Naturally Occurring Insecticides from the Marine Sponge *Xestospongia testudinaria* to Control the Whitefly *Bemisia tabaci* (Genn.) and the Aphid *Aphis gossypii* (Glover)

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The insecticidal potential of the marine sponge *Xestospongia testudinaria* extracts against the whitefly *Bemisia tabaci* and the aphid *Aphis gossypii* under laboratory conditions were examined after 24 and 72 hrs. of treatments. The bioassay-guided isolation afforded nine structurally diverse marine compounds: xestosterol (1), 18-bromoocotadeca-(9E,17E)-dien-7,15-dinoic acid (2), 18,18-dibromo-(9E)-octadeca-9,17-dien-5,7-diynoic acid (3), 16-bromo(7E,11E,15E)hexadeca-7,11,15-trien-5,13-dinoic acid (4), methyl 18-bromoocotadeca-(9E,17E)-dien-7,15-dioate (5), 2-methylmaleimide-5-oxime (6), maleimide-5-oxime (7), 2'-deoxythymidine (8) and 2'-deoxyuridine (9). The chemical structures were determined on the basis of detailed spectroscopic analyses (ESI-MS and ¹H, ¹³C-NMR). Maleimide-5-oxime (7) was the most potent one against 3rd instar nymphs of *B. tabaci* under laboratory conditions after 24 and 72 hrs of treatments, with LC₅₀ values of 175.26 and 7.62 ppm, respectively. While against *Aphis gossypii*, 18-bromoocotadeca-(9E,17E)-dien-7,15-diynoic acid (2) recorded the highest LC₅₀ values of 1.54 and 0.001 ppm after 24 and 72 hrs of treatments against *Aphis gossypii*, respectively. Structure-activity relationship revealed the effectiveness of maleimides and brominated polyacetylenic lipids.

**Keywords:** Marine extracts, Insecticidal activity, Sucking pests

**INTRODUCTION**

Among cotton sucking pests, whiteflies *Bemisia tabaci* and aphid *Aphis gossypii* are of major importance. These sucking pests are responsible for indirect and direct yield losses by sucking the sap from leaves and also by transmitting different viruses. Both species secrete honeydew on leaves resulting in sooty mold growth, which can reduce the quality and marketability of harvested products. Honeydew falling on open bolls make the lint sticky which creates problems during ginning (Torres et al. 2003; Zidan, 2012).

The high pesticides toxicity and their residues in soil, water resources and crops as a result of their non-biodegradable properties create hazard impacts on human health, environment and beneficial non-targeted organisms (Joseph et al. 2010; Dawidar et al. 2014; Mostafa et al. 2017).

Consequently, there is a great interest in developing new sustainable and eco-friendly insecticides to manage pest populations with less toxicological risks through using natural products (Mostafa et al. 2017). The marine environment is an exceptional rich reservoir of biologically active metabolites, which may be safer alternatives in contrast to synthetic pesticides as they are known to have minimal environmental impact and danger to consumers (Peng et al. 2003; Abou-Elela et al. 2009; Joseph et al. 2010).

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The barrel sponge *Xestospongia testudinaria* (Haplosclerida, Petrosiidae) contains diverse interesting secondary metabolites such as brominated polyacetylenic lipids (Jiang *et al.* 2011; Liu *et al.* 2011; El-Gamal *et al.* 2016), alkaloids, sterols, terpenoids and xestoquinones (Jiang *et al.* 2011). Considering these components, the sponge was reported to possess remarkable biological activities, including antifungal, antibacterial, Na+/K+ ATPase inhibition and HIV-1 integrase inhibition (Liang *et al.* 2014), cytotoxic activity (El-Gamal *et al.* 2016), anti-inflammatory, antioxidant and immunomodulatory effects (El-Shitany *et al.* 2015).

The current study aimed to assess the potential of marine natural products extracted from the sponge *Xestospongia testudinaria* as naturally occurring insecticidal agents and to identify the bioactive ingredient of these extracts.

