Insecticidal and Enzyme Inhibition Activities of Leaf/bark Extracts, Fractions, Seed Oil and Isolated Compounds From *Triadica Sebifera* (L.) Small Against *Aphis Craccivora* Koch

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Abstract

Aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae) is a major sap-sucking insect pest of leguminous crops and also transmit plant viruses leading to economic yield loss. Insecticidal activities of *T. sebifera* have not been reported against *A. craccivora* and other insect pests till **date**. In this study, residual toxicity of leaf/bark extracts, seed oil, fractions, isolated compounds and its combinations; synergistic activity of leaf/ bark extract with seed oil and its enzyme inhibition activities against *A. craccivora* was studied. Among extracts, ethanolic bark extract 80% ($LC_{50}=5115.98 \text{ mg L}^{-1}$) was more effective against *A. craccivora* than other extracts. Among fractions, *n*-hexane fraction of leaf ($LC_{50}=425.73 \text{ mg L}^{-1}$) and ethyl acetate fraction of bark ($LC_{50}=813.45 \text{ mg L}^{-1}$) was more effective. Among compounds, gallic acid was most effective ($LC_{50}=1303.68 \text{ mg L}^{-1}$) than shikimic acid and quercetin ($LC_{50}=1725.09$ and $1855.93 \text{ mg L}^{-1}$, respectively). Seed oil ($LC_{50}=850.94 \text{ mg L}^{-1}$) is more superior than extracts/fractions/compounds. All the combinations showed toxicity and synergistic activity. Leaf/bark extracts and seed oil ($0.25\text{-}2 \text{ mg L}^{-1}$) significantly inhibits the AChE and GST activity in *A. craccivora*. Based on field bio-efficacy studies, the leaf extract/seed oil or their combinations can be recommended for development of botanical formulation for the control of aphid.

Introduction

Aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae) is the major sap-sucking insect pest of leguminous crops. Adults and nymphs suck the plant sap from leaves/stem/pods which affects the growth. In severe infestation, stunted growth of plants, secretion of honeydew on leaves/pods which attracts the development of sooty mould fungus which affects the photosynthesis of plants. *A. craccivora* also transmits plant viruses leading to economic yield loss¹. Currently, farmers are using synthetic insecticides of different classes for the control of aphids. Non-judicious and repeated application of insecticides for control of aphids led to the development of resistance². Due to the harmful effect of chemical insecticides on the environment, public, customers, non-target organisms, there is an essential substitute for the effective management of aphid.

Chinese tallow, *Triadica sebifera* (L.) Small (Euphorbiaceae) is the world's most invasive species common in China³. It is used for traditional medicine in China, Taiwan, and Japan. Due to the rapid growth, widespread invasion of native ecosystems, still it is used as a popular ornamental due to its beautiful foliage that changes from yellow to crimson colour. Seeds were used for herbal medicines, candles, soap, cocoa butter, lamps, heating oils, cosmetics, and pharmaceuticals⁴⁻⁵. The seed oil is used to treat skin disorders, purgatory, and root bark decoctions caused by dyspepsia⁶. The leaves and roots having the properties of depurative, diuretic, and laxative. The decoction is used to cure edema, constipation. The root and bark extracts are used for the treatment of snake bites and skin ulcers⁷. *T. sebifera* has the properties of anti-bacterial, anti-viral, antioxidant, analgesic, anti-inflammatory, anti-hypersensitivity⁸⁻¹¹.

Insecticidal activities of *T. sebifera* have not been reported against aphid and other insect pests till **date**. Therefore, in the present study, we have explored *T. sebifera* for 1). Chemical analysis of *n*-hexane fractions of leaf/bark ethanolic aqueous extracts of *T. sebifera*; 2). Isolation of compounds from ethyl acetate and *n*-butanol fractions of leaf; 3). Residual toxicity of leaf/bark ethanolic/methanolic aqueous extracts, seed oil and its combinations, fractions, isolated compounds; 4). Combination of seed oil with leaf/bark extracts for **its synergistic interaction** against *A. craccivora* and 5). Detoxification enzyme activities of LEE, BEE and SO against *A. craccivora*

Methods

Plant material

The leaves at the vegetative stage, bark, and seeds of *T. sebifera* used in the study was collected in and around CSIR-IHBT Campus, Palampur, Himachal Pradesh (32°06'05"N, 76°34'10"E) located in the Dhauladhar range of the western Himalayas. The authentication of plant material was done by a Taxonomist of CSIR-IHBT, Palampur (H.P.), India, and voucher specimen were deposited in the herbarium (voucher no. PLP 18563) of the institute. The plant material was dried under shade for 15 days and was used for further processing.

Statement to confirm the methods:

All methods were carried out in accordance with relevant guidelines and regulations.

Statement for collection of the plant material

Institutional permission was taken for the collection of plant material and the voucher specimens (PLP 18563) were deposited in the herbarium of the institute.

