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BIOCHEMICAL AND INSECTICIDAL EFFICACY OF CLOVE AND BASIL ESSENTIAL OILS AND TWO PHOTOSENSITIZERS AND THEIR COMBINATIONS ON *Aphis gossypii* GLOVER (HEMIPTERA: APHIDIDAE)

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Abstract

The present study investigates the insecticidal and biochemical effects of two essential oils (EOs) and two photosensitizers against cotton aphids in a laboratory setting. The EOs evaluated were clove (*Syzygium aromaticum* L.) and basil (*Ocimum basilicum*), while the photosensitizers were rose bengal and rhodamine B. The individual median lethal concentrations (LC_{50}) revealed that clove was ~4.44 times more potent than basil, and rhodamine B was ~1.34 times more potent than rose bengal. The mortality rates increased using higher concentrations of the photosensitizers and prolonging exposure time to sunlight. The most effective combination against adult aphids was found to be a mixture of sub-lethal concentrations of clove and rhodamine B, resulting in a mortality rate of 92.31%. Conversely, the combination of basil and rose bengal exhibited the lowest efficacy with a mortality rate of 33.33%. Biochemical analyses indicate that Rhodamine B, basil, and the basil-rhodamine B mixture (mixture C) significantly reduced trehalase activity. However, the protease activity significantly increased in aphids treated with rose bengal, clove, and the clove-rose bengal mixtures (mixtures A and B). The lipase activity is notably decreased upon treatment with rhodamine B and clove. Glutathione S-transferase (GST) activity decreased in aphids treated with rose bengal and the basil-rhodamine B mixtures (mixtures C and D), suggesting that GST did not play a role in detoxifying these compounds, thereby explaining the susceptibility of A. gossypii. Overall, the combination of essential oils and photosensitizers has demonstrated a synergistic effect in controlling *Aphis gossypii*, offering great potential as an effective strategy for aphid management.

Keywords: *Aphis gossypii*. Basil. Clove. Joint action. Rhodamine B. Rose Bengal.

1. Introduction

The cotton aphid, *Aphis gossypii*, is a polyphagous insect and a major pest of various plants of different families, such as Malvaceae, Rutaceae, and Cucurbitaceae (Wang et al. 2016). Nymphs and adults attack the lower surface of their leaves. They suck sap from the phloem, excrete sugary substances (honeydew), enhancing the sooty mold development, inoculate toxins and are vectors for viruses in many plants, such as the vermilion and vein mosaic form (Michelotto and Busoli 2003). Large colonies of this

aphid are mainly produced due to its intrinsic biotic characteristics, such as rapid development and parthenogenic reproduction, which are enhanced when coupled with plants under water stress and high temperature (Foster et al. 2007). The rapid multiplication of this aphid requires the producer to maintain short timespans of pesticide application, which can lead to the selection of a resistant population (Barros et al. 2006). The traditional control of *A. gossypii* mainly depends on the heavy application of synthetic insecticides, especially during the growing season. Moreover, there are many drawbacks, such as the disruption of natural enemies, toxic residues in the crops, resistance development, and undesirable impacts on non-target organisms (Isman 2000). Consequently, there has been an increasing demand for novel active pest control treatments and compounds with less harmful environmental effects (Rodríguez-González et al. 2019).

Due to their lack of side effects, low cost, high availability, and high toxicity against various insect pests, including aphids, botanical insecticides have recently emerged as key tools for pest control. In addition, they are unlikely to result in insecticide resistance due to their molecular complexity (Bedini et al. 2020). Combining insecticide applications can increase the effectiveness of pest management by lowering the risks of environmental pollution, limiting the amount of insecticide used, and preventing the development of resistance (Usha et al. 2014).

Essential oils (EOs) and their constituents can be used as alternatives to control many insects as they are selective and biodegrade into non-toxic products without effects on non-target organisms or the environment (Liu et al. 2012). Over the past 20 years, there has been a noticeable increase in research studies looking into the possible contribution of EOs derived from plants toward the management of aphids. EOs should be seriously considered as ecologically-friendly aphicides because they have the potential to be very successful at controlling pests (Atanasova and Leather 2018).

According to recent research studies, two EOs have demonstrated great potential as control agents against *A. gossypii*. These are basil (*Ocimum basilicum* L.) and clove (*Syzygium aromaticum*) (Atanasova and Ganchev 2018; El-Solimany and Aboelfadel 2022). Basil has been developed as an insecticide against mosquito species (Bhatnagar et al. 1993). The EO of clove has been widely implemented for its insecticidal, repellent, and deterrent activities against many insect pests (Corte´s-Rojas et al. 2014). In addition, one promising non-conventional insecticide using photosensitizer substances for controlling insect pests has been reported, which are converted into toxic photoproducts (Spikes 1986; Filiberti et al. 2009). Photosensitization includes the activation of light-sensitive compounds, which produce chemical reactions that cause damage or destroy cells. Photosensitization is catalyzed upon irradiation with sunlight or an artificial light source (Ben Amor et al. 2000). These photosensitizers include rose bengal and rhodamine B, which belong to the halogenated xanthenes. They have shown to be effective photo-insecticides against at least two-dozen insect species. Many insects are particularly susceptible to the photodynamic activity of dyes (Ben Amor and Jori 2000).

The aim of this study was to evaluate the insecticidal and biochemical efficacy of two EOs: basil (*O. basilicum*) and clove (*S. aromaticum*) and two photosensitizers: Rose bengal and rhodamine B, and their combinations against an adult cotton aphid (*A. gossypii*) under laboratory conditions.

2. Material and Methods

Aphis gossypii Culture

A field population of *A. gossypii* was collected from pepper plants cultivated in Mariouteya, Giza, governorate, Egypt (30.0220° N latitude and 31.2055° E longitude). Colonies were kept in a rearing room at 25 ±2 °C, 70 ±5% RH, and 14:10 h (L:D).

