarly, the inhibition of invertase activity was observed in all treatments compared to control (Fig. 4) and this inhibition increased over time. The higher concentration 18% of WSO and MPE caused pronounced inhibition of invertase activity with values (-20.57, -34.13 and -42.93%) and (-24.31, -41.53 and -44.94%), respectively, compared to 1% RMP (-31.40, -49.89 and -55.95%) over the period (1, 3 and 7 days). Notably, higher concentrations generally led to greater reductions in enzyme activity compared to lower concentrations. It is worth mentioning that the activities of amylase and invertase enzymes were found to be concentration and time dependent. In the same context, treated M. cartusiana with used frying soybean oil showed lower activity of the amylase and invertase enzymes (Farag and Sabry, 2017). The decrease in enzyme activity was attributed to the toxicants direct effect caused by irregularity of enzyme activity (Sobhi, 2020; Sakla et al., 2021).

Table 3. Effect of WSCO and MPE on juveniles and adults of *E. vermiculata* snail compared to RMP

Different superscripts significantly differ at P=0.05 level



Fig. 3. The amylase activities in *E. vermiculata* snail adults after exposure to different concentrations of WSCO, MPE and RPM by using dipping technique over time (1, 3 and 7 days). SA – Specific activity (mg glucose/g. b. wt./min.) and RA% – (Relative activity %) – [(Treatment - Control)/Control] × 100.



Fig. 4. The invertase activities in *E. vermiculata* snail adults after exposure to different concentrations of WSCO, MPE and RPM by using dipping technique over time (1, 3 and 7 days). SA – Specific activity as (mg glucose/ g. b. wt./min.) and RA% – (Relative activity %) – [(Treatment - Control)/Control] × 100.

## CONCLUSION

Waste cooking oil can be used as a raw material to synthesis maltose polyester, which

is explored for its potential as an eco-friendly alternative molluscicide in managing *E. vermiculata* snail. The toxicological experiments revealed its significant impact on *E. vermiculata* snail juveniles and adults, showing increase mortality percentages as compared to the control group. Moreover, notable decrease in the examined snail's amylase and invertase enzymes activity as compared to the control group. The implications of this research offer a novel avenue for controlling *E. vermiculata* snail.

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