Preparation of leaf/bark extracts and fractions

The well-dried leaves and bark samples were powdered using a grinder. About 1 kg of samples was extracted separately by maceration (3 x 5 L) 12 h intervals using a concentration of 50%, 80% and 100% ethanol: water at room temperature. The macerated samples were filtered, and then the solvent was removed under low pressure at 45°C in a Rotatory evaporator (Buchi, R-210), accordingly, leaf/bark, ethanolic/methanolic extracts at a concentration of 50%, 80%, and 100% were also prepared. The yields obtained for 50%, 80% and 100% leaf ethanolic aqueous extracts (LEE) were 78.8, 90.5, and 51.4 g, respectively. Similarly, the yield obtained for 50%, 80% and 100% leaf methanolic aqueous extracts (LME) were 77.3 g, 72.6 g, and 48.6 g, respectively. The yield obtained for 50%, 80% and 100% bark ethanolic aqueous extracts (BEE) were 77.5, 85.6, and 53.2 g, respectively. In the same way, the yield obtained for 50%, 80% and 100% bark methanolic aqueous extracts (BME) were 67.5 g, 78.5g, and 63.3g, respectively, and kept <4°C until further analysis starts.

The dried ethanolic aqueous extract 80% from leaf (75 g) and bark (65 g) was dissolved in a minimum volume of distilled water and sequentially fractionated (500 mL x 5) from lower to higher polarity of the solvents (*n*-hexane, ethyl acetate, *n*-butanol and water) and evaporated separately at 45°C using Rotary evaporator and then lyophilized to get respective fractions. The yields obtained for the *n*-hexane, ethyl acetate, *n*-butanol and water fractions of leaf were 25 g, 21.5 g, 18.4 g and 8.1 g, respectively. Similarly, the yields obtained for the *n*-hexane, ethyl acetate, *n*-butanol and water fractions of bark were 10 g, 8.58 g, 19.7 g and 8.3 g, respectively and kept <4°C until further analysis (Fig. 2).

Extraction of seed oil

About 10 kg seeds were dried under the shade for 15 days. The seed coat was stripped manually, defatted by mixing them in hot water (70°C) using a mini extractor. The defatting process was regulated in the mini extraction system for 3.5 h/percolation 6 times, for the appearance of black kernels and then cleaned with hot water then transferred to a hot air oven (45°C) for drying. When the kernels were dehydrated, 2kg of kernels were grounded in a blender and dissolved in *n*-hexane (3 L x 4 times) in a percolator. The eluted solvent was collected, filtered, and removed under low pressure at 45°C in Rotavapor (Buchi, R-210) which yields 1 L of seed oil (SO) which was kept in <4°C until further analysis.

Isolation of compounds

The ethyl acetate fraction (8 g) was taken and subjected for column chromatography over 60-120 mesh size silica gel. The column was packed in pure chloroform and eluted with methanol in the increasing order of polarity. A total of 50 fractions were collected (100 ml each) using a gradient elution of chloroform and methanol mixture. All the fractions were then divided into seven main fractions (i.e., F_A-F_G) after TLC analyses. Fractions F_C-F_D eluted at polarity (20:80) MeOH: CHCl₃ and then again subjected for column chromatography over 60-120 mesh size silica gel, results in the isolation of compound **1** (128 mg) at polarity (10:90) MeOH: CHCl₃ and compound **2** (150 mg) at polarity (15:85) MeOH: CHCl₃.

n-butanol fraction (6 g) was subjected to column chromatography for isolation of pure molecules. Mesh size 60-120 silica was used with solvent system chloroform: methanol: formic acid for column chromatography. At different polarities and TLC-based analysis, five major fractions (i.e., BF_A-BF_E) were collected. BF_A fraction was again subjected to 230-400 silica for isolation. The solvent system used for isolation was chloroform: methanol with 0.01% formic acid. Compound **3**, (124 mg) was isolated at 3% methanol: chloroform with 0.01% formic acid and compound **4** (108 mg) was isolated at 5% methanol: chloroform with 0.01% formic acid.

Preparation of fatty acid methyl esters (FAMES)

The FAMES were prepared as per the method followed by Adebisi et al.²². Briefly, 1 mL of *n*-hexane was added into 0.1 mL oil and 1 mL of sodium methoxide (1.5 g NaOH in 50 mL methanol). The mixture was stirred vigorously using vortex and kept untouched for 10 min to segregate the transparent fatty acid methyl ester solution from the opaque aqueous layer and was stored at 4°C until further use.

Gas chromatography-mass spectrophotometry (GC-MS) analysis

The GC-MS analysis of *n*-hexane fractions of leaf and bark ethanolic aqueous extracts and seed oil were carried out using a Shimadzu QP 2010 and DB–5MS (J & W Scientific, Folsom, USA) capillary column (30 m × 0.25 mm, i.d., 0.25 µm thickness). The GC oven temperature was programmed at 70°C for 4 min and then increased to 220°C at the 4°C/ min and held for 5 min. Injector temperature was 240°C, and interface temperature was 250°C, acquisition mass range of 800-50 amu, ionization energy was 70eV. Helium is used as a carrier gas. Compounds were identified using a library search of the National Institute of Standards and Technology (NIST) database³⁵, and mass spectral fragmentation patterns with those reported in the literature³⁶.