Chemicals

Two photosensitizers were used in our study: Rose bengal ($C_{20}H_2Cl_4I_4K_2O_5$) and rhodamine B ($C_{28}H_{31}ClN_2O_3$), which were obtained from Alfa Company in Benha, Egypt. All chemicals used for our biochemical analyses were purchased from Sigma-Aldrich, Germany.

Extraction of basil (*O. basilicum*) and clove (*S. aromaticum*) EOs

Fresh leaves of both *O. basilicum* and *S. aromaticum*, obtained from the National Research Center, Dokki, Giza, Egypt, were washed and the EOs extracted using hydro-distillation performed on a Clevenger-type apparatus at 75 °C. 30 g of crushed leaves of basil and 25 g of the dried plant buds of clove were placed in a steam flask and the distillation step conducted over 4 h. The collected distillate was further extracted with n-hexane using a separatory funnel and filtered after separation. Sodium sulphate (Na_2SO_4) was added to remove any water and the solutions were concentrated using a rotary evaporator (YUHUA, Shanghai, China). The final yield of basil EO was 0.5 mL per 30 g and the final yield of clove EO was 0.35 mL per 25 g.

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of both the basil (*O. basilicum*) and clove (*S. aromaticum*) EOs was studied on a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness) (Abd El-Kareem et al. 2016). WILEY 09 and NIST14 mass spectral databases were used to identify their components.

Bioassay

To determine the susceptibility of *A. gossypii* to the chemicals studied, a series of four concentrations (8×10^{-5}, 5×10^{-5}, 3×10^{-5}, and 1×10^{-6} M) of the two photosensitizers, and seven concentrations (2.5, 5, 10, 25, 50, 100, and 150 mg/L) of the two EOs were prepared in dechlorinated water. For the photosensitizers, a group of 50 adults (5 replicates × 10 adults) was used for each concentration. For the Eos, a group of 50 adults (5 replicates × 10 adults) was used for each concentration. Using a small plastic sprayer, 1 mL of each concentration was applied to a single pepper leaf, which was placed in a plastic cup with a transparent cover, and the cups were exposed to sunlight. The same experiment was repeated, but the cups were kept under dark conditions. For the control samples, the leaves were sprayed with dechlorinated water only. The cups were exposed to direct sunlight for 1.5 h. The mortality was investigated and recorded after 24 and 48 h of treatment to calculate the lethal concentrations (LCs) to be used in our further experiments.

The mortality was calculated and statistically analyzed using probit analysis (Finney 1971). The LC values of the photosensitizers and EOs were determined using “LdP-line” software. In addition, the toxicity index (Sun 1950) and relative potency (Zidan and Abdel-Mageed 1988) were calculated as follows:

Toxicity index = (LC_{50} of the most effective compound/LC_{50} of the tested compound) × 100

Relative potency = LC_{50} of the lowest toxic compound /LC_{50} of the tested compound

Joint action

To evaluate the joint action of the compounds studied, four mixtures were prepared: Mixture A comprised of the LC_{30} of rose bengal × the LC_{10} of basil EO; mixture B comprised of the LC_{30} of rose bengal × LC_{10} of clove EO; mixture C, comprised of the LC_{30} of rhodamine B × LC_{10} of basil EO; mixture D comprised of the LC_{30} of Rhodamine B × LC_{10} of clove EO.

Comparisons were made with the untreated samples exposed to direct sunlight (UL) or untreated samples exposed to dark conditions (UD). Each mixture was applied to five replicates (10 adults each). The mortality was calculated after 0.5, 1.0, and 1.5 h of treatment.

Biochemical analysis

After 48 h of treatment with the LC_{50} of the photosensitizers, EOs, and their mixtures, the surviving adults were collected. Each sample was replicated three times. The adults were homogenized in 0.2 M

phosphate buffer (pH 7.0) using a chilled glass and centrifuged at 8000 rpm for 15 min at 5 °C. The supernatants were kept at –20 °C prior to the following analyses.

Trehalase enzyme activity

To determine the trehalase activity, the sample solution (20 µL) was incubated with 3% trehalose solution (250 µL) and phosphate buffer (230 µL, 0.1 M, pH = 5.4) at 30 °C for 10 min. To stop the reaction, 250 µL of 3,5-dinitrosalicylic acid (DNS) reagent was added to each tube in a water bath (95 °C for 5 min). The samples were then cooled, diluted using 2.5 mL of water, and observed at 550 nm on a UV-spectrophotometer 1201 (Beckman, USA). The trehalase activity levels were calculated using glucose standard curves (Ishaaya and Swirski 1976).

Determination of the protease activity

The protease activity was determined as indicated by Tatchell et al. (1972) with slight modification. The method was based on measuring the increase in free amino acids split from albumin during incubation for 1 h at 30 °C. The reaction mixture contained 100 µL of insect homogenate, 1 mL of phosphate buffer (0.1 M, pH = 8) with 100 µL of bovine serum albumin (0.5 %). To stop the reaction, 1.2 mL of 20% trichloroacetic acid (TCA) was added. After 15 min, the mixtures were centrifuged for 20 min at 3000 rpm, and the supernatant collected to measure amino acids according to Lee and Takabashi (1966). The mean level of amino acids was calculated using DL-alanine as a standard.

Determination of the lipase activity

The lipase activity was investigated in accordance with Beisson et al. (2000) using a Spectrum diagnostic kit. DGMRE, a synthetic substrate, was split by lipase to yield methyl resorufin, a colored final product. The increasing absorbance of the red color of methyl resorufin was spectrophotometrically measured at 578 nm against air.