**MATERIALS AND METHODS**

**Instruments**

NMR spectra were recorded on either Bruker AMX 400 or JEOL 500 MHz, $^{13}$C-NMR spectra were recorded at JEOL 125 MHz. Chemical shifts are given in δ (ppm) relative to TMS as internal slandered. ESI*MS* spectra were performed on UPLC MS-MS $^{1}$H$^{2}$O* “USA* with Bruker microOTOF and TQ detector. GC/MS analysis was conducted on a Varian GC interfaced to Finnegan SSQ 7000 for MS identification of the GC components, Mass Selective Detector (SMD) with ICIS V2.0 data system.

**Chemicals**

Normal column chromatography was performed on silica gel Merck grain size 0.2-0.063 mm; thin layer chromatography and preparative TLC were carried out on silica gel Merck GF 254 precoated plates on aluminum sheets 20×20 cm. Pet. ether (60 -80°C), methylene chloride, ethyl acetate and methanol solvents were purchased from Adwic company, Egypt.

**Sponge sample**

The sponge *Xestospongia testudinaria* was collected from deep water (fifteen m depth) of Sharm Obhur (21°29′31″N 39°11′24″E), Jeddah, Saudi Arabia, and was characterized from Faculty of Maritime Studies, King Abdulaziz University. A Voucher sample (JAD 02013) has been charged at the Chemistry Department, Faculty of Science, King Abdulaziz University.

**Extraction and isolation**

The freeze-dried sponge (180 g) was extracted using solvent mixture CH$_2$Cl$_2$/MeOH (1:1 v/v, 2 x 6L) at room temperature. A viscous reddish oil extract (15 g) was obtained. A part of the extract (8 g) was subjected to normal silica gel column chromatography, eluted with gradient increasing polarities from petroleum ether to ethyl acetate then methanol. The effluent was incorporated to five main fractions (A-E), based on their TLC pattern. Fraction A was obtained with pet. ether/ethyl acetate (1:1) purified on silica gel PTLC, developed by pet. ether/ethyl acetate (17:1) to afford five compounds, (1) (42 mg, $R_f$ = 0.69), (2) (37 mg, $R_f$ = 0.38), a binary mixture of (3), (4) (28 mg, $R_f$ = 0.26) and (5) (24 mg, $R_f$ = 0.92). Fraction C was obtained by 100% ethyl acetate purified on PTLC silica gel using CH$_2$Cl$_2$/MeOH (9:1), to yield two compounds (6) (31 mg, $R_f$ = 0.47) and (7) (35 mg, $R_f$ = 0.39). Fraction D was obtained by ethyl acetate/MeOH (9:1) separated using the same previous solvent system in fraction C to yield two compounds (8) (36 mg, $R_f$ = 0.27) and (9) (27 mg, $R_f$ = 0.20).

**Xestosterol (1)**

White powder. $^1$H NMR (CDCl$_3$): δ$_t$ 6.36 (3H, s, H-18), 1.00 (3H, s, H-19), 0.94 (3H, d, J=6.6 Hz, H-21), 1.82 (1H, m, H-25), 0.80 (6H, t, J=7.3 Hz, H-29, H-30), 3.52 (1H, m, H-3), 5.35 (1H, brs, H-6), 2.27 (1H, m, H-4a), 2.23 (1H, m, H-4b), 4.68 (1H, brs, H-28a), 4.76 (1H, brs, H-28b). El-MS: $m/z$ (rel. int.) 426 (5%) [M$^+$], 411 (6%) [M-CH$_3$]$^+$, 314 (100%) [C$_{22}$H$_{34}$O]$^+$, 281 (43%) [C$_{21}$H$_{29}$]$^+$, 271 (28%) [C$_{19}$H$_{27}$O]$^+$, 229 (33%) [C$_{17}$H$_{26}$]$^+$, 173 (13%) [C$_{13}$H$_{17}$]$^+$, 55 (33%) [C$_4$H$_7$]$^+$.