Test insects

A. craccivora was collected from the open field conditions and maintained/reared on live French bean, *Phaseolus vulgaris* L. under controlled conditions (25 ± 2°C temperature; 60 ± 5% humidity and photoperiod 16:8 Light: Day) for more than 100 generations. The fresh nymphs of 3-4 days old were selected for experiments.

Statement for confirmation of experimental protocols

Experimental protocols are approved by the institutional committee (Publication committee) vide reference number 4903. However, we have not used any experimental animals for the present study.

Preliminary screening

Preliminary screening of leaf/bark extracts, fractions, seed oil, and compounds was carried out at higher concentrations (5000-10000 mg L⁻¹) for their efficacy against nymphs of *A. craccivora*. **Based on preliminary data, 5-6 concentrations were prepared and evaluated against aphid in the final bioassay experiments.**

Residual toxicity of leaf/bark aqueous ethanolic/methanolic extracts under laboratory

Residual toxicity of leaf/bark extract/fractions and seed oil was tested against nymphs of *A. craccivora* by the Potter spray method as per the standard method³⁷. Briefly, five concentrations of LEE/LME/BEE/BME (500-20000 mg L⁻¹), leaf and bark fractions (250-10000 mg L⁻¹), SO (500-10000 mg L⁻¹) and compounds (312.5-5000 mg L⁻¹) were prepared. The leaf discs of 3 cm diameter were prepared and pressed over the water-agar medium on Petri plates to maintain the freshness of leaf discs. About 10 nymphs/leaf disc were released in each Petri plate. Each treatment was replicated thrice. The test solution (2 ml) was sprayed under Potter's spray tower operated at 1.1 kg/cm² pressure and incubated under controlled conditions. Observations on dead insects were recorded at 24 h intervals and up to 96 h. The commercial neem-based formulation i.e., Indo-neem (Azadirachtin 0.15% EC and neem oil 35%) used as a positive control for comparison.

Residual toxicity of binary mixtures of leaf/bark extracts and seed oil under laboratory

Based on preliminary study, the blends/mixtures of seed oil with leaf /bark ethanol aqueous extract of *T. sebifera* was prepared in five concentrations (62.5-1000 mg L⁻¹) and proportions as 1:3 (SO: LEE), 1:1 (SO: LEE), 3:1 (SO: LEE), 1:1:1 (SO: LEE: BEE), {(1+1): 2} (LEE+SO: BEE), {(1+1): 2} (LEE+BEE: SO) and (1+1): 2 (SO+BEE: LEE) for bioassay and synergistic activity against *A. craccivora* under laboratory conditions. Five concentrations (62.5-1000 mg L⁻¹) were prepared and sprayed against nymphs of *A. craccivora* as described above. Each treatment was replicated thrice. Observations on mortality were recorded at 24, 48, 72, and 96 h after treatment. The fractional effect indices (FEI) were calculated to study the joint action of binary mixtures/combinations. FEI=fractional effect of A + fractional effect of B, in which fractional effect of A=LC₅₀ mixture /LC₅₀ of A and fractional effect of B=LC₅₀ mixture / LC₅₀ of B³⁸. The FEIs were interpreted based on Bassole et al.³⁹ classifications as being synergistic if FEI < 0.5, additive if FEI ≥ 0.5 and ≤ 1.0, indifferent if FEI > 1.0 and ≤ 4.0 or antagonistic if FEI > 4.0.

Residual toxicity of leaf/bark extracts and its binary mixtures under plant growth chamber

The residual toxicity of leaf/bark ethanolic aqueous extract, seed oil at six concentrations (625-20000 mg L⁻¹) and its combinations of *T. sebifera* (SO+LEE, SO+BEE) in a 1:1 ratio was prepared in five concentrations (125-2000 mg L⁻¹) and evaluated against *A. craccivora* under controlled conditions (26 ± 2°C, 70 ± 5% relative humidity and photoperiod of 16 h light and 8 h dark) in a plant growth chamber (Percival Scientific, Perry, Iowa). The *Phaseolus vulgaris* plants were raised on plastic pots (7 x 9 cm) filled with the potting mixture (1:1:1 ratio of vermiculate: cocopeat: perlite). One-week old plants (3-4 leaf stage) were inoculated with two days old nymphs of *A. craccivora* and allowed to settle for 24 h. After settling, test solutions/concentrations were sprayed on plants using a hand sprayer. Observations of the number of dead insects/plants were recorded at 24, 48, 72, and 96 h after treatment. There were six treatments and each treatment was replicated five times. The fractional effect indices (FEI) were calculated as mentioned above section to study the joint action of binary mixtures/combinations.

