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Nutritional Responses of the Black Cutworm, *Agrotis ipsilon* (Hufn.), Larvae under Toxicity Effects of Five Wild Botanical Extracts from Sinai, Egypt

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Authors' contributions

This work was carried out in collaboration among all authors. Author EEN done the procedures on insects, statistical analysis, original draft, and reviewers' modifications to the manuscript. Authors SST and AIAS administered and interpreted laboratory analyzes of the tested oils. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To identify the chemical components of five wild Botanical oils (BOs) and their larvicidal influences on the anti-nutritional indices of the 4th instar larvae of *Agrotis ipsilon*.
Study Design: A comparative study with the randomized design, using five plant Extracts replicated five times.
Place and Duration of Study: *Mentha longifolia*, *Artemisia judaica*, *Majorana hortensis*, *Origanum syriacum*, and *Achillea santolina* were collected from the Sinai desert, Egypt. Study procedures were done at the Laboratory of Botany and Zoology, Faculty of Science, Zagazig University, Egypt, between December 2018 and June 2020.
Methodology: An analysis of the tested BOs components was done using a Shimadzu GC-9A gas chromatograph. Five sub-lethal concentrations of each plant were prepared (5 replicate/treatment) to evaluate medium lethality against *A. ipsilon* larvae (20 larvae/replicate). Untreated larvae were used distilled water only as a control. Ten larvae of each treatment were treated with only one LC_{50} to estimate the effect of different tested BOs on anti-nutritional Activities.

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Results: The main component of the *M. longifolia* and *A. judaica* oil was Piperitone at 39.79 and 37.55%, respectively; whereas the *M. hortensis*, *O. syriacum*, and *A. santolina* oil was Terpinen-4-ol, Thymol, and Fragranyl acetate at 29.82, 31.21, and 25.67%, respectively. According to LC_{50} of the tested BOs, the toxicity of *A. judaica*, *M. longifolia*, *O. syriacum*, respectively, were the most effective oils, while *M. hortensis* and *A. santolina* oils were the least susceptibilities. The more toxic oils reduced food consumption, causing a significant decrease in relative consumption rate (RCR), growth rate (RGR), and efficiency of conversion of ingested food (ECI)/digested (ECD). The previous oils also showed a significant increase in metabolic cost (MC) and anti-feeding activities against *A. ipsilon* larvae compared to *M. hortensis* and *A. santolina*.
Conclusion: It is suggested that *A. judaica* and *M. longifolia* extracts contain high Piperitone content and could be accepted as toxicants to control *A. ipsilon*.

Keywords: Organic composition; wild plants; larvicidal; consumption; metabolism; growth rate; antifeedant; black cutworm.

1. INTRODUCTION

The black cutworm, *Agrotis ipsilon* (Hüfn.), (Lepidoptera: Noctuidae) is a common destructive phytophagous insect-pest. In Egypt, it causes severe damage not only; on cotton but also; to a large number of field and vegetable crops [1]. Moreover, *A. ipsilon* larvae are not a climbing cutworm, so most of their feeding occurs below or at the soil surface level. The early instar larvae only attack the epidermis of the leaves and stem of the young seedling at night, reducing crop yield and causing substantial economic losses [2]. They can feed on more than 400 square centimeters of crops during their growth, but more than 80% of the consumption records after beginning newly molted fourth instar larvae [3,4]. In previous years, the intensive use of chemical pesticides was led to undesirable impacts on non-target insects, wildlife, and insecticide-resistant strains [5], also hazardous to animals and humans by pesticide residues and environmental pollution [6]. Therefore, more attention is given to safer methods to control such pests as an optional alternative to conventional insecticides.

Essential oils as botanical extracts were considered one of these approaches that reduced the different problems of synthetic insecticides; because they contain a wide-ranging of bioactive chemicals, which are easy to use, non-dangerous, and qualitative in their work [7,8]. The Egyptian Deserts, especially the Sinai Peninsula, are distinguished by the abundance of wild plants that; are widely used in folk remedies for different diseases. These plants belong to more than fifty families, many of which are used as pesticides [9]. Desert plant-derived oils consist of a definitive group of volatile compounds that give a distinct flavor or odor to these plants. They also principally contain sesquiterpenes, monoterpenes, and their oxygenated derivatives as plant secondary metabolites [10]. Furthermore, they often include several types of molecules; most of them are natural mixtures of phenylpropanoids and turbines, which are helpful due to their biological activity [11,12].

In this regard, the results of previous studies indicated that botanical extracts and their terpenes could be accepted as toxicants to control *A. ipsilon*. Jeyasankar [13] showed that gaultheria oil was more effective than eucalyptus oil on insecticidal activities against *A. ipsilon* larvae. Also, Elhosary et al. [14] achieved a noticeable decrease in some growth indices after treatment 4th instar larvae of *A. ipsilon* with mango seed extracts and water fleabane leaves. Sharaby and Elnujiban [15] reported that the mixture of some essential oils and terpenes improved their toxicity toward *A. ipsilon* Larvae, resulting in larval deformation and growth inhibition. Elbadawy et al. [16] indicated that *jojoba* oil was most effective against the 4th instar larvae of *A. ipsilon*, causing 60% mortality. Additionally, botanical extracts as antioxidants have been applied to many other lepidopterous insects such as *Spodoptera littoralis* [17], *Spodoptera frugiperda* [18].

Within the scope of previous studies, no study compared the activity of BOs for *Mentha longifolia*, *Artemisia judaica*, *Majorana hortensis* Moench., *Origanum syriacum* L. subsp. sinaicum, and *Achillea santolina* L. as toxicants against *A. ipsilon*. So the present study aimed to identify the chemical components of the tested BOs and their larvicidal influences on the anti-nutritional indices of the 4th instar larvae of *A. ipsilon*.