Determination of the GST activity

The GST activity was investigated in accordance with Habig et al. (1974) using 1-chloro 2,4-dinitrobenzene (CDNB) as a substrate. A mixture of potassium phosphate buffer, GSH, enzyme solution, and the substrate were incubated at 30 °C for 5 min. The increase in the absorbance observed at 340 nm was then recorded versus a blank mixture. The nanomole conjugated substrate/min/mg protein was then determined.

Statistical analyses

The biochemical parameters were statistically analyzed using one-way ANOVA utilizing the SPSS package (V20), followed by LSD post-hoc multiple comparisons (Turner and Thayer 2001) at a probability of <0.05.

3. Results

Gas chromatography-mass spectrophotometry (GC-MS) analysis

GC-MS analysis of basil and clove EOs showed the presence of various bioactive components in different amounts (Table 1 and 2, respectively). The chemical structures of the major compounds are shown in Figure 1. Table 1 shows Basil EO was comprised of four main compounds: 1,6-Octadien-3-OL,3,7-dimethyl (8.88%), estragole (35.42%), methyleugenol (7.61%), and dotriacontane (10.38%). On the other

hand, clove EO was mainly rich in Monoterpenes, including Eucalyptol (27.01%) and Lupeol (17.16%) (Table 2).

Table 1. Chemical compounds of basil (*Ocimum basilicum*) EO as identified by (GC-MS).

RT	Area %	Compound name	Match Factor (MF)
5.40	3.57	Eucalyptol	905
6.91	8.88	1,6-Octadien-3-OL,3,7-Dimethyl	945
8.90	1.15	Terpinen-4-ol	854
9.34	35.42	Estragole	944
10.52	2.02	(-)-Carvone	871
14.22	1.59	Alloaromadendrene	838
14.53	7.61	Methyleugenol	937
14.94	1.64	Bicyclo[7.2.0] Undec-4-Ene,4,11,11-Trimethyl-8-Methylene-, [1R (1R*,4E,9S*)]-	919
15.29	4.47	Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)	943
17.21	2.17	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1à,4aá,8aà)	919
17.88	1.18	cis-à-Bisabolene	879
18.81	1.78	(-)-5-Oxatricyclo[8.2.0.0(4,6)]Dodecane,,12-Trimethyl-9-Methylene	911
20.21	2.80	Tau.-Cadinol	902
32.35	2.84	Ethanol, 2-(9-octadecenyloxy)-, (Z)	836
35.69	2.73	13-Heptadecyn-1-ol	798
37.42	10.38	Dotriacontane	836
40.79	4.06	Dotriacontane	827
41.48	2.28	1-Heptatriacotanol	931
44.24	3.42	Isochiapin B	806

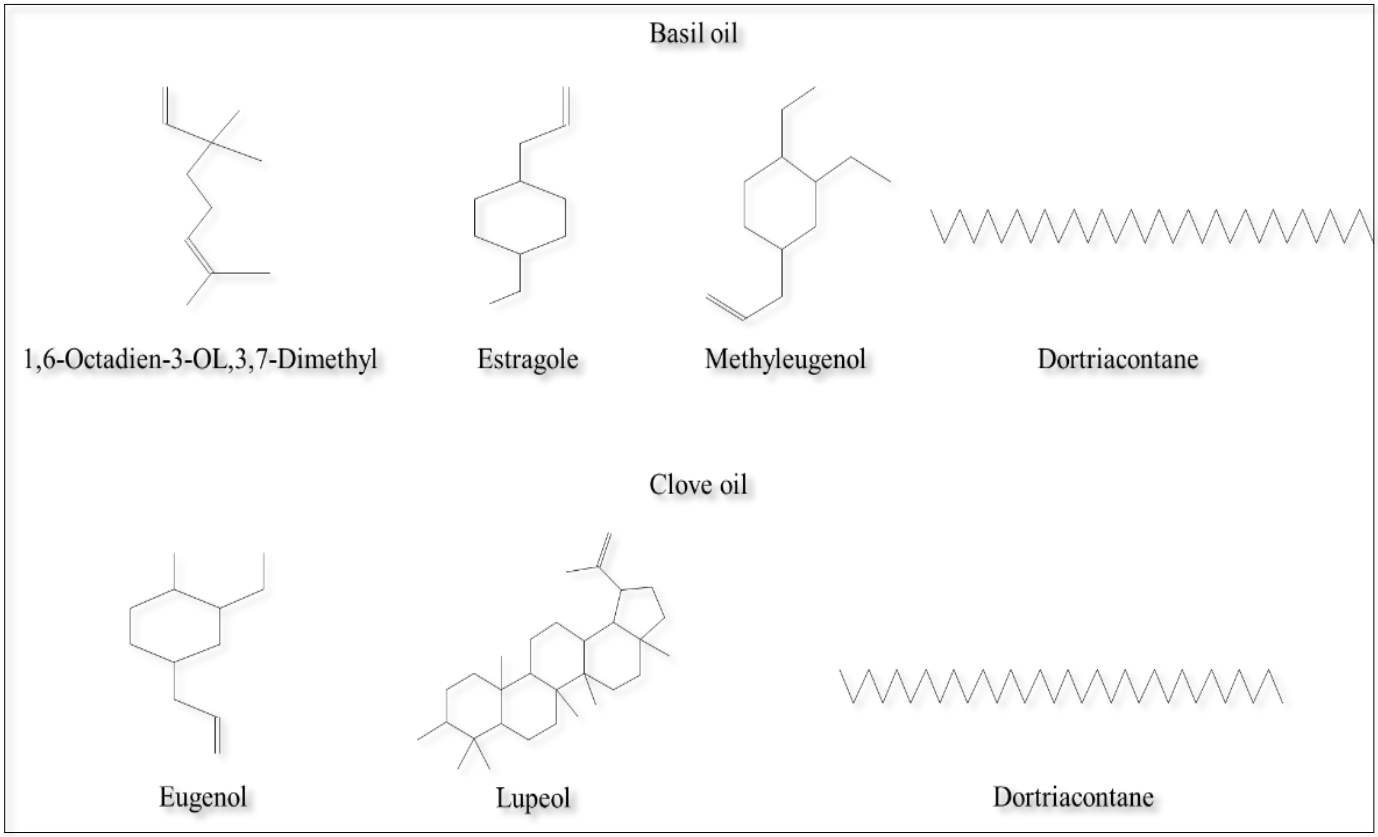

Figure 1. Chemical structure of the main bioactive compounds of basil and clove EOs.