Enzyme inhibition activities of LEE, BEE and SO against *A. craccivora*

Four different concentrations of LEE, BEE and SO (0.25, 0.5, 1 and 2%) were chosen for enzyme activity³² according to the residual toxicity assay as described above. The nymphs of *A. craccivora* of each concentration after 48 h of treatment were homogenised and centrifuged at 15000 rpm at 4°C for 30 minutes. The pellet was discarded and the supernatant was used for the enzyme activity. Before proceeding with enzyme activity, the total protein concentration was measured using the Bradford reagent. For the enzyme assay of glutathione S-transferase (GST) and acetylcholine esterase (AChE), the assay kits were procured from Cayman Chemicals and Abcam, respectively.

Data analysis

The mortality data of aphids based on residual toxicity of extracts, fractions, seed oil, combinations, and pure compounds were compiled. The median lethal concentration values (LC₅₀) and other regression

parameters were determined by Probit⁴⁰ using SPSS 10 software, version 16. The FEI for binary mixtures/combinations were calculated to study the joint action studies (synergistic, additive, indifferent, and antagonistic). Similarly, percent mortality data against aphids were also analysed by one-way analysis of variance, and means were compared by Tukey's post hoc test.

Results

Fatty acid composition of seed oil of *T. sebifera*

The fatty acid composition and distribution of *T. sebifera* are presented in Table 1. The SO of *T. sebifera* gives a yield of 50% (v/w). The saturated fatty acids are present in the SO were palmitic acid (4.99%) and stearic acid (1.38%). The unsaturated fatty acids are oleic acid (8.78%), linoleic acid (15.42%), and palmitoleic acid (2.13%). The distribution of saturated fatty acids is less (6.73%) as compared to unsaturated fatty acids (26.33%).

Table 1

Chemical composition of fatty acid methyl esters present in seed oil of *Triadica sebifera*; Note: RI^a = Calculated Retention Indices; RI^b = Retention Indices from literature

Sr. No.	Compound's name	Area (%)	RI ^a	RI ^b	Mass fragmentation
1	9-Hexadecenoic acid, methyl ester (Palmitoleic acid, C ₁₆ : 1)	2.13	1933	1931 ⁴¹	268 [M ⁺], 236, 207, 194, 152, 138, 123, 97, 74, 69, 55, 41
2	Hexadecanoic acid, methyl ester (Palmitic acid, C ₁₆ : 0)	4.99	1986	1984 ⁴²	270 [M ⁺], 239, 227, 199, 185, 171, 143, 129, 101, 87, 74, 57, 41
3	9,12-Octadecadienoic acid (Z, Z), methyl ester (Linoleic acid, C ₁₈ : 2)	15.42	2092	2094 ⁴³	294 [M ⁺], 262, 178, 164, 150, 135, 123, 109, 95, 81, 67, 55, 41
4	9-Octadecenoic acid, methyl ester (Oleic acid, C ₁₈ : 1)	8.78	2100	2101 ⁴⁴	296 [M ⁺], 264, 222, 180, 166, 152, 137, 123, 97, 83, 69, 55, 41
5	Octadecanoic acid, methyl ester (Stearic acid, C ₁₈ : 0)	1.38	2121	2128 ⁴⁵	298 [M ⁺], 255, 213, 199, 143, 129, 101, 87, 74, 57, 43, 41

Structure Elucidation Of Isolated Compounds

Compound 1 was isolated as a white amorphous powder. The ESI-QTOF-MS of the compound showing the molecular ion peak at m/z 471.08 (M+Na)⁺ indicated the molecular formula as C₂₁H₂₀O₁₁ (Fig. S1). Aromatic signals observed in proton spectrum at δ_H 7.98 d (2H, $J=8.4$ Hz), 6.81 d (2H, $J=8.4$ Hz), 6.30 s (1H), and 6.11 s (1H) showing the presence of two sets of equivalent protons and two singlet protons

which confirms the presence of two aromatic rings in the structure. Also, the presence of a doublet signal at δ_H 5.16 d (1H, $J=7.2$ Hz) which showing the HSQC correlation with δ_C 104.2 indicates the presence of a β -D-glucose moiety, which was also confirmed by COSY and HMBC correlations. The carbon value at δ_C 179.4 showed the presence of the C=O group. Hence from the above spectral data and comparison with literature data¹², compound **1** was identified as Kaempferol-3-O-glucoside (Fig. S2).

Kaempferol-3-O-glucoside: White amorphous powder; 128 mg; mp. 178°C; ESI-QTOF-MS (Positive) m/z : 471.08 (M+Na)⁺ ¹H-NMR (600 MHz, CD₃OD) (Fig. S3 and Table S1), ¹³C-NMR (150 MHz, CD₃OD) (Fig. S4 and Table S1).