**18-Bromoocotadeca-(9E,17E)-dienen-7,15-diynoic acid (2)**

White powder. ESI-MS (m/z), [M-H]$^-$ 349 (C$_{18}$H$_{20}$O$_2$Br$^-$), 351 (C$_{18}$H$_{22}$O$_2$Br$^-$). $^1$H NMR (CDCl$_3$): δ$_t$ 6.57 (1H, d, J=14 Hz, H-18), 6.16 (1H, dt, J=14, 2.2 Hz, H-17), 6.01 (1H, m, H-10), 5.45 (1H, brd, J=15.7 Hz, H-9), 2.46 (2H, t, J=7.3 Hz, H-2), 2.30 (2H, m, H-6), 2.25 (2H, m, H-14), 2.09 (H, q, J=6.9 Hz, H-11a), 2.27 (H, q, J=6.9 Hz, H-11b), 1.67 (2H, m, H-3). $^{13}$C-NMR: 167.0 (C-O), 110.2 (C-9), 142.6 (C-10), 88.6 (C-7), 79.2 (C-8), 117.9 (C-17), 117.2 (C-18), 92.7 (C-15), 77.4 (C-16), 34.6 (C-2), 24.6 (C-3), 28.2 (C-4), 28.3 (C-5), 19.3 (C-6), 32.4 (C-11), 28.0 (C-12), 27.7 (C-13), 19.2 (C-14).

**18,18-dibromo-(9E)-octadeca-9,17-dienoic,5,7-dynoic acid (3)**

White solid. ESI-MS (m/z), [M-H]$^-$ 427 (C$_{18}$H$_{22}$O$_2$Br$_2$). $^1$H NMR (CD$_3$OD): δ$_t$ 6.48 (1H, t, J=7.4 Hz, H-17), 6.24 (dt, J=15.8, 7.1 Hz, H-10), 5.48 (brd, J=15.8 Hz, H-9), 2.26 (t, 2H, J=7.3 Hz, H-2), 2.30 (2H, td, J=7.1, 1.5 Hz, H-16), 1.78 (2H, quin, J=7.3 Hz, H-3), 2.30 (2H, td, J=7.3, 1.5 Hz, H-4), 2.11 (2H, m, H-11), 1.43 (4H, m, H-12, 15).

**16-bromo (7E,11E,5E)hexadeca-7,11,15-triene-5,13-dynoic acid (4)**

White solid. ESI-MS (m/z), [M-H]$^-$ 318.97 (C$_{16}$H$_{22}$O$_2$Br$^-$). $^1$H NMR (CD$_3$OD): δ$_t$ 6.79 (1H, d, J=14 Hz, H-16), 6.37 (1H, dd, J=14, 2.3 Hz, H-15), 6.14 (1H, dt, J=16, 6.8 Hz,
H-11), 5.95 (1H, dt, J=15.9, 6.8 Hz, H-8), 5.61 (1H, dd, J=16, 2.2 Hz, H-12), 5.49 (1H, brd, J=15.9 Hz, H-7), 1.80 (2H, quin, J=7.3 Hz, H-3), 2.35 (2H, t, J=7.3 Hz, H-4), 2.19 (4H, m, H-9, 10).

**Methyl-18-bromoocatodeca-(9E,17E)-dione-7,15-diynoate (5)**

White powder. 1H-NMR (CDCl3): δH 6.50 (1H, d, J=14 Hz, H-18), 6.10 (1H, d, J=14 Hz, H-17), 5.95 (1H, dt, J=15.6, 6.6 Hz, H-10), 5.38 (1H, brd, J=15.6 Hz, H-9), 2.26 (2H, m, H-6), 2.26 (2H, m, H-14), 1.63 (2H, m, H-3), 3.59 (3H, s, OCH3).