2. MATERIALS AND METHODS

2.1 Insects and Rearing Technique

Newly moulted 4th instar larvae of the *A. ipsilon* were provided from a laboratory culture at the Plant Protection Research Institute, Giza, Egypt. Castor bean leaves (*Ricinus communis* L.) were used as larval diets under laboratory conditions at 25±2°C, 60-70% RH, and 12 h light per day. The larvae were reared individually in clear plastic cups (7 cm deep by 5 cm diameter) to prevent larval cannibalism habits. In each treatment, fresh castor leaves were cleaned daily with sterile water and dried before treated with tested BOs or eaten by untreated larvae. The rearing technique was applied along the following larval stages until pupation happened. The newly formed pupae were kept inside glass jars until the moth's emergence, where female and male moths (1:1) were moved into a glass jar (2L) which was provided with a suspended piece of cotton soaked with a 15% sucrose solution (2 days/period). Strips cloth set in the muslin cap were applied as hanging places for egg deposition, which daily collected.

2.2 Plant Materials and BOs Isolation Process

Wild plants were collected from various places of the south Sinai desert, Egypt. Characterization and site localization of the tested plants are shown in Table 1. Plant material was distinguished at the Laboratory of Botany Department, Faculty of Science, Zagazig University, Egypt.

The plant materials were air-dehydrated at 23±3°C with suitable ventilation for 10 -14 days until they became brittle and then milled into a fine powder form. The air-dehydrated sample was subjected to hydro-distillation using a Clevenger type apparatus for 4 hrs. (250g sample/1000ml distilled water), according to Louni et al. [19]. Anhydrous sodium sulphate was used to extract oil-free water and saved in a fridge at; 5°C until needed. The BOs content was estimated as a relative percentage (v/w). The used parts for extraction, physical properties, and yield ratios of the BOs derived from current plant species are shown in Table 2.

2.3 Identification of BOs Compounds Using (GC-MS) Analysis

An analysis of the tested BOs components was performed using a Shimadzu GC-9A gas chromatograph attached to the mass spectrometer detector. The GC-MS analysis was carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30m length x 0.25mm diameter, 0.25µm film thickness). The oven temperature was initially installed for 5 minutes at 40°C and then programmed to increase the temperature of 4°C per minute until 250°C. The detector and injector temperature was 260°C. Helium was used as a carrier gas using 31.5 cm/sec linear velocity, 1.1 mL/min flow rate, and 1/60 split ratio, 70 eV ionization energy for 1 sec scan time, 40-350 amu mass range. The elements of the tried BOs were recognized by correlation of their Kovat's records and mass-spectra designs with those accessible in the library data set (NIST, WILEY). Kovat's indices were specified by co-injection of the samples with a solution, including a homologous sequence of n-alkanes (C8-C22) under the same conditions described by Adams [20]. The relative concentration of each constituent of the BOs was computed by the analysis program based on the peak area integrated without using correction factors.

2.4 Toxicity of the Tested BOs against *A. ipsilon* Larvae

Newly molted 4th instar larvae of *A. ipsilon* were the most susceptible stage to some of the botanical extracts, according to ELhosary et al. [14] and El-Badawy et al. [16]. Five concentrations; of each BO were prepared (125,

Table 1. Species list of the tested wild plants and their site localization

Scientific name	Family	English name	Site (Wadi)	GPS location	
				Latitude N	Longitude E
Mentha longifolia	Lamiaceae	Horse mint	El-Raha	28°34'07.2"	33°57'28.2"
Artemisia judaica	Asteraceae	Wormwood	Al-Arbaeen	28°32'30.2"	33°57'34.3"
Majorana hortensis	Lamiaceae	Marjoram	Um Adawi	28°05'13.8"	34°22'46.0"
Origanum syriacum	Lamiaceae	Oregano	El-Deir	28°33'25.2"	33°58'32.6"
Achillea santolina	Asteraceae	milfoil	El-Raha	28°33'52.8"	33°57'34.4"

Table 2. Physical Properties and yield ratios of the tested BOs derived from five plant species

Plants	Part used	Physical Characteristic			Oil yield (%)
		Odor	Color	Density(g/mL)	
M. longifolia	Leaves	Minty	Pale yellow	0.795	0.59
A. judaica	Aerial parts	Herbal	Light yellow	0.912	0.88
M. hortensis	Leaves	spicy herbal	Light yellow	0.871	1.37
O. syriacum	Aerial parts	Minty	Colorless	0.931	0.71
A. santolina	Aerial parts	Fragrant	yellowish green	0.845	0.46

250, 500, 1000, and 2000 ppm). Castor leaves were dipped in each concentration level; of each BO for 15 seconds and air-dried on filter papers at room temperature. Equal discs of treated leaves (3 cm in diameter) were supplied for 48 h to newly molted 4th instar larvae and then replaced daily by another fresh one until pupation. The previous step was repeated using distilled water only as control. Larvae were starved for four h before being transferred individually into plastic cups. The tested BOs were distributed into five groups of cups, each of which was divided into five subgroups to be treated with one of the previously prepared concentrations. Each concentration was replicated five times (20 larvae/replicate). Another group was identified without any treatments as control [21]. The mortalities were checked daily and corrected by Abbott's formula [22].

2.5 Anti-nutritional Activities of the Tested BOs against *A. ipsilon* Larvae

Ten larvae of each treatment were treated with only one LC50 value to evaluate the effect of different tested BOs on anti-nutritional Activities. Each treatment was replicated five times. Each cup of the control sample was supplied by known weighed disks of fresh castor leaves, treated with the sub-lethal concentration of each treatment, according to Shaurub et al. [23]. All the larvae, food supplied, food unconsumed, and larvae feces were weighed daily before and after feeding until the pupation was done. All above weights were expressed as dry weight percentages using an oven at 60°C for 48 h. Fresh and dry weights of these components were registered daily to evaluate the feeding indices. The methodology recommended by Truzi et al. [24], based on Scriber and Slansky [25], was used to estimate the quantitative nutritional parameters of the 4th instar larvae of *A. ipsilon* as follows: the weight of feces produced (F), food ingested (I)= F+A, food assimilated (A)= I−F, food metabolized (M) = (I−F)−B, weight gain by larvae (B)= (I−F)−M, duration of feeding period (T), mean weight of larvae during feeding period (L), relative consumption (RCR)= I/(L*T), metabolic (RMR)= M/(L*T), and growth rates (RGR)= B/(L*T), the efficiency of conversion of ingested food(ECI) = (B/I)*100, the efficiency of conversion of digested food (ECD)= (B/I−F)*100, approximate digestibility (AD)= ((I−F)/I)*100, metabolic cost (MC)= 100−ECD, anti-feeding Activity= [(I control−treated)/I control]*100.