Compound **2** was isolated as a white amorphous powder. The molecular formula C₂₁H₂₀O₁₁ was calculated after ESI-QTOF-MS, which was showing the molecular ion peak at m/z 487.08 (M+Na⁺) (Fig. S5). Compound **2** was identified by comparing observed data with literature-reported data¹³. After comparison of observed data with literature, compound **2** was identified as Quercetin-3-O-glucoside (Fig. S2).

Quercetin-3-O-glucoside: White amorphous powder; 150 mg; mp. 226°C; ESI-QTOF-MS (Positive) m/z : 487.08 (M+Na⁺). ¹H-NMR (600 MHz, CD₃OD) (Fig. S6 and Table S1), ¹³C-NMR (150 MHz, CD₃OD) (Fig. S7 and Table S1).

Compound **3** was obtained as a white powder. The molecular ion peak was observed at m/z 341.03 (2M+H)⁺ in ESI-QTOF-MS (Fig. S8), which indicates the formation of a non-covalent dimer in ESI-MS¹⁴. Hence the stable molecular ion peak was formed in the form of a non-covalent dimer (2M+H)⁺. From spectral analysis and literature reports¹⁵, compound **3** was identified as gallic acid (Fig. S2).

Gallic acid: White powder; 124 mg; mp. 260°C; ESI-QTOF-MS (Positive) m/z : 341.03 (2M+H)⁺; ¹H-NMR (600 MHz, CD₃OD) (Fig. S9 and Table S2), ¹³C-NMR (150 MHz, CD₃OD) (Fig. S10 and Table S2).

Similarly, compound **4** was also isolated as a white powder. The molecular ion peak for compound **4** was obtained at m/z 349.32 (2M+H)⁺ (Fig. S11), which was also the molecular ion peak of the non-covalent dimer peak of compound **4**. ESI-QTOF-MS analyses of compound **4** were indicated its molecular formula C₇H₁₀O₅ and after analysis and comparison of observed data with literature¹⁶, compound **4** was identified as Shikimic acid (Fig. S2).

Shikimic acid: White powder; 108 mg; mp. 184-185°C, ESI-QTOF-MS (Positive) m/z : 349.32 (2M+H)⁺; ¹H-NMR (600 MHz, CD₃OD) (Fig. S12 and Table S2), ¹³C-NMR (150 MHz, CD₃OD) (Fig. S13 and Table S2).

Gas chromatography-mass spectrophotometry (GC-MS) analysis of *n*-hexane fractions

The metabolites and their mass fragmentation, identified through GC-MS in the *n*-hexane fractions of leaf and bark were presented in Table 2. In the *n*-hexane fraction of the leaf, the major metabolites were *n*-

Hexadecanoic acid (15.61%) and were followed by octadecanoic acid, ethyl ester (9.85%) and neophytadiene (5.87%). In the case of the *n*-hexane fraction of the bark, the major metabolites were galaxolide (44.73%) and were followed by ethyl phthalate (28.43%) and 1-Octadecene (2.69%).

Table 2

Chemical composition of *n*-hexane fraction from leaf and bark of *Triadica sebifera*; Note:RI^a = Calculated Retention Indices; RI^b = Retention Indices from literature

Metabolites	Molecular formula	Area (%)	RI ^a	RI ^b	Mass fragmentation
Leaf					
1,8-Cineole	C ₁₀ H ₁₈ O	1.61	1037	1037 ⁴⁶	<i>m/z</i> 154 (M ⁺), 139, 125, 108, 84, 81, 69, 43, 41
Fenchyl acetate	C ₁₂ H ₂₀ O ₂	0.70	1230	1223 ⁴⁷	<i>m/z</i> 154 (M ⁺), 136, 121, 108, 95, 80, 69, 43, 41, 27
Neophytadiene	C ₂₀ H ₃₈	5.87	1841	1836 ⁴⁸	<i>m/z</i> 137 (M ⁺), 123, 109, 95, 82, 68, 43, 41
<i>n</i> -Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	15.61	1961	1962 ⁴⁹	<i>m/z</i> 256 (M ⁺), 227, 213, 199, 185, 171, 157, 143, 129, 115, 98, 85, 73, 60, 43, 41
cis, cis, cis-7,10,13-Hexadecatrienal	C ₁₆ H ₂₆ O	3.06	1992	1989 ⁵⁰	<i>m/z</i> 264 (M ⁺), 222, 149, 135, 121, 108, 95, 79, 67, 55, 41
Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	9.85	2189	2189 ⁵¹	<i>m/z</i> 312 (M ⁺), 269, 213, 157, 143, 129, 115, 101, 88, 70, 57, 43, 41
Bark					
Hexanoic acid	C ₆ H ₁₂ O ₂	0.31	984	989 ⁵²	<i>m/z</i> 87 (M ⁺), 73, 60, 43, 41, 40
2-Decenal	C ₁₀ H ₁₈ O	0.23	1263	1260 ⁵³	<i>m/z</i> 121 (M ⁺), 98, 84, 70, 57, 43, 41, 40
Ethyl phthalate	C ₁₂ H ₁₇ O ₄	28.43	1587	1585 ⁵⁴	<i>m/z</i> 222 (M ⁺), 177, 149, 121, 105, 93, 76, 65, 50
1-Octadecene	C ₁₈ H ₃₆	2.69	1803	1800 ⁵⁵	<i>m/z</i> 125 (M ⁺), 111, 97, 83, 57, 41, 40
Galaxolide	C ₁₈ H ₂₆ O	44.73	1834	1837 ⁵⁶	<i>m/z</i> 258 (M ⁺), 243, 213, 185, 171, 157, 143, 128