Toxicity of the photosensitizers and EOs to *A. gossypii*

Table 3 and 4 show there was a positive correlation between mortality percentages and concentrations of the photosensitizers and EOs. Fig. 2 shows the results indicate that rose bengal at a concentration of 1×10^{-6} M caused a decrease in the adult survival by 21.43% under open environmental

conditions and the mortality percentages and concentration positively correlated after 1 h of exposure to sunlight. Similarly, rhodamine B had a lethal effect on *A. gossypii*. The adult mortality percentages were significantly increased at higher concentrations and reached their maximum value (96%) at a concentration of 8×10^{-5}M after exposure to sunlight for 1 h.

The results presented in Fig. 3 reveal the EO concentration-dependent mortality. 48 h post-treatment of the adult aphids, a concentration of 150 ppm of clove EO and basil EO exhibited a mortality of 100 and 96.67%, respectively.

Table 2. Chemical compounds of clove (*Syzygium aromaticum*) EO as identified by (GC-MS).

RT	Area %	Compound name	Match Factor (MF)
7.10	0.68	Cyclohexanol,1-Methyl-4-(1-Methylethenyl)-, cis-	882
9.43	0.62	Benzene,1-Methoxy-4-(1-Propenyl)-, (Z)	856
10.58	2.75	D-Carvone	922
13.10	0.31	1,2,3-Propanetriol,Triacetate	860
13.34	27.01	Eugenol	942
14.16	0.91	2-Propenoic acid, 3-Phenyl-,Methyl Ester	840
14.97	0.21	Alloaromadendrene	878
15.32	0.31	(R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene	785
17.30	1.63	2-Allyl-6-Methoxyphenol	893
17.53	0.60	Undecanoic acid, 10-Methyl-, Methyl Ester	907
18.64	0.58	3-Amino-4-[(1-Benzyl-2-Methoxy-2 Oxoethyl) Amino]-4-Oxobutanoic acid	689
18.73	0.62	(-)-Spathulenol	790
18.96	1.13	Diethyl Phthalate	892
20.22	0.50	Tau.-Cadinol	903
20.38	0.28	Cyclopentaneacetic acid,3-Oxo-2-Pentyl-, Methyl Ester	860
22.06	0.28	Cyclopentanetridecanoicacid, Methyl Ester	727
24.17	0.29	i-Propyl 12-Methyl-Tridecanoate	772
24.41	1.15	Neophytadiene	930
24.51	0.25	1,3,5-Triazine-2,4-Diamine,6-Chloro-N-Ethyl-	818
24.90	0.33	Cholestan-3-ol, 2-Methylene-,(3á,5à)-	827
25.27	0.39	Ethyl(9Z,12Z)-9,12-Octadecadienoate #	820
26.20	1.59	Hexadecanoic acid, Methyl Ester	900
27.03	0.66	Hexadecanoic acid	830
27.71	0.73	Ethylene brassylate	867
29.33	0.22	6,9-Octadecadienoic acid,Methyl ester	796
29.47	0.67	11-Octadecenoic acid, methyl ester	831
29.66	0.37	01297107001 Tetraneurin - A –Diol	795
30.27	0.88	6,9,12,15-Docosatetraenoic acid,Methyl Ester	810
32.11	0.53	3à,5à-Cyclo-ergosta-7,9(11),22t-triene-6á-ol	743
33.12	0.29	2-(7-Hydroxymethyl-3,11-dimethyldodeca-2,6,10-trienyl)-[1,4]benzoquinone	752
33.80	1.53	1-Phenanthrenemethanol,1,2,3,4,4a,9,10,10aoctahydro-1,4a-dimethyl-7-(1-methylethyl)-,[1S-(1à,4aà,10aá)]-	878
34.39	1.92	á-Sitosterol	852
36.74	1.52	24-Noroleana-3,12-diene	824
38.12	0.93	Methyl commate B	795
38.72	0.61	1-Heptatriacotanol	825
39.61	17.16	Lupeol	842
40.06	0.61	1-Heptatriacotanol	823
40.18	1.05	9,10-Secocholesta-5,7,10(19)-Triene-3,24,25-Triol, (3á,5Z,7E)-	833
40.52	1.79	4,8,13-Cyclotetradecatriene-1,3-Diol,1,5,9-Trimethyl-12-(1-Methylethyl)-	834
41.01	0.30	1-Heptatriacotanol	840
41.37	2.39	Dotriacontane	841
42.11	1.07	1-Heptatriacotanol	843
42.77	8.00	LUP-20(29)-ENE-3,28-Diol, (3á)-	859
43.60	6.19	Dotriacontane	851
43.88	2.05	1-Heptatriacotanol	804
44.62	3.79	Isochiapin B	802
45.03	1.53	1-Heptatriacotanol	832
45.16	0.79	Tricyclo[20.8.0.0 (7,16)] triacontane, 1(22),7(16)-Diepoxy-	816