**2-Methylmaleimide-5-oxime (6)**

Colourless crystals. 1H-NMR (CD3OD): δH 7.20 (1H, d, J=1Hz, H-4), 1.80 (d, J=1 Hz, CH3).

**Maleimide-5-oxime (7)**

Colourless crystals. 1H NMR (CD3OD): δH 5.59 (1H, d, J=7 Hz, H-3), 7.39 (1H, d, J=7 Hz, H-4). 13C NMR (CD3OD): 167.4 (C=O), 101.7 (C-3), 143.6 (C-4), 153.5 (C-5).

**2'-deoxythymidine (8)**

Colourless needles, 1H NMR (CD3OD): δH 1.85 (3H, d, J=0.9 Hz, CH3), 7.79 (1H, d, J=1Hz, H-4), 6.25 (1H, t, J=7 Hz, H-7), 2.20 (1H, m, H-8a), 2.18 (1H, m, H-8b), 4.37 (1H, quin, J=3.4 Hz, H-9), 3.87 (1H, q, J=3.4 Hz, H-10), 3.76 (1H, dd, J=12, 3.4 Hz, H-11a), 3.69 (1H, dd, J=12, 3.4 Hz, H-11b).

**2'-deoxyuridine (9)**

Colourless needles, 1H NMR (CD3OD): δH 5.67 (1H, d, J=8.1 Hz, H-3), 7.96 (1H, d, J=8.1 Hz, H-4), 6.25 (1H, t, J=6.8 Hz, H-7), 2.20 (1H, m, H-8a), 2.18 (1H, m, H-8b), 4.36 (1H, quin, J=3.4 Hz, H-9), 3.90 (1H, q, J=3.4 Hz, H-10), 3.75 (1H, dd, J=12.1, 3.4 Hz, H-11a), 3.69 (1H, dd, J=12.1, 3.4 Hz, H-11b), 13C NMR (CD3OD): 166.2 (C-2), 102.6 (C-3), 142.5 (C-4), 86.6 (C-7), 41.3 (C-8), 72.3 (C-9), 88.9 (C-10), 62.8(C-11).

**Insect collection and rearing**

The culture of *B. tabaci* was maintained on unsprayed potted plants of cotton variety, var. Giza 86 caged in a separate muslin cloth cages (1.5x1.5 x 1.5 m) in the greenhouse of Faculty of Agriculture farm, Mansoura University, Egypt. For this purpose, whitefly adults were collected from the field using an aspirator and were released on the caged cotton plants in the greenhouse. These cages were preserved in a greenhouse at 25-35°C, 55-75% R.H. and natural light.

*A. gossypii* were collected from naturally infested cotton plants which were unsprayed before with any pesticides in greenhouse of the farm of Faculty of Agriculture, Mansoura University, Egypt.

**Bioassays**

Bioassays were conducted on 3rd instar nymphs of *B. tabaci* using spray method technique as described by Cruz-Estrada *et al.* (2013). Cotton leaves with nymphs were detached and rounded sections (~2 cm in diameter) that had at least 30 nymph individuals were marked. All fractions and isolated compounds were formulated as an emulsion in water containing 0.1% triton X-100. A series of five diluted concentrations of each naturally components were prepared and sprayed immediately after preparation using a plastic hand atomizer. Control treatment was sprayed with 0.1% triton X-100 in water. The marked leaves of each treatment and control were deposited individually after spraying process on a bed of 2% agar in 9 cm-diameter Petri dishes to keep moisturized. Each treatment was replicated three times. Petri dishes containing leaves were incubated under laboratory conditions at 24 ± 3°C, 75 ± 8 % R.H. and 12:12 hrs L:D. Mortality evaluations were carried out after 24 and 72 hrs of extracts application.