2.6 Statistical Analysis

The lethality values were evaluated based on probit analysis [26]. To determine the LC50 values, Fiducial limits (95%) of each treatment, and slope, under IBM-SPSS software version 25.00, significant differences among the treatments were analyzed using one-way ANOVA. Means were significantly ordered by using Fisher's LSD test at 0.05 levels [27].

3. RESULTS AND DISCUSSION

3.1 Chemical Constituents of Tested BOs Derived from Wild Plants

The chemical composition was analyzed of the tested BOs of *M. longifolia*, *A. judaica*, *M. hortensis*, *O. syriacum*, and *A. santolina*. The principal ingredients of each oil and Kovats index were also; organized in Table 3. The qualitative composition results showed the main components of the *M. longifolia* oil were Piperitone, 1,8-Cineole, Pulegone, Limonene, L-menthone, Caryophyllene, germacrene-D, and Terpinen-4-ol 39.79, 9.85, 7.12, 5.76, 4.95, 4.81, 3.79, and 3.01%, respectively. Besides, the principal components of *A. judaica* oil were Piperitone, Camphor, E-ethyl cinnamate, Terpinen-4-ol, and Spathulenol 37.55, 21.19, 15.34, 5.75, and 4.08%, respectively. The main components of the *M. hortensis* oil were Terpinen-4-ol, γ-Terpinene, E-sabinene hydrate, and α-Terpinene was recorded 29.82, 15.15, 11.35, and 9.71%, respectively. Additionally, *O. syriacum* oil scored (31.21, 15.37, 12.07, 9.64,

6.03, 5.96, 4.73, and 4.11%, respectively) for Thymol, γ-Terpinene, Terpinen-4-ol, Carvacrol, trans-β-ocimene, α-Terpinene, p-Cymene, and Sabinene. Finally, the prime components of *A. santolina* oil were Fragranyl acetate, 1,6-Dimethyl-1,5-cyclooctadiene, Fragranol, β-Thujone, and 1,8-Cineole at 25.67, 16.71, 10.27, 8.39, and 7.26%, respectively.

Table 3. Chemical components of the BOs were extracted from the tested plants by hydro-distillation using analyzed by GC-MS

Components	mol wt. (g/mol)	KI	Composition (%)				
			M. longifolia	*A. judaica*	*M. hortensis*	*O. syriacum*	*A. santolina*
α-Thujene	136	931	0.19	-	0.97	1.36	-
α-Pinene	136	939	1.93	0.35	-	0.91	0.49
Camphene	136	953	0.32	0.47	-	-	0.75
Sabinene	136	976	1.54	0.91	2.99	4.11	0.45
β-Pinene	136	980	0.85	0.39	-	-	0.13
Myrcene	136	991	0.97	0.76	1.31	2.05	-
α-Phellandrene	136	1005	-	2.63	0.95	-	-
α-Terpinene	136	1018	-	0.45	9.71	5.96	1.02
p-Cymene	134	1026	0.31	-	3.15	4.73	2.15
Limonene	136	1031	5.76	-	-	-	-
1,8-Cineole	154	1033	9.85	0.72	-	-	7.26
trans-β-ocimene	136	1050	-	-	-	6.03	-
γ-Terpinene	136	1062	0.87	-	15.15	15.37	0.91
Terpinolene	136	1088	-	-	3.62	1.56	0.18
Linalool	154	1098	1.33	-	-	-	0.40
(E)-sabinene hydrate	154	1115	-	-	11.35	-	1.31
β-Thujone	152	1143	-	-	-	-	8.39
Camphor	152	1145	-	21.19	-	-	5.17
L-menthone	154	1154	4.95	-	-	-	-
Isoborneol	154	1156	2.62	0.64	-	-	3.85
Terpinen-4-ol	154	1177	3.01	5.75	29.82	12.07	5.11
α-Terpineol	154	1189	1.98	0.98	2.03	0.42	3.05
Fragranol	154	1196	-	-	-	-	10.27
Verbenone	150	1204	-	0.77	-	-	-
Pulegone	152	1237	7.12	-	-	-	-
Geranial	152	1270	-	1.36	0.18	-	-
piperitone	152	1282	39.79	37.55	0.37	-	0.35
Thymol	150	1290	-	-	0.25	31.21	0.58
Carvacrol	150	1298	-	0.55	-	9.64	0.31
Fragranyl acetate	196	1335	-	-	-	-	25.67
α-Cubebene	204	1351	0.54	-	-	-	-
α-Humulene	204	1451	0.85	-	0.43	0.10	-
caryophyllene	204	1454	4.81	1.88	3.61	0.75	0.25
β-Farnesene	204	1458	0.34	0.47	-	-	-
(E)-ethyl cinnamate	176	1460	-	15.34	-	-	-
γ-Muurolene	204	1477	-	-	-	-	-
germacrene-D	204	1480	3.79	-	0.95	-	0.70
β-Selinene	204	1485	-	-	-	-	-
Bicyclogermacrene	204	1494	-	-	0.09	0.47	0.67
1,6-Dimethyl-1,5-cyclooctadiene	136	1503	-	-	-	-	16.71
γ-Cadinene	204	1513	-	-	-	-	-
δ-Cadinene	204	1524	-	-	-	-	-
cis-Nerolidol	222	1534	-	-	0.11	-	-
spathulenol	220	1575	0.59	4.08	1.89	ND	0.34
caryophyllene oxide	220	1581	-	-	-	-	0.49