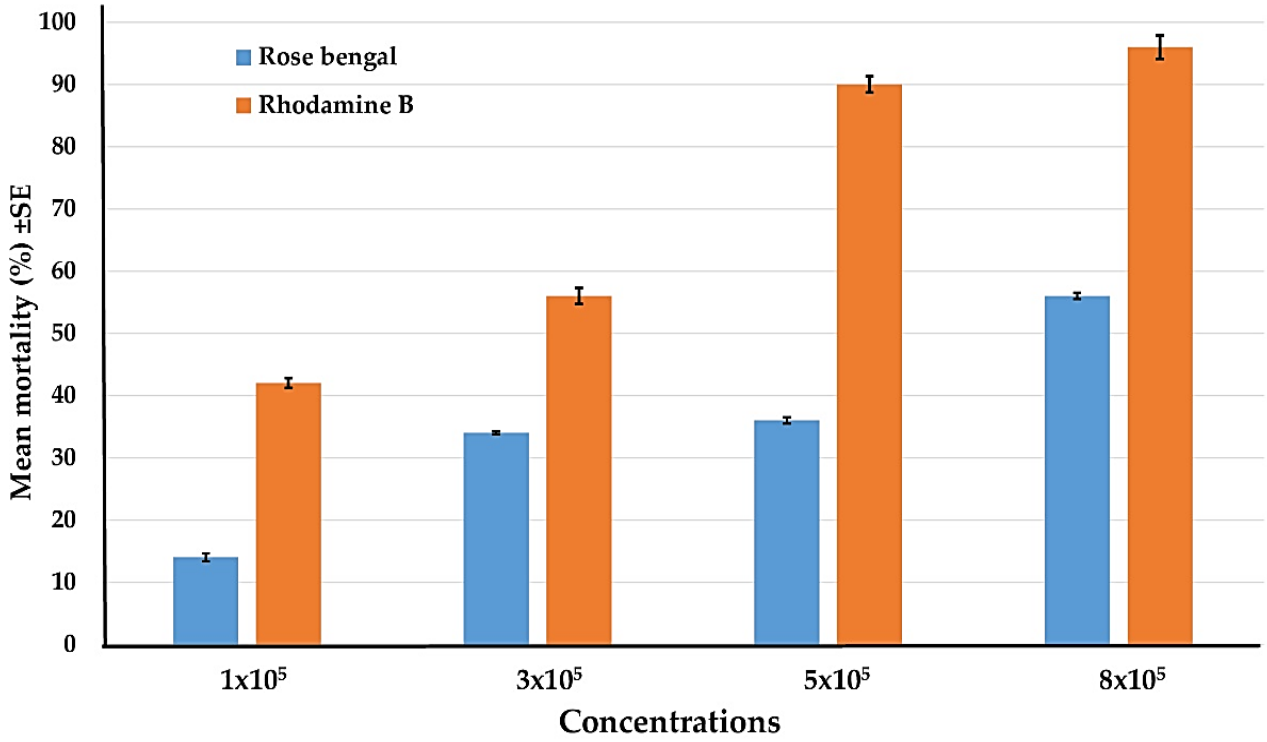

Figure 2. Mortality percentage of *Aphis gossypii* treated with different concentrations of Rose bengal and Rhodamine B in direct sunlight for 1 hour.