One hundred individuals of *A. gossypii* were counted on an infested leaf and then placed in a Petri-dish (15 cm in diameter). Each infested leaf with the chosen number was treated using spraying method as described before in *B. tabaci* bioassay. Each treatment was replicated three times. Mortality was evaluated after 24 and 72 hrs of treatment.

**Statistical analysis**

The average of mortality percentages were corrected using Abbott's formula (Abbott, 1925) and statistically analyzed according to Finney (1971) to determine LC50, LC90 and slope values. Toxicity index was calculated using Sun's equation for different fractions or isolated compounds by comparing these materials with the most effective one (Sun, 1950).

**RESULTS AND DISCUSSION**

Our interest of identifying new leads that could serve as prototype for eco-friendly natural insecticides has motivated us to examine the insecticidal activity of the marine sponge *X. testudinaria* natural components. Strong insecticidal properties of marine sponge, *X. testudinaria* were obtained after 24 and 72 hrs of exposure with LC50 728.53 and 31.15 ppm for *B. tabaci*, and 3.41 and 0.019 for *A. gossypii*, respectively. Bioassay-guided fractionation has led to separate nine marine natural products, identified and examined for their insecticidal activity against 3rd instar nymphs of *B. tabaci* and *A. gossypii*.

Column and thin layer chromatographic separation techniques led to isolate nine compounds (Fig.1) belonging to different classes. Five compounds belonging to sterols and brominated polyacetylenic lipids were
isolated from fraction A, purified and identified using different spectral analysis.

Compound (1) was isolated as a white powder, with a molecular formula C₃₀H₅₀O obtained from EI-MS spectrum at m/z 426 [M⁺]. Examination of ¹H NMR spectrum as well as the MS fragmentation pattern suggested that compound (1) is Xestosterol that was reported previously by El-Gamal et al. (2016) from the same sponge.

X. testudinaria is rich in brominated polyacetylenic compounds which possess a wide range of biological activities (Liu et al. 2011). ¹H and ¹³C NMR spectra of compound (2) contained signals of two units of trans disubstituted ethylene, adjacent to an acetylenic bond, in between four adjacent methylene groups, beside, a pentamethylene group accommodated between a carboxyl and an acetylenic groups. The NMR data together with [M-H]⁺ ions at m/z 349 and 351, in approximately equal abundance, in the negative ESI-MS (corresponding to the molecular formula C₁₉H₂₇BrO₂) allow us to confirm that compound (2) is 18-bromoocadeca-(9E,17E)-diene-7,15-diynoic acid (2). Spectral analyses of compound (2) were identically matched with that previously reported from X. testudinaria by Pham et al. (1999) and Bourguet-Kondracki et al. (1992).

¹H NMR spectrum of ester (5) contained signals identical to those of the acid (2) except one singlet signal appeared at δ 5.39 (3H, s, OCH₃), confirming that compound (5) is the methyl ester of (2) so, (5) is methyl 18-bromoocadeca-(9E,17E)-diene-7,15-diynoate, which was identified from the same sponge previously by Qu and Tucker (1991).

The obtained binary mixture of (3) and (4) was examined by negative ESI-MS that gave [M-H]⁺ ions at m/z 426.99, 428.87 and 430.82 in ratio (1:2:1) corresponding to the molecular formula (C₁₉H₂₇BrO₂) of (3), and [M-H]⁺ ions at m/z 318.97 and 321 in ratio (1:1) corresponding to the molecular formula (C₁₉H₂₇BrO₂) of (4). The assignments of the ¹H NMR signals revealed the presence of isolated terminal trisubstituted ethylene and endyne systems corresponding to the molecular formula of (3) while compound (4) was characterized by additional ethylenic system beside the trimethylene group between a carboxyl and an acetylenic groups. This (3) and (4) were identified as 18,18-dibromo-(9E)-octadeca-9,17-dien-5,7-diynoic acid, previously published by Akiyama et al. (2013), and 16-bromo(7E,11E,15E)hexadeca-7,11,15-trien-5,13-diynoic acid, published before by El-Gamal et al. (2016), respectively.