Components	mol wt. (g/mol)	KI	Composition (%)				
			M. longifolia	*A. judaica*	*M. hortensis*	*O. syriacum*	*A. santolina*
1-epi-cubenol	222	1613	-	0.95	-	-	-
Tau-Cadinol	222	1630	1.93	0.53	0.08	0.15	-
Torreyol	222	1645	-	-	-	-	-
β-Eudesmol	222	1649	-	-	-	-	0.22
α-Cadinol	222	1653	0.41	-	0.31	-	-
Shyobunol	222	1689	-	-	-	-	-
Unidentified	--	--	3.35	1.28	10.68	3.11	2.82

Compounds were listed in the order of their elution; mol wt.: molecular weight; KI: Kovat's indices confirmed by comparison with Kovats index on DB5 column (Adams, 1995)

The main ingredients in Table 3 were observed in more than one plant, such as α-Terpinene, p-Cymene, 1,8-Cineole, γ-Terpinene, Camphor, Terpinen-4-ol, and piperitone, but others were special to the plant species. The main components of the oils were divided into four basic categories: monoterpene hydrocarbons (α-Pinene, Camphene, Sabinene, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, trans-β-ocimene, γ-Terpinene, Terpinolene, 1,6-Dimethyl-1,5-cyclooctadiene), oxygenated monoterpenes (1,8-Cineole, Linalool, (E)-sabinene hydrate, β-Thujone, Camphor, L-menthone, Isoborneol, Terpinen-4-ol, α-Terpineol, Fragranol, Verbenone, Pulegone, Geranial, piperitone, Thymol, Carvacrol, Fragranyl acetate, and (E)-ethyl cinnamate), sesquiterpene hydrocarbons (α-Cubebene, α-Humulene, caryophyllene, β-Farnesene, γ-Muurolene, germacrene-D, β-Selinene, Bicyclogermacrene, γ-Cadinene, andδ-Cadinene) and oxygenated sesquiterpenes (cis-Nerolidol, spathulenol, caryophyllene oxide, 1-epi-cubenol, Tau-Cadinol, Torreyol, β-Eudesmol, α-Cadinol, and Shyobunol).

The different chemical categories of the identified compounds are already in Table 4. The identified components ratio scored 96.65, 98.72, 89.32, 96.89, and 97.18%, respectively, of the total composition oils. Oxygenated monoterpenes were the most abundant constituents, recording 70.65, 84.85, 44.00, 53.34, and 71.72%, respectively, of the BOs. Moreover, monoterpene hydrocarbons recorded the second order with 12.74, 5.96, 37.85, 42.08, and 22.79%, respectively. In contrast, the sesquiterpene hydrocarbons and oxygenated sesquiterpene were secondary components.

Essential oils as natural mixtures contained many components at different concentrations. A few of these composites are identified by high concentrations related to other compounds when analyzing these oils. In general, the majority of these BOs oils are terpenoids and terpenes with diverse functions [28]. There is a real variability in the chemical constitution of tested BOs collected from Egyptian plants if compared with the same species from other areas, depending on many differences including climate, geology, season, used part, vegetative cycle, geographical location, and the used method to extract the BO [29]. The main compounds obtained from an analysis of the BOs are similar to those of Salama et al. [30] stated that Piperitenone, Cispipertone, and limonene were classified as the most valuable chemical

Table 4. The BO components were derived from the tested plants with constituent categories

Components	Composition (%)				
	M. longifolia	*A. judaica*	*M. hortensis*	*O. syriacum*	*A. santolina*
Monoterpene hydrocarbons	12.74	5.96	37.85	42.08	22.79
Oxygenated monoterpenes	70.65	84.85	44.00	53.34	71.72
Total Monoterpenes	83.39	90.81	81.85	95.42	94.51
Sesquiterpene hydrocarbons	10.33	2.35	5.08	1.32	1.62
Oxygenated sesquiterpenes	2.93	5.56	2.39	0.15	1.05
Total Sesquiterpenes	13.26	7.91	7.47	1.47	2.67
Number of identified components	25.00	22.00	22.00	17.00	28.00
identified components ratio	96.65	98.72	89.32	96.89	97.18

constituents in *M. longifolia* oil from Egypt. The results are also in agreement with previous studies from other countries that have recorded Piperitone and Limonene as the significant components of the *M. longifolia* oil [31,32], Piperitone component [33], and Menthon and Piperiton components [34]. However, the classification of monoterpene piperitone as the principal compound in *M. longifolia* oil contrasts sharply with the other findings as the oil contained carvone or ciscarveol [35,36] as the main ingredient.

The previous investigations on the compound constituents of *A. judaica* oil gathered from the Sinai have concurred with the current results. Where; it was deep in piperitone (27-46%), camphor (16-23%), transethyl cinnamate (8–13%), and ethyl cinnamate (5-6%) [37]. Conversely, chrysanthenol and camphor concentrations were presented in low amounts (0.14 and 0.38%, respectively). Also, Abdelgaleil et al. [38] proved that the Egyptian *A. judaica* oil contained piperitone followed by trans-ethyl cinnamate; as the major constituents of the oil. Moreover, the main ingredients of *A. Judaica* oil were Piperitone, trans-ethyl cinamate, and ethyl-3-phnyl propionate [39]. The principal components of oils studied by Abu-Darwish et al. [40] in Jordan were Piperitone, camphor ethyl cinnamate, comparable to those detected in the same plant in Egypt.