Residual toxicity of leaf/bark extracts and seed oil of *T. sebifera* against *A. craccivora*

The residual toxicity of LEE, LME, BEE, BME and SO of *T. sebifera* against aphid with respect to LC₅₀ values are presented in Table 3. Bark extracts are most effective than leaf extracts. Among leaf extracts,

LEE 80% was found most effective ($LC_{50} = 9590.49 \text{ mg L}^{-1}$) against *A. craccivora* after 72 h and was followed by LME 100%, 80%, and 50% ($LC_{50} = 9627, 10800, \text{ and } 11540 \text{ mg L}^{-1}$, respectively) as compared to LEE 100% ($LC_{50} = 14100 \text{ mg L}^{-1}$). Similarly, 96 h after treatment also, LEE 80% was found more effective ($LC_{50} = 6756.42 \text{ mg L}^{-1}$) and was followed by LME 80 and 100% ($LC_{50} = 7120.27 \text{ and } 7528.56 \text{ mg L}^{-1}$, respectively) as compared to LME 50% and LEE 100% ($LC_{50} = 7579.55 \text{ and } 8702.07 \text{ mg L}^{-1}$, respectively).

Table 3

Efficacy of ethanolic/methanolic leaf/bark aqueous extracts and seed oil of *Triadica sebifera* against *Aphis craccivora*; Note: LC₅₀=Lethal concentration to kill 50% of test insect; Mean of three replications; n=150 insects per treatment; LC₅₀ was calculated for fractions showing > 50% mortality using Probit analysis

Leaf extracts	LC ₅₀ (mg L ⁻¹)	Confidence limits (mg L ⁻¹)	Slope ± SE	Chi square	P value
LEE 100% (72 h)	14100.0	11764.41 – 17527.10	2.31 ± 0.53	0.36	0.99
LEE 100% (96 h)	8702.07	6880.35 – 10137.00	3.08 ± 0.52	0.61	0.99
LEE 80% (72 h)	9590.49	7706.54 – 11128.43	2.91 ± 0.52	1.81	0.87
LEE 80% (96 h)	6756.42	5342.84 – 7885.95	3.97 ± 0.58	1.84	0.87
LEE 50% (72 h)	38860.0	24892.46 – 341196.32	2.07 ± 0.72	0.67	0.98
LEE 50% (96 h)	28570.0	21383.79 – 71689.83	2.51 ± 0.71	0.68	0.98
LME 100% (72 h)	9627.0	6881.53 – 11725.61	2.07 ± 0.49	2.02	0.85
LME 100% (96 h)	7528.56	5691.95 – 8931.12	3.04 ± 0.52	5.07	0.41
LME 80% (72 h)	10800.0	8603.89 – 12754.79	2.45 ± 0.50	2.24	0.81
LME 80% (96 h)	7120.27	5593.04 – 8326.45	3.63 ± 0.55	6.95	0.22
LME 50% (72 h)	11540.0	9689.45 – 13331.76	2.86 ± 0.53	3.76	0.58
LME 50% (96 h)	7579.55	6239.59 – 8676.68	4.15 ± 0.58	5.80	0.33
Seed oil (72 h)	2504.59	1675.92 – 3562.91	1.18 ± 0.21	0.86	0.97
Seed oil (96 h)	850.938	533.52 – 1171.05	1.69 ± 0.25	3.28	0.66
Bark extracts					
BEE 100% (72 h)	8325.46	6958.41 – 9455.76	4.05 ± 0.57	7.39	0.19
BEE 100% (96 h)	5228.89	4038.43 – 6165.80	4.88 ± 0.78	3.83	0.57
BEE 80% (72 h)	7300.57	5889.44 – 8435.86	3.97 ± 0.57	6.17	0.29
BEE 80% (96 h)	5115.98	3613.44 – 6219.77	4.04 ± 0.75	1.31	0.73
BEE 50% (72 h)	10650.0	8244.02 – 12743.89	2.25 ± 0.50	3.73	0.59
BEE 50% (96 h)	7098.41	5159.65 – 8546.32	2.91 ± 0.52	7.32	0.20
BME 100% (72 h)	8737.64	6586.26 – 10377.96	2.61 ± 0.50	2.12	0.83
BME 100% (96 h)	5701.69	4147.42 – 6880.23	3.73 ± 0.63	2.34	0.67
BME 80% (72 h)	9490.58	7504.78 – 11085.89	2.78 ± 0.51	0.45	0.99