Compounds (6) and (7), as colorless crystals, were isolated from fraction C. ¹H and ¹³C NMR spectra indicate the maleimide structure. So the maleimide oximes (6) and (7) were identified to be 2-methyl maleimide-5-oxime (6) and maleimide-5-oxime (7), published previously by El-Gamal et al. (2016).

Purification of fraction D resulted in separation of two compounds (8) and (9), as colorless needles. Careful examination of ¹H and ¹³C NMR spectra gave a pyrimidine base moiety in accordance with the nucleosides 2'-deoxythymidine (8) and 2'-deoxyuridine (9) (Kamori et al. 1980), which reported for the first time from X. testudinaria.

Fig. 1: Isolated compounds from Xestospongia testudinaria

Insecticidal activity of Xestospongia testudinaria fractions and isolated compounds to 3rd instar nymphs of whiteflies Bemisia tabaci

Natural products of marine origin are actually being studied as alternative eco-friendly insecticides to manage crop pests (Peng et al. 2003; El Sayed et al. 1997).

Among the different X. testudinaria fractions which evaluated against 3rd instar nymphs of B. tabaci under laboratory conditions, Fraction C was the most potent at LC₉₀ and LC₅₀ levels (Table 1) followed by fractions A, D, Crude extract, B and finally E, respectively after 24 hrs of treatment. While after 72 hrs of treatment Crude extract exhibited the highest degree of efficiency followed by fractions D, C, E, A then the lowest active fraction B, respectively (Table 1).
The insecticidal activity of natural extracts may be attributed to chemical composition of their secondary metabolites content. The most effective fractions A, C and D were investigated chemically by using chromatographic and spectral analyses to search for promising active ingredients could serve as natural insecticides. Nine isolated and purified compounds were examined against 3rd instar nymphs of B. tabaci under laboratory conditions after 24 and 72 hrs of treatments. Results obtained in Table 2 revealed that compound (7) was which isolated from fraction C (the most effective fraction) was the most potent on the basis of toxicity index followed by compounds (6), (3, 4), (2), (5), (9), (8) and (1) respectively after 24 hrs of treatment. The potency arrangement after 72 hrs of treatment were compound (7) followed by compounds (6), (3, 4), (2), (5), (1), (9) and (8), respectively (Table 2).

**Table (1): Toxicity of Xestospongia testudinaria fractions against 3rd instar nymphs of Bemisia tabaci after 24 and 72hrs of treatment**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>LC50 (ppm) and confidence limits at 95%</th>
<th>Slope ± SE</th>
<th>Toxicity index*</th>
<th>LC50 (ppm) and confidence limits at 95%</th>
<th>Slope ± SE</th>
<th>Toxicity index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Extract</td>
<td>728.53/1005.37 (24 hrs), 624.68/9059.49 (72 hrs)</td>
<td>1.528±0.304</td>
<td>27.77</td>
<td>31.15</td>
<td>138.66/488.64</td>
<td>1.547±0.453</td>
</tr>
<tr>
<td>Fraction A</td>
<td>369.68/4213.14 (24 hrs), 900.00/5716.60 (72 hrs)</td>
<td>1.213±0.263</td>
<td>54.70</td>
<td>83.33</td>
<td>112.51</td>
<td>488.34/4500.91</td>
</tr>
<tr>
<td>Fraction B</td>
<td>787.22/10510.07 (24 hrs), 257.60/7265.60 (72 hrs)</td>
<td>1.139±0.276</td>
<td>25.70</td>
<td>123.15</td>
<td>75.76</td>
<td>172.10</td>
</tr>
<tr>
<td>Fraction C</td>
<td>122.80/2413.89 (24 hrs), 148.02/2756.60 (72 hrs)</td>
<td>1.00±0.00</td>
<td>100.00</td>
<td>7.61</td>
<td>17.11</td>
<td>76.88</td>
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<tr>
<td>Fraction D</td>
<td>387.57/2755.42 (24 hrs), 328.67/872.68 (72 hrs)</td>
<td>1.05±0.387</td>
<td>52.22</td>
<td>27.77</td>
<td>47.28</td>
<td>328.67</td>
</tr>
<tr>
<td>Fraction E</td>
<td>618.35/3055.12 (24 hrs), 230.91/6562.62 (72 hrs)</td>
<td>0.81±0.298</td>
<td>24.72</td>
<td>9.96</td>
<td>19.18</td>
<td>76.63</td>
</tr>
</tbody>
</table>