In *M. hortensis* oil, Twenty-two components were distinguished, addressing 89.32% of the oil. The significant constituents of this oil, Terpinen-4-ol, γ-Terpinene, (E)-sabinene hydrate, and α-Terpinene, were similar to that described by other authors [41,29]; however, the concentrations of the significant components produced from the oil could change possibly due to both genetic differences and environmental factors [42]. In the *O. syriacum* oil, seventeen compositions were identified, expressing 96.89% of the total components, the main ingredients being thymol, γ-terpinene, Terpinen-4-ol, Carvacrol, and trans-β-ocimene. Hence, this structure is similar to the *O. syriacum* oil grown in Egypt, in which thymol and γ-terpinene were the principal constituents [29]. In contrast, many studies have publicized that carvacrol and thymol were the two main components of *O. Syriacum* oil [43,44].

The chemical profile of the Egyptian *A. santolina* displayed that oil contained Twenty-eight components, corresponding to 97.18% of the total composition. The essential oils derived from *A. santolina* in the current study coincided with the previous reports [45,46]. On the other hand, differences were observed in the fundamental parts of the oils of Achillea species like *A. millefolium* [47], *A. biebersteinii* [48,46], and *A. fragrantissima* [45] compared to *A. santolina*. Furthermore, quite differences were showed between the same plant extract main components; in different countries of origin: Iran [49] and Algeria [50].

3.2 Toxicity of the Tested BOs Against *A. ipsilon* Larvae

The results in Fig. 1 indicated the mortality rate of *A. ipsilon* larvae under various concentrations of the examined BOs. The outcomes introduced that the most noteworthy mortalities were related to the highest BOs concentrations at 2000 ppm. They recorded 82, 75, 67, 48, and 40% mortalities for *A. judaica*, *M. longifolia*, *O. syriacum*, *M. hortensis*, and *A. santolina*, respectively. The low mortality percentages were observed at 125 ppm 25, 20, 18, 13, and 11%, respectively, at above mention tested BOs. The data likewise reported that the most elevated cumulative increase in larval mortality was 25, 33, 49, 71, and 82%, respectively, according to increasing the concentrations of *A. judaica* oil except; at 250 and 500 ppm concentrations. While the least cumulative increase in mortality was (11, 19, 21, 42, and 40%, respectively); when treated with various concentrations of *A. santolina* oil. Furthermore, this figure showed that *M. longifolia* oil had a higher larval mortality than in *O. syriacum* oil and then *M. hortensis* oil when treated with previous concentrations. The achieved results confirmed that *A. judaica* oil had higher mortalities and more successful than other tested BOs. On the contrary, *A. santolina* was the lowest one.

These results are similar to studies conducted on other plant extracts to control the same insect; Elbadawy et al. [16] exhibited that the mortalities were compatible with the increasing concentrations and exposure time. At the highest concentration, the mortality percentage was (3.33 - 30%) after 48 h and increased to (33.3 - 46.6%) for seven days after treated the larvae with the tested plant oils. Moreover, ELhosary et al. [14] declared that the larval mortalities differed under- tested plant extracts. The highest mortality rates were 66.6 and 80% when the larvae were uncovered to water flea and water

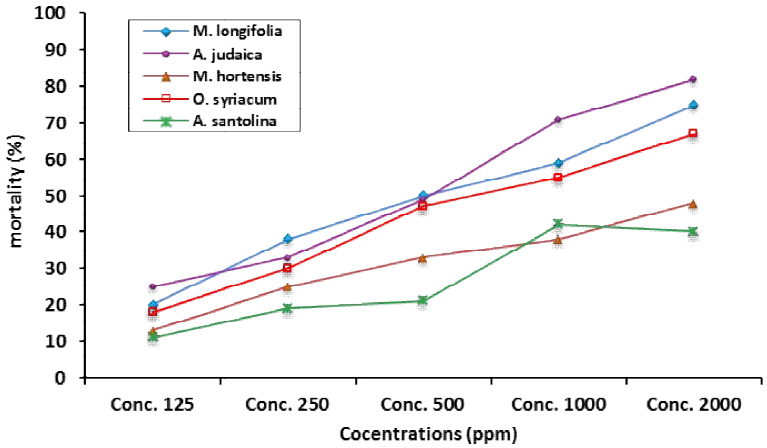

Fig. 1. Larval mortality of *A. ipsilon* under various concentrations of the tested BOs

mango extracts, respectively. The previous reports of other insects are consistent with the mortality rate after using one or more of the current plant extracts, as Al-Sharook et al. [51] stated that the mortalities of treated insects induced by plant oils, causing their failure to consume enough amounts of air to separate the used cuticle by a new one at ecdysis. Also, plant oils may increase a metamorphosis inhibiting according to the hormonal regulation disturbance. Also, Louni et al. [19] indicated that the mortalities of *Ephestia kuehniella* larvae were 84% on the 1st day when treated with *M. longifolia* oil at 40000 ppm. The highest mortality up to 16 days of the *S. littoralis* larvae exposed to *A. judaica* mixed with Chromafenozide was 70 and 77% at 72 and 96 hrs, respectively [17].

The recorded data in Table 5 reviewed that the LC_{50s} of the tested BOs against the 4th instars were 600.70, 486.33, 1883.2, 744.45, and 1970.2 ppm, respectively. The current outcomes revealed that the toxicity of *A. judaica* oil was the best one compared to other BOs; meanwhile, *M. longifolia* oil was less toxic than *A. judaica* oil, followed by *O. syriacum* and *M. hortensis* oil. The LC_{50} value of *A. santolina* oil has less susceptibility against *A. ipsilon* larvae. Thus, there were differences among the toxicity indices of the tested BOs according to their LC_{50} values.