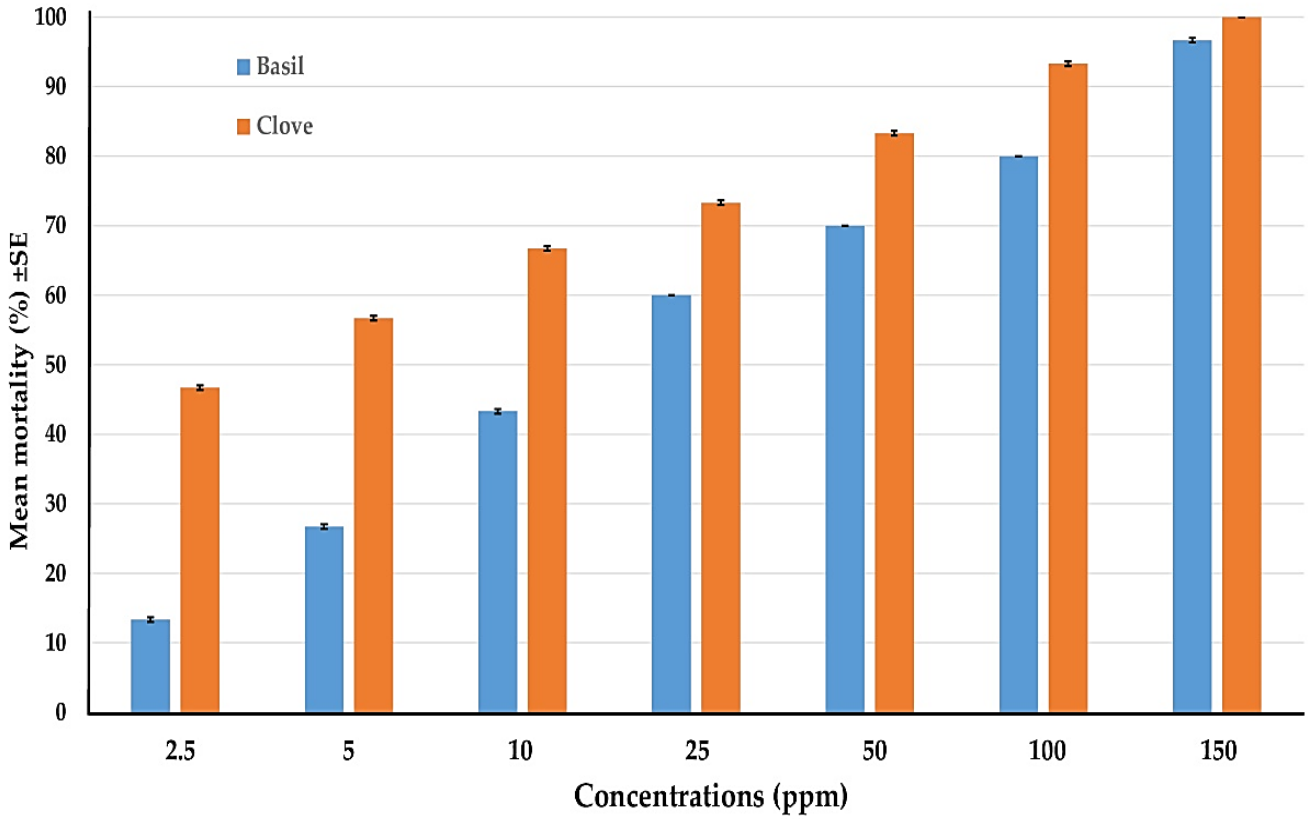

Figure 3. Mortality percentage of *Aphis gossypii* 48h post-treatment with different concentrations of basil (*Ocimum basilicum*) EO, and clove (*Syzygium aromaticum*) EO.

Table 3 shows the LC_{30} and LC_{50} of rose bengal and rhodamine B were (1.6×10^{-5} & 5.24×10^{-7}M) and (2.00×10^{-4} & 2.68×10^{-6}M), respectively. The data also indicate that rhodamine B was more toxic than rose bengal. Rose bengal recorded a toxicity index of 74.63% when compared to rhodamine B (100%).

In addition, rhodamine B was ~1.34 times more potent than rose bengal. On the other hand, clove EO was ~4.44 fold more toxic than basil EO, which had a toxicity index of 22.45%.

Table 3. Toxicity of photosensitizers (1h post-treatment) and EOs (48 hours' post-treatment) to *Aphis gossypii*.

Photosensitizer	LC_{30} (Molar) confidence limits	Toxicity index (%)	Relative potency	LC_{50} (Molar) confidence limits	LC_{90} (Molar) confidence limits	Slope±SE	χ2
Rose bengal	1.6×10^{-5} (1×10^{-5}- 1×10^{-4})	74.63	1.00	2.00×10^{-4} (4×10^{-5}- 1.5×10^{-3})	0.211 (0.1-1)	0.43±0.09	6.45
Rhodamine B	5.24×10^{-7} (1×10^{-7}- 1×10^{-6})	100	1.34	2.68×10^{-6} (1×10^{-6}- 1×10^{-5})	1×10^{-4} (1×10^{-4}- 1×10^{-3})	0.74±0.09	41.38
Essential Oil	LC_{10} (ppm) confidence limits	Toxicity index (%)	Relative potency	LC_{50} (ppm) confidence limits	LC_{90} (ppm) confidence limits	Slope±SE	χ2
Basil oil (*O. basilicum*)	1.73 (1.00-2.99)	22.54	1.00	15.46 (8.93-26.74)	137.94 (79.73-238.64)	1.41±0.12	0.45
Clove oil *(S. aromaticum)*	0.13 (0.05-0.31)	100	4.44	3.48 (1.49- 8.12)	90.202 (38.68-210.31)	0.91±0.18	1.00

Joint action

The photodynamic effect of various combinations of sub-lethal concentrations of the photosensitizers and EOs on the mortality of *A. gossypii* adults after 0.5, 1, and 1.5 h of exposure to sunlight is summarized in Table 4. After 30 min of exposure to sunlight, the mortality percentages of the adults were 12.96, 48.08, 21.15, and 71.15% for mixtures A, B, C, and D, respectively. After 1.5 h of exposure to sunlight, the mortality percentages of the adults were 33.33, 67.31, 43.31, and 92.31% for mixtures A, B, C, and D, respectively when compared with the untreated groups (UL and UD). The mortality rates, in descending order, were as follows: Mixture D > mixture B > mixture C > mixture A. It is also worth noting that there was a positive correlation between the exposure time and mortality rate for all mixtures studied.

Table 4. Photodynamic effect of mixtures of sub-lethal concentrations of photosensitizers and EOs on adult mortality percentage of *Aphis gossypii* after 0.5, 1 and 1.5 hours of sunlight exposure.

Sunlight exposure time (h)	Mortality %± SE				UL	UD
	A	B	C	D		
0.5	12.96±0.24^{a}	48.08±0.31^{b}	21.15±0.37^{c}	71.15±0.24^{d}	0.0^{e}	0.0^{e}
1.0	24.07±0.24^{a}	57.69±0.31^{b}	30.77±0.37^{c}	82.69±0.24^{d}	0.0^{e}	0.0^{e}
1.5	33.33±0.24^{a}	67.31±0.3^{b}	42.31±0.40^{c}	92.31±0.24^{d}	0.0^{e}	0.0^{e}

Data are shown as mean ± SE. Data followed by the same letter in each row are not significantly different at P<0.05 by LSD's multiple range test.

Biochemical analysis

Table 5 indicates that when *A. gossypii* adults were treated with rhodamine B, basil EO, and mixture A or C, the trehalase activity was significantly decreased when compared with the control sample. The corresponding changes were –24.35, –15.67, –24.61, and –12.23, respectively. The other treatments had no significant effect on the trehalase activity.

The protease activity of the adults treated with rhodamine B and mixture D significantly decrease to 3.37 ±0.19 and 2.93 ±0.12 µg D,L-alanine/min/mg protein, respectively. The corresponding changes were –39.52 and –47.31%, respectively when compared with the control samples. In contrast, the protease activity significantly increased after treatment with rose bengal (9.57 ±0.33), clove EO (12.63 ±0.90), mixture A (8.53 ±0.08), and mixture B (9.53 ±0.39) when compared to the control sample (5.57 ±0.23).