**Table (2): Toxicity of Xestospongia testudinaria isolated compounds against 3rd instar nymphs of Bemisia tabaci after 24 and 72hrs of treatment**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Isolated compound</th>
<th>LC50 (ppm) and confidence limits at 95%</th>
<th>Slope ± SE</th>
<th>Toxicity index*</th>
<th>LC50 (ppm) and confidence limits at 95%</th>
<th>Slope ± SE</th>
<th>Toxicity index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(1) 679.22/7996.47 (24 hrs), 3290.75/3467.9E4 (72 hrs)</td>
<td>0.13±0.389</td>
<td>14.79</td>
<td>40.48</td>
<td>211.22</td>
<td>603.35/662.62</td>
<td>1.373±0.398</td>
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<td>(2) 190.12/603.03 (24 hrs), 985.37/6100.00 (72 hrs)</td>
<td>0.13±0.367</td>
<td>81.34</td>
<td>157.51</td>
<td>40.43</td>
<td>301.45</td>
<td>1.037±0.306</td>
</tr>
<tr>
<td></td>
<td>(3, 4) 718.96 (24 hrs), 463.93/9758.7E3 (72 hrs)</td>
<td>0.12±0.267</td>
<td>79.85</td>
<td>14.57</td>
<td>30.51</td>
<td>90.85</td>
<td>518.31</td>
</tr>
<tr>
<td>C</td>
<td>(5) 170.24/1572.29 (24 hrs), 2018.83/1474.6E4 (72 hrs)</td>
<td>0.08±0.255</td>
<td>56.10</td>
<td>3.91</td>
<td>37.77</td>
<td>106.33</td>
<td>619.94</td>
</tr>
<tr>
<td>D</td>
<td>(6) 6618.10 (24 hrs), 5291.98/6399.2E3 (72 hrs)</td>
<td>0.08±0.258</td>
<td>85.03</td>
<td>19.09</td>
<td>17.96</td>
<td>54.65</td>
<td>557.75</td>
</tr>
<tr>
<td></td>
<td>(7) 4574.79 (24 hrs), 963.66/1148.4E3 (72 hrs)</td>
<td>0.09±0.259</td>
<td>89.03</td>
<td>3.91</td>
<td>17.96</td>
<td>54.65</td>
<td>557.75</td>
</tr>
<tr>
<td></td>
<td>(8) 939.85 (24 hrs), 7670.49 (72 hrs)</td>
<td>1.50±0.468</td>
<td>18.65</td>
<td>455.98</td>
<td>255.35</td>
<td>1515.1</td>
<td>3273.84</td>
</tr>
<tr>
<td></td>
<td>(9) 812.99 (24 hrs), 493.81/2359.20 (72 hrs)</td>
<td>1.14±0.278</td>
<td>21.56</td>
<td>267.40</td>
<td>267.40</td>
<td>3861.77</td>
<td>1.103±0.356</td>
</tr>
</tbody>
</table>