According to the current lethality values results, *A. Judaica* oil was more active as an insecticide. Meanwhile, *A. santolina* oil had less insecticidal activities against the 4th instars. These results are partially similar to El-Sabrout et al. [52] when using *A. Judaica* oil for controlling *S. littoralis* larvae, containing monoterpenes Limonene, 1,8-Cineole, α-Phellandrene, and Camphor at most elevated concentrations in the total composition. Formulation of the *A. judaica* oil comprised a majority share of piperitone and Camphor, which gave the most effective oil in the insecticidal activity. In the same trend, Piperitone and transethyl cinnamate are considered to be the principal components of *A. Judaica* oil, which played an active role in the control of *S. littoralis* [38]. These results are similar to El-Massry et al. [39], who proved that Piperitone, ethyl cinnamate, and spathulenol are the chief components of the *A. judaica* oil.

The Chemical analysis of *M. longifolia* oil specified that the foremost compound was piperitenone oxide, which could be responsible for the higher efficiency of larvicidal activity. This compound belongs to the group of epoxyketone monoterpene [53]. Due to the lack of previous studies in controlling the lepidopteran insects using the *M. longifolia* extracts, Many studies of other insects have been shown a higher efficacy of piperitenone from *Mentha* species on these insects compared to other tested essential oils, such as *M. spicata* against *Anopheles stephensi* [54], *M. microphylla* against *Tribolium castaneum and Sitophilus oryzae* [55], *M. spicata*, *M. suaveolens*, and *M. longifolia* against *Culex pipiens* [56], and different *Mentha* L. Species against *Culex quinquefasciatus* [33].

Although some essential oils act as neurotoxic agents, the mechanism of the insecticide activity of *M. hortensis* oil is unclear [57]. Previous studies showed an increase in the toxicity activities of this oil compared to some of the main components isolated from it, particularly c-terpinene and terpinen-4-ol against the 4th instar

Table 5. Lethality values and toxicity indices of the tried BOs against *A. ipsilon* 4th instars

Treatments	Lethality variables					Toxicity index
	LC50 (ppm)	95% Fiducial limits (Lower – Upper)	Regression equation (y= a.x + b)	R^2	Slope ± SE	
M. longifolia	600.70	(281.00–1284.13)	Y=1.23x+1.58	0.956	1.23 ±0.16	81.0
A. judaica	486.33	(250.94–942.50)	Y=1.41x+1.19	0.969	1.41 ±0.14	100
M. hortensis	1883.2	(687.00–5162.65)	Y=0.98x+1.76	0.898	0.98 ±0.22	25.8
O. syriacum	744.45	(350.57–1580.91)	Y=1.26x+1.38	0.977	1.26 ±0.16	65.3
A. santolina	1970.2	(801.91–4840.49)	Y=1.16x+1.15	0.919	1.16 ±0.19	24.7

LC50: Lethal Concentration which causes 50% mortalities of the larvae during a specific trial period, Y: value of the predicted dependent variable, a: slope coefficient, x: value of the experimental variable, b: the y-intercept of the regression line, R2: The r-squared coefficient, SE: standard error

larvae of *S. littoralis* and adults of *Aphis fabae* with LC_{50} values of 2.48 µg and 1.86 g/l in the topical application and rapid dipping assays, respectively [41]. Moreover, the insecticide activities of some essential oils against other order insects, including three of them in the present study, were evaluated against adult insects of *S. oryzae* and *T. castaneum*. According to LC_{50} values of these oils, the *M. hortensis* was less toxic than *A. Judaica* oil in the insecticidal potential against both insects; however, it was more effective than *A. santolina* oil against these insects [55]. These results are similar to the toxic activity of some tested oils in the present study.

Although no previous study of the insecticidal activity of *O. syriacum* oil has been testified against *A. ipsilon*, this oil proved highly effective as an insecticide against other insects, such as Kaya et al. [58], who declared that *O. syriacum* oil produced the top toxicity at the lowest concentration (30 µg ml) compared to other oils, and it had the lowest value LC_{50} (11.2 µg mL) against *C. maculatus*. This result is confirmed by the current study, where the *O. syriacum* oil scored the third point of toxicity (65.3%) among the tested BOs against the *A. ipsilon* larvae. Despite; the differences in the tested insects, the similarity of the main ingredients in *O. syriacum* oil with previous studies helped realize the importance of using it as a powerful insecticide. These components (carvacrol, 1.8 cineole, menthol, camphor, terpene, and thymol) have sub-lethal deterrent actions on various insect species [58,59].

Some previous reports have studied the toxic effects of the insecticides extracted from *A. santolina*, which have different efficacy against many insects when; its insecticidal activities differ according to the order of insects and used method. Therefore, some authors proved a highly toxic activity against *Trogoderma granarium* larvae [46]. On the contrary, the extract of *A. santolina* oil offered the lowest toxicity value at LC_{50} (4033 ppm) against *Sitotroga cerealella* larvae when; compared with nine plant extracts collected from North Sinai, Egypt [60]. The current study agreed with the previous findings that *A. santolina* oil was the least toxic among the other tested BOs under investigation.

Finally, although the above-mention plant oils have not been examined against *A. ipsilon* in the previous reports, the current results indicated variant degrees among the toxicity indices of the tested BOs, where the *A. Judaica* oil was the most toxic activity, followed by *M. longifolia*, *O. syriacum*, and *M. hortensis*, respectively. Moreover, *A. santolina* oil scored in the last point of the toxicity index against the 4th instars of *A. ipsilon*.

3.3 Toxicity Effects of the Tested BOs on the Nutritional Indices of *A. ipsilon*

Data in Table 6 proved the effect of the tested BOs on the amount of ingested food, produced feces, weight gain, assimilated and metabolized food of the bollworm 4th instar larvae, where the least amount was found in *A. judaica* at 143.42, 37.49, 42.94, 105.93, and 62.99 mg, respectively. However, the highest amounts were detected in *A. santolina* at 351.25, 72.72, 176.79, 278.53 mg, respectively, excluding Metabolized food. The results also indicated that there are significant differences between most of the tested BOs compared to the untreated sample, while no differences were found between *A. santolina* and the control, *M. longifolia* and *A. judaica* in produced feces; *M. longifolia* and *O. syriacum*, *M. longifolia*, *A. santolina*, and *M. hortensis* in metabolized food.