A significant decrease in the lipase activity was noticed in the adults treated with rhodamine B, basil EO, and clove EO when compared with the untreated group (Table 5). The decrease was –23.97, –38.01,

and –22.91%, respectively when compared to the control. However, a significant increase in lipase activity was observed upon treatment with rose Bengal, in which the elevation was +8.02%.

Table 5 also shows that rose bengal, mixture C, and mixture D significantly reduce the GST activity to 8.10 ±0.21, 5.83 ±0.22, and 6.00 ±0.25 mmol sub. conjugated/min/mg protein, respectively. The corresponding reduction was –14.74, –38.63, and –36.84%, respectively when compared with the control sample. In contrast, both EOs, mixture A, and mixture B cause a significant elevation in the GST activity (17.23 ±0.62, 17.07 ±0.30, 10.60 ±0.38, and 12.27 ±0.37 mmol sub. conjugated/min/mg protein, respectively). The corresponding elevation was +81.37, +79.68, +11.58, and +29.16%, respectively.

Table 5. Effect of Photosensitizers, EOs and their mixtures on trehalase, protease, lipase, and Glutathion-S- transferases activities in *Aphis gossypii* adults.

Treatments	Mean ±SE			
	Trehalase (µg glucose/min/ mg protein)	Protease (µg D, L-alanine /min/mg protein)	Lipase (mU/mg protein)	GST (mmole sub. Conjugated/min/mg protein)
Control	89.10±1.70^{a}	5.57±0.23^{a}	9.47±0.24^{a}	9.50±0.40^{a}
Rose bengal	85.47±1.24^{a}	9.57±0.33^{b}	10.23±0.33^{b}	8.10±0.21^{b}
Rhodamine B	67.40±2.12^{b}	3.37±0.19^{c}	7.20±0.15^{c}	9.43±0.18^{a}
Basil oil	77.03±1.24^{c}	6.03±0.18^{a}	5.87±0.18^{d}	17.23±0.62^{c}
Clove oil	92.78±1.14^{a}	12.63±0.90^{d}	7.30±0.15^{c}	17.07±0.30^{c}
Mixture A	67.17±1.69^{b}	8.53±0.08^{b}	9.37±0.08^{a}	10.60±0.38^{d}
Mixture B	84.37±6.79^{a}	9.53±0.39^{b}	8.37±0.18^{a}	12.27±0.37^{e}
Mixture C	78.20±1.25^{c}	7.60±1.11^{a}	8.63±0.08^{a}	5.83±0.22^{f}
Mixture D	87.80±1.11^{a}	2.93±0.12^{c}	9.16±0.86^{a}	6.00±0.25^{f}

Data followed by the same letter in each column are not significantly different at P<0.05 by LSD's multiple range test.

4. Discussion

The effectiveness of photodynamic sensitizers as insecticidal agents depends on light, dye, and oxygen. Oxygen is necessary for the damage that occurs in various biological systems via the production of different reactive oxygen species (ROS) (Paillous and Fery-Forgues 1994). The current results reveal that rhodamine B causes a high mortality rate (96%) in cotton aphid adults at the highest concentration of 8×10^{-5}M. However, rose bengal exerts a relatively low rate (53.85%) at the same concentration. This finding was in agreement with Fondren and Heitz (1978) who reported that rhodamine B was highly effective against the face and house fly in the absence of light. Rhodamine B, unlike rose bengal, doesn't contain any halogen atoms, but two oxygen atoms are replaced by alkyl-amino groups. These amines may be responsible for the higher toxicity observed. The higher toxicity of rhodamine B can also be attributed to being a cationic dye, which is more toxic than the anionic dye, rose bengal. On the contrary, Rose bengal is more effective than rhodamine B against 4th instar larvae of *Culex pipiens* (El-Shourbagy et al. 2018) and *Spodoptera littoralis* larvae (EL-Bendary and El-Helaly 2022). In our study, rhodamine B exhibited a toxicity index of 100 and was taken as a reference. The rapid metabolism of this dye may form the basis of its high toxicity. It was nearly 1.34 times more potent than rose bengal. When the concentrations of both dyes and sunlight exposure time increase, the mortality of adults increases until it reached 96% after 1 h of treatment with rhodamine B.

When the adults were treated with EOs, the highest concentration (150 ppm) results in a mortality rate of 100% for clove and 96.67% for basil. These findings were consistent with those of Zohry et al. (2020) against the Granary Weevil, *Sitophilus granaries*. Similar results were also obtained by Pumnuan et al. (2017) who reported that clove EO caused remarkably high mortalities against adult *A. gossypii*.

Previous studies have suggested that different insect species respond differently to the extract of plants. For instance, the toxicity evaluation of seven plant extracts to four insects belonging to 4 different orders including the aphid, *Acyrthosiphon pisum*, showed that it was the most susceptible to all of the extracts tested with 100% mortality observed after 24 h (Khan et al. 2017). The efficacy of some plant EOs, including coriander, lavender, fennel, oregano, juniper, and clove against *A. gossypii* has been evaluated and it was found that all of the tested EOs show a high insecticidal effect on cotton aphid (Atanasova and

Leather 2018). The findings of Sayed et al. (2020) suggest that *Ochradenus baccatus* can be used as an effective insecticide in IPM programs against *Aphis craccivora*. Plant extracts and EOs are new technology in insecticide formulations that can be utilized in pest control (Moustafa et al. 2021; Awad et al. 2022; Aly 2023). Our results show that the toxicity indices of clove and basil Eos were 100 and 22.45%, respectively. The high toxicity index of clove EO can be attributed to the rapid penetration of the oil through the aphid's cuticle, thus contacting the trachea nerve endings leading to rapid death due to a neurotoxic effect (Bessette et al. 2013). Our findings also show that clove EO was ~4.44 times more potent than basil EO.