**Table (4): Insecticidal activity of Xestospongia testudinaria fractions and isolated compounds to Aphis gossypii**

The results showed that the most toxic fraction was A followed by C, B, Crude extract, D and finally E, respectively at LC50 level after 24 hrs of initial application. Taking the toxicity index into consideration after 72 hrs of treatment Crude extract was the most toxic against A. gossypii followed by fractions A, C, B, E and D, respectively (Table 3). Table (4) showed the susceptibility of A. gossypii to the nine isolated compounds after 24 and 72 hrs of treatment. Data revealed that compound (2) which was isolated from fraction A (most active fraction) exhibited a high degree of efficiency at LC50 level against A. gossypii after 24 hrs of treatment, followed by compounds (9), (3, 4), (5), (8), (7), (6) and the least one (1), respectively. Comparing the toxicity indexes of the isolated compounds after 72 hrs of exposure, it was found that compound (2) was the most effective followed by compounds (3, 4), (9), (5), (8), (7), (1) and the least one (6), respectively.
Table (3): Toxicity of Xestospongia testudinaria fractions against Aphis gossypii after 24 and 72hrs of treatment

<table>
<thead>
<tr>
<th>Fractions</th>
<th>LC50 (ppm) and confidence limits at 95%</th>
<th>LC90 (ppm) and confidence limits at 95%</th>
<th>Slope ± SE</th>
<th>X²</th>
<th>Toxicity index</th>
<th>LC50 (ppm) and confidence limits at 95%</th>
<th>LC90 (ppm) and confidence limits at 95%</th>
<th>Slope ± SE</th>
<th>X²</th>
<th>Toxicity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>3.41</td>
<td>203.20</td>
<td>0.722±0.161</td>
<td>2.07</td>
<td>61.69</td>
<td>0.019</td>
<td>4.19</td>
<td>0.549±0.162</td>
<td>0.95</td>
<td>100.00</td>
</tr>
<tr>
<td>Extract</td>
<td>0.93</td>
<td>7.23</td>
<td>3.73</td>
<td>0.966</td>
<td>1968.61</td>
<td>0.0001</td>
<td>1.24</td>
<td>63.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction A</td>
<td>0.18</td>
<td>5.61</td>
<td>0.581±0.157</td>
<td>1.86</td>
<td>100.00</td>
<td>0.0001</td>
<td>5.02</td>
<td>4103.33</td>
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<tr>
<td>Fraction B</td>
<td>0.67</td>
<td>5.48</td>
<td>0.793±0.165</td>
<td>1.12</td>
<td>80.25</td>
<td>0.101</td>
<td>4.88</td>
<td>0.760±0.149</td>
<td>0.73</td>
<td>19.00</td>
</tr>
<tr>
<td>Fraction C</td>
<td>0.19</td>
<td>6.25</td>
<td>0.563±0.156</td>
<td>1.93</td>
<td>90.02</td>
<td>0.0002</td>
<td>11.42</td>
<td>60981.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction D</td>
<td>9.26</td>
<td>101.75</td>
<td>1.231±0.355</td>
<td>0.28</td>
<td>22.70</td>
<td>0.099</td>
<td>62.94</td>
<td>0.712±0.174</td>
<td>4.13</td>
<td>1.90</td>
</tr>
<tr>
<td>Fraction E</td>
<td>7.89</td>
<td>52.98</td>
<td>0.587±0.159</td>
<td>0.39</td>
<td>10.69</td>
<td>0.0004</td>
<td>15.80</td>
<td>737.94</td>
<td></td>
<td>1.23</td>
</tr>
</tbody>
</table>

Structure-activity relationship of the isolated nine compounds revealed that the natural product class type and substitutions were significant. 3rd instar nymphs of whiteflies B. tabaci was strongly affected by maleimide class than brominated polyacetylenic lipids then nucleoside and sterol classes, respectively, while in case of A. gossypii the most potent class was brominated polyacetylenic lipids followed by nucleoside, maleimide and finally sterol classes.

Another observation should be taken in considerations that methyl substituted classes is less effectiveness than the unsubstituted classes also, the activity of free brominated polyacetylenic acids is more toxic than the methyl ester derivatives.

REFERENCES


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