The use of natural plant extracts in insect pest control programs has received much attention in recent years due to the environmental pollution, pest resistance, and adverse effects on the organisms resulting from the irregular use of pesticides. Food utilization efficiencies are useful for measuring the growth rate and development of the consumer [25] also; food quality of different host plants plays a crucial role in insect performance [61]. The least amount of assimilated food due to part of the ingested food was used by the larvae for transformation into biomass or energy for metabolism. Furthermore, the decreased amount of metabolized food could be owing to the highest amount of food used for growth and not metabolic energy. Shekari et al. [62] proved that the reduced food consumption; due to a stress of the chemical components of the botanical on the enzyme expression system to synthesize new and higher amounts of detoxification enzymes.

The data presented in Table 7 presented that the most toxic oils reduced larval weight without significant differences between *M. longifolia and O. syriacum* at 95.30 and 101.92 mg, respectively. These previously oils caused a notable decrease in RCR without differences between *O. syriacum and M. hortensis* at 0.567 and 0.644 mg/mg/d, respectively; meanwhile, the RMR showed a non-significant reduction in most tested BOs except *M. longifolia*. The RGR decrease was observed significantly between *A. judaica* and *O. syriacum* at 0.117 and 0.202 mg/mg/d, respectively, while *A. santolina* and *M. hortensis* oil showed a non-significant decrease with control.

The current results showed that RCR was significant reduced with *A. judaica* and *M. longifolia;* this may be due to a low food intake or a toxic effect caused by the tested BOs. Furthermore, the RGR showed significant inhibition in *A. judaica*, *M. longifolia*, and *O. syriacum* indicated that these BOs were more effective and may act as an inhibitor. These results coincided with Senthil-Nathan [63] proved that *Melia azedarach* decreased the RCR and RGR of *Cnaphalocrocis medinalis* 4th instar larvae (Lepidoptera: Pyralidae). Moreover, the methanol extract of *S. marianum* diminished the RGR of *Pieris rapae* larvae [64]. Furthermore, El-Sabrout et al. [52] detected that *A. judaica*, *O. vulgare*, *Citrus lemone*, *Rosmarinuc officinalis,* and *Schinus molle* reducing the RGR of *S. littoralis* larvae, reduced RGR may have come from severe damage in the cell surface of the midgut lumen. The RMR demonstrating the amount of food consumed in metabolism by larva per gram of body weight per day may help clear the metabolic capacity that can affect the growth. The obtained results exhibited that RMR non-significantly decreased in all BOs. That is maybe due to less food consumption or a toxic effect caused by the tested plant extracts, resulting in using the food for purposes other than growth, such as detoxification enzymes synthesis. These results concord with Carvalho et al. [65] cleared that RMR for treated *S. frugiperda* larvae with a trypsin inhibitor (isolated from *R. communis* leaves) non-significantly affected.

The current results in Table 8 showed that ECI values decreased when the larvae were exposed to the most toxic oils compared to the control at 28.89, 29.94, and 35.53% in the direction of *M. longifolia*, *A. judaica*, and *O. syriacum*, respectively, while those values increased for *M. hortensis* and *A. santolina* at 47.09 and 50.33%, respectively. Likewise, the ECD values for the same previous oils were 36.23, 40.54, and 45.54% for the most toxic oils, 59.52 and 63.47% for the least efficient oils, respectively. Simultaneously, the outcomes didn't show significant differences between *M. longifolia* and *A. judaica* oils when used to reduce ECI and ECD. The AD revealed no significant reduction in all tested BOs. However, the MC recorded a highly significant increase in *M. longifolia, A. judaica,* and *O. syriacum* at 63.77, 59.46, and 54.46%, respectively. Moreover, the antifeedant index indicated that A. judaica, *M. longifolia*, and *O. syriacum* were the highest feeding inhibitors at 66.45, 52.54, and 42.54%, respectively.

ECD reflects metabolic efficiency and can be reduced by lowered the ECD or enhanced metabolic cost. As a response to decreased assimilation, more food is ingested, which increases RCR [24]. Both *M. hortensis* and *A. santolina* increased the food conversion efficiencies ECI and ECD. That may be attributed to the treated 4th instars at LC_{50} values, which required large amounts of energy to deal with the toxicity of two tested BOs. These results concurred with [64,66]. However, both ECI and ECD exhibited a significant decrease for *A. judaica* and *M. longifolia* compared with control. These results agreed with Mordue (Luntz) & Blackwell [67] stated that the reduction in ECI indicated that most food is converted into energy while less is converted to body tissue growth. ECD also diminished as the proportion of

Table 6. Effect of the tested BOs at LC_{50} values on the amount of food consumption of the 4th instars

Treatments	Weights (mg ± SD)				
	Ingested food	Produced feces	Weight gain	Assimilated food	Metabolized food
M. longifolia	202.91±14.42^{e}	41.08±3.77^{ef}	58.63±5.34^{e}	161.83±11.15^{e}	103.2±8.21^{bc}
A. judaica	143.42±10.97^{f}	37.49±2.83^{f}	42.94±3.95^{f}	105.93±9.87^{f}	62.99±5.38^{d}
M. hortensis	304.87±22.56^{c}	63.65±7.22^{c}	143.57±12.64^{c}	241.22±17.23^{c}	97.65±7.13^{c}
O. syriacum	245.66±18.31^{d}	54.01±4.41^{d}	87.28±7.22^{d}	191.65±15.05^{d}	104.37±9.66^{b}
A. santolina	351.25±24.93^{b}	72.72±6.95^{a}	176.79±14.71^{b}	278.53±18.50^{b}	101.74±8.09^{c}
Control	427.53±31.66^{a}	70.81±7.1^{ab}	194.31±16.7^{a}	356.72±25.75^{a}	162.41±13.5^{a}
F-value	228.4**	70.03*	320.1**	272.3**	126.1**