The effect of various EO combinations with the photosensitizers were also studied in this work. It was found that a combination of clove EO and Rhodamine B (mixture D) exhibits the highest potency against adult aphids causing a mortality rate of 92.31%. While basil EO in combination with rose bengal (mixture A) exhibited the lowest toxicity effect with a mortality rate of 33.33%.

Chitin, as an essential constituent of cuticle and peritrophic membrane, has a vital role in insect development and growth regulation. Chitin biosynthesis and degradation in insects are dynamic and complex processes that are regulated by various types of enzymes including trehalase, which is important in chitin metabolism. As reported in many previous studies, the inhibition of such enzymes may have potential application in insect pest control (Adhav et al. 2018).

Our results show that rose bengal, clove EO, mixed rose bengal and clove EO (mixture B), and mixed rhodamine B and clove EO (mixture D) have no effect on the trehalase activity in cotton aphid adults. However, rhodamine B, basil EO, mixed rose bengal and basil EO (mixture A), and mixed rhodamine B and basil EO (mixture C) result in a significant reduction in trehalase activity. In the same context, trehalase in the tomato leafminer *Tuta absoluta* was the most affected enzyme with a significantly high level after treatment with clove EO (Taha et al. 2015). The reduction in trehalase activity observed in adult aphids treated with rhodamine B during exposure to sunlight indicates that the generated reactive oxygen species (ROS) induce further damage to the enzyme. In addition, the decrease in enzyme activity can also be attributed to the damage in tissues responsible for its secretion, primarily the midgut.

Proteases are activated during stressful conditions, indicating a possible relationship between the reduction in energy production, inactivation of oxidative enzymes, and acceleration of proteolysis (Henderson et al. 1985). In general, the higher protease activity indicates a deep protein loss that causes disassembly and disorganization of structural proteins in tissues during toxicity. Our results have revealed a significant increase in the protease activity of cotton aphids treated with rose bengal, clove EO, and mixtures A and B when compared with the control sample. Nonetheless, our results reveal that the protease activity significantly decreases in the aphids treated with rhodamine B or mixed rhodamine B and clove EO (mixture D). An investigation stated a significant reduction in the protease activity of the rice leaf roller, *Cnaphalocrocis medinalis*, fed on a diet containing a botanical extract, including neem seed and *Vitex negundo* leaf extract (Senthil-Nathan et al. 2006). Similar findings indicate that the protein content reduces in *Culex pipiens* larvae treated with some plant extracts due to their interference with natural protein synthesis (Farag et al. 2021).

Lipases hydrolyze the outer linkages of fat molecules (Yazdani et al. 2014). In the present study, there was no significant decrease in lipase activity of aphid adults treated with mixures A, B, C, and D when compared to the control sample. However, the decrease was obvious in those treated with Rhodamine B, basil EO, and clove EO. The reduction in the midgut lipase activity can be attributed to the disturbed digestion and absorption processes (Napoleão et al. 2013). On the contrary, the lipase activity of adult aphids treated with rose bengal increased. This finding was in accordance with Sujatha et al. (2010), who noted that *Pedalium murex* L. extract increased *the* lipase activity in *Spodoptera litura*.

GSTs are detoxification enzymes and protect cells from chemical toxicants and oxidative stress by assisting the excretion of lipophilic and electrophilic compounds out of the cell (Hayes and Pulford 1995). By detoxifying xenobiotic compounds, detoxification enzymes, such as GST, are widely known for assisting plant-eating insects in maintaining their physiological functions (Feyereisen 2012). Examples of xenobiotics are pesticides and harmful secondary metabolites produced by host plants (Vashist and Ahmad 2011). Multiple modes of action of plant extracts and EOs against insects include neurotoxicity, suppression of insect development and digestive enzymes, and the inhibition of GST enzymes (Park and Tak 2016).

In this study, the GST activity increases in adult aphids treated with the EOs and photosensitizers when compared to the control sample. Basil EO, clove EO, and mixtures A and B revealed a significant elevation in the GST activity. These results were consistent with Metwally et al. (2022) who evaluated the insecticidal activity of EOs derived from *Proserpinaca palustris* and *Terminalia chebula* against *Aphis fabae* and found a significant increase in the reduction percentage of GST enzyme in the treated aphid adults. Therefore, it was confirmed that GSTs have a role in the detoxification and/or metabolism of basil EO, clove EO, and mixtures A and B in cotton aphid adults.

Contrarily, treating aphid adults with rose bengal, mixture C, and mixture D reduces the GST activity. Similar results were obtained by Abd El-Aziz and El-Sayed (2009), in which a reduction in GST activity was observed in the last larval instar of the confused flour beetle, *Tribolium confusum* treated with six EOs. In addition, Phankaen et al. (2017) found that *Tribolium castaneum* GST was inhibited by caffeine from a *Coffea arabica* extract. Similarly, *Tagetes minuta* oil prevented *Plutella xylostella* GST from being produced (Dolma et al. 2021). The GST reduction recorded in our study and elsewhere suggests that GST has no role in the detoxification of the above-mentioned compounds and thus, explains the susceptibility of *A. gossypii* to them.

5. Conclusions

The EOs of basil and clove, as well as two photosensitizers, rose bengal and Rhodamine B, alone and in combination, have considerable toxic effects against *Aphis gossypii*. In addition, these compounds affected some biochemical parameters such as the trehalase, protease, lipase, and GST activities. The clove EO and rhodamine B mixtures exhibited the greatest effectiveness against adult aphids and showed a synergistic effect. Synergistic effects were produced by the interaction of the EOs and photosensitizers. Accordingly, our results provide a good base for developing insecticides based on these two EOs and the two photosensitizers. These combinations may be the subject of additional research in the future to determine how well they work in integrated pest management programs.

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