Leaf extracts	LC ₅₀ (mg L ⁻¹)	Confidence limits (mg L ⁻¹)	Slope ± SE	Chi square	P value
BME 80% (96 h)	5779.72	4187.11 – 7007.96	3.55 ± 0.58	1.42	0.92
BME 50% (72 h)	10580.0	8431.88 – 12452.44	2.51 ± 0.51	1.43	0.92
BME 50% (96 h)	5233.81	2941.71 – 6869.58	2.51 ± 0.53	7.29	0.20
Azadirachtin (72 h)	2642.32	2013.70 – 3816.64	1.53 ± 0.22	0.99	0.80
Azadirachtin (96 h)	1174.22	973.61 – 1416.60	2.28 ± 0.25	5.16	0.16

Among bark extracts, BEE 80% (LC₅₀=7300.57 mg L⁻¹) was most effective against aphid after 72 h, then BEE and BME 100% (LC₅₀=8325.46 and 8737.64 mg L⁻¹, respectively) as compared to BME 80%, 50% and BEE 50% (LC₅₀=9490.58, 10580 and 10650 mg L⁻¹, respectively). Similarly, 96 h after treatment, BEE 80% (LC₅₀=5115.98 mg L⁻¹) was found to be more effective and was followed by BEE 100% (LC₅₀=5228.89 mg L⁻¹) as compared to BME 50%, 100%, 80%, and BEE 50% (LC₅₀=5233.81, 5701.69, 5779.72 and 7098.41 mg L⁻¹, respectively).

The SO of *T. sebifera* in the current study was found to be more effective after 72 h and 96 h of treatment (LC₅₀=2504.59 and 850.94 mg L⁻¹, respectively) than BEE and LEE (LC₅₀=5115.98-6756.42 mg L⁻¹). All the leaf and bark extracts were not superior to the positive control, Indo-neem (Azadirachtin 0.15% EC) after 72 h and 96 h (LC₅₀= 2642.32 and 1174.22 mg L⁻¹, respectively). However, the SO is more superior to leaf and bark extracts.

Residual toxicity of the combination of seed oil with leaf/bark extract of *T. sebifera* and its synergistic activity against *A. craccivora* under laboratory

The residual toxicity of the combination of SO with 80% LEE/BEE and its synergistic activity of *T. sebifera* against *A. craccivora* under laboratory conditions were presented in Table S3-S4. Results showed that among combinations studied, a mixture of LEE 80% + SO and BEE 80% {(1+1):2} was found more effective (LC₅₀=239.94 mg L⁻¹) against nymphs of *A. craccivora* after 72 h and was followed by a mixture of BEE + SO (1:1) (LC₅₀=263.56 mg L⁻¹), SO + LEE at 1:1 ratio (LC₅₀=303.29 mg L⁻¹) and LEE+BEE+SO at 1:1:1 ratio (LC₅₀=337.33 mg L⁻¹) as compared to other mixtures/blends. Based on the fractional effect index (FEI), all the combinations/blends evaluated against *A. craccivora* showed synergistic interaction after 72 h. Among them, LEE+BEE at 1:3, 3:1, and 1:1 ratio showed the most significant synergistic interaction (FEI= 0.088, 0.092 and 0.122, respectively), as compared to other blends/combinations. Similarly, 96 h after treatment, BEE + SO (1:1 ratio) was found to be more effective (LC₅₀=144.26 mg L⁻¹) against *A. craccivora* and was followed by SO+LEE at 1:1 ratio (LC₅₀=168.9 mg L⁻¹), LEE+SO+BEE at {(1+1):2} ratio (LC₅₀=170.46 mg L⁻¹), LEE +BEE at 1:3 ratio (LC₅₀=179.31 mg L⁻¹) as compared to other mixtures/blends. Based on FEI, all the combinations/blends evaluated against *A.*

craccivora showed a synergistic effect. Among them, LEE+BEE at 1:3, 3:1, and 1:1 ratio showed significant synergistic interaction (FEI= 0.061, 0.070, and 0.100, respectively), as compared to other blends/mixtures.

Residual toxicity of seed oil, leaf/bark extracts and its binary mixtures of *T. sebifera* and its synergistic activity against *A. craccivora* under plant growth chamber

Residual toxicity of seed oil, leaf/bark extracts, and its binary mixtures (1:1) of *T. sebifera* against *A. craccivora* under plant growth chamber conditions are presented in Table S5. Results showed that the binary mixture of SO+LEE (1:1 ratio) was found to be more effective against *A. craccivora* ($LC_{50}=264.05 \text{ mg L}^{-1}$) after 72 h and was followed by SO+BEE ($LC_{50}=362.76 \text{ mg L}^{-1}$) as compared to SO, LEE, and BEE ($LC_{50}=1100.22, 1840.84$ and $5073.99 \text{ mg L}^{-1}$, respectively). Based on FEI values, both the blends (SO+LEE and SO+BEE) showed synergistic interaction (FEI=0.38 and 0.40) against *A. craccivora* (Table S5). Similarly, 96 h after treatment, binary mixtures of SO+LEE were more effective against *A. craccivora* ($LC_{50}=223.82 \text{ mg L}^{-1}$) and was followed by SO+BEE ($LC_{50}=247.54 \text{ mg L}^{-1}$) as compared to LEE, SO, and BEE ($LC_{50}=685.47, 706.53$ and $2328.79 \text{ mg L}^{-1}$, respectively). Based on FEI values, SO+BEE showed synergistic interaction (FEI=0.46). A binary mixture of SO+LEE showed additive interaction after 96h of treatment.