*The values attached to the same letter within each column do not indicate statistical differences between them, SD: standard Deviation, *: at P ≤ 0.05, **: at P ≤ 0.01*

Table 7. Effect of the tested BOs at LC_{50} values on the consumption, metabolism, and growth rates of the 4th instars

Treatments	Larval wt. (mg ± SD)	Relative Rates (mg/mg/d ±SD)		
		RCR	RMR	RGR
M. longifolia	95.30±7.25^{e}	0.481±0.08^{e}	0.244±0.05^{bc}	0.139±0.03^{cd}
A. judaica	81.83±6.74^{f}	0.389±0.07^{f}	0.171±0.04^{c}	0.117±0.02^{d}
M. hortensis	113.53±8.99^{c}	0.644±0.10^{cd}	0.206±0.06^{c}	0.303±0.08^{a}
O. syriacum	101.92±9.23^{de}	0.567±0.08^{d}	0.241±0.07^{c}	0.202±0.05^{bc}
A. santolina	135.01±12.26^{b}	0.665±0.10^{bc}	0.193±0.05^{c}	0.335±0.10^{a}
Control	148.49±13.01^{a}	0.825±0.14^{a}	0.313±0.09^{a}	0.375±0.13^{a}
F-value	64.79*	24.36*	6.56*	18.76*

*RCR: Relative consumption rate, RMR: Relative metabolic rate, RGR: Relative growth rate, the values attached to the same letter within each column do not indicate statistical differences between them, SD: standard Deviation, *: at P ≤ 0.05, **: at P ≤ 0.01*

Table 8. Effect of the tested BOs at LC_{50} values on the food utilization, absorption, metabolic, and antifeedant activities of the 4th instar larvae of *A. ipsilon*

Treatments	Nutritional indices (%)				Anti-feeding %
	ECI	ECD	AD	MC	
M. longifolia	28.89±1.76^{f}	36.23±2.90^{e}	79.75±8.15^{a}	63.77±4.85^{a}	52.54±3.94^{b}
A. judaica	29.94±2.02^{ef}	40.54±4.11^{de}	73.86±6.43^{a}	59.46±3.93^{b}	66.45±5.15^{a}
M. hortensis	47.09±3.66^{bc}	59.52±6.25^{a}	79.12±7.87^{a}	40.48±3.86^{e}	28.69±1.98^{d}
O. syriacum	35.53±2.55^{d}	45.54±3.96^{c}	78.01±6.99^{a}	54.46±5.05^{c}	42.54±3.65^{c}
A. santolina	50.33±4.75^{a}	63.47±5.38^{a}	79.30±8.24^{a}	36.53±4.17^{f}	17.84±1.56^{e}
Control	45.45±3.39^{c}	54.47±6.06^{b}	83.44±7.75^{a}	45.53±3.91^{d}	0.00±0.00^{f}
F-value	84.41*	48.68*	1.65	63.31*	566.2**

*ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food, AD: Approximate digestibility, MC: Metabolic cost, the values attached to the same letter within each column do not indicate statistical differences between them, SD: standard Deviation, *: at P ≤ 0.05, **: at P ≤ 0.01*

digested food converted into energy increased; it exhibited a post-ingestion toxic effect, which can be considered secondary phagodeterrence responsible for the reduced RCR, RGR, and RMR. Some previous studies showed a reduction in ECI and ECD of some Lepidoptera larvae treated with various botanical extracts [63,52].

AD denotes; the degree of food utilization depends on the digestibility of ingested food and the efficiency with which digested food (assimilated) converted into biomass [68]. It is based on variances between the weight of ingested food and feces, and it indicates the ability of an insect to absorb stored or metabolized food through the stomach wall. The achieved results showed a non-significant reduction of AD in all tested BOs, which may be due to the low percentage of excretion of consumed food by larvae because of the inhibitory effect of these tested BOs as compared

to control. These results agreed with some stated results of inhibited AD of various insects by some botanical extracts, for instance, *Pieris rapae* larvae treated with methanol extract *of Silybium marianum* [64], *S. littoralis* 4th instar larvae treated with an alcohol extract of *Conyza dioscoridis* [66]. On the contrary, some results showed a rise in AD, such as *Glyphodes pyloalis* 4th instar larvae treated with *Thymus vulgaris* and *O. vulgare* [69], *Plutella xylustella* 3rd instar larvae treated with *O. vulgare* [70].

Antifeedant and growth activity inhibitors reduce pest damage by certain botanical products but without killing the pest. The present data manifested that the antifeedant index was highly significantly increased with *A. judaica, M. longifolia,* and *O. syriacum,* which proved that these BOs were the highest feeding inhibitors. These results coincided with Chennaiyan et al. [71] stated that *Barleria longiflora* leaves affect the *S. litura* larvae feeding behavior. Gvozdenac et al. [72] observed that *Aesculus hippocastanum* had a highly antifeeding activity against *Lymantria dispar* larvae. However, *Ambrosia artemisiifolia*, *Daucus carota,* and *Elodea canadensis* exhibited no antifeedant activity. Moreover, El-Sabrout et al. [52] showed that *A. judaica*, *O. vulgare*, *C. lemone*, *R. officinalis,* and *S. molle* had potent antifeedant effects on *S. littoralis* larvae.

4. CONCLUSION

Under our toxicity results, usage of *A. judaica*, *M. longifolia*, and *O. syriacum* oils play a significant role in anti-nutritional activities, showing extensive deterrence potency and harmful impact on the food consumption, absorption, digestion, assimilation, and conversion, reflecting on growth and population of *A. ipsilon* larvae compared to *M. hortensis* and *A. santolina* oils. Accordingly, we would infer that these wild botanical extracts can be accepted as another successful choice in contrast to conventional synthetic pesticides and may assume a more influential part in the integrated pest control procedures against this insect later on.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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