Residual toxicity of leaf and bark fractions of *T. sebifera* against *A. craccivora*

The residual toxicity of leaf/bark fractions of *T. sebifera* against *A. craccivora* was presented in Table 4. Results showed that, among leaf fractions, *n*-hexane fraction ($LC_{50}= 425.73 \text{ mg L}^{-1}$) was more effective than ethyl acetate and *n*-butanol ($LC_{50}=838.89$ and $1527.84 \text{ mg L}^{-1}$, respectively) as compared to water fraction ($LC_{50}= 2702.82 \text{ mg L}^{-1}$) after 72 h. Similarly, 96 h after treatment, *n*-hexane fraction ($LC_{50}=196.61 \text{ mg L}^{-1}$) was the most promising efficacy than ethyl acetate ($LC_{50}=367.75 \text{ mg L}^{-1}$) as compared to water and *n*-butanol fraction ($LC_{50}= 864.68$ and $1527.84 \text{ mg L}^{-1}$, respectively).

Table 4

Efficacy of fractions of *Triadica sebifera* leaf and bark against *Aphis craccivora*; Note: LC₅₀=Lethal concentration to kill 50% of test insect; Mean of three replications; n=150 insects per treatment; LC₅₀ was calculated for fractions showing > 50% mortality using Probit analysis

Leaf fractions	LC ₅₀ (mg L ⁻¹)	Confidence limits (mg L ⁻¹)	Slope ± SE	Chi square	P value
<i>n</i> -hexane (72 h)	425.73	196.38 – 679.40	1.09 ± 0.21	1.55	0.82
<i>n</i> -hexane (96 h)	196.61	76.21 – 316.54	1.57 ± 0.33	2.45	0.65
Ethyl acetate (72 h)	838.89	558.64 – 1178.77	1.39 ± 0.21	3.96	0.41
Ethyl acetate (96 h)	367.75	230.65 – 503.57	1.88 ± 0.32	0.75	0.94
<i>n</i> -butanol (72 h)	1527.84	1123.04 – 2093.81	1.60 ± 0.22	1.14	0.89
<i>n</i> -butanol (96 h)	990.22	746.43 – 1294.96	1.92 ± 0.25	2.68	0.61
Water (72 h)	2702.82	1799.12 – 4556.10	1.12 ± 0.19	1.34	0.85
Water (96 h)	864.68	643.83 – 1133.25	1.89 ± 0.25	2.07	0.72
Bark fractions					
<i>n</i> -hexane (72 h)	1659.98	1211.70 – 2310.56	1.54 ± 0.21	3.62	0.46
<i>n</i> -hexane (96 h)	1130.95	867.74 – 1467.86	2.02 ± 0.26	1.95	0.75
Ethyl acetate (72 h)	3629.52	2322.33 – 6984.03	1.05 ± 0.19	3.60	0.46
Ethyl acetate (96 h)	813.45	613.57 – 1052.33	2.04 ± 0.27	0.80	0.94
<i>n</i> -butanol (72 h)	3539.63	2343.17 – 6296.58	1.15 ± 0.20	1.92	0.75
<i>n</i> -butanol (96 h)	1071.81	762.69 – 1472.81	1.52 ± 0.22	5.26	0.26
Water 72 h)	3049.50	2087.70 – 4995.85	1.24 ± 0.20	5.94	0.20
Water (96 h)	915.15	684.74 – 1198.41	1.90 ± 0.25	2.55	0.63
Azadirachtin (72 h)	2642.32	2013.70 – 3816.64	1.53 ± 0.22	0.99	0.80
Azadirachtin (96 h)	1174.22	973.61 – 1416.60	2.28 ± 0.25	5.16	0.16

Among bark fractions, *n*-hexane fraction (LC₅₀=1659.98 mg L⁻¹) was most promising against *A. craccivora* after 72 h than water (LC₅₀=3049.50 mg L⁻¹) as compared to *n*-butanol and ethyl acetate (LC₅₀=3539.63 and 3629.52 mg L⁻¹, respectively). Similarly, 96 h after treatment, ethyl acetate fraction was most effective (LC₅₀=813.45 mg L⁻¹) than water (LC₅₀=915.15 mg L⁻¹) as compared to *n*-butanol and *n*-hexane (LC₅₀=1071.81 and 1130.95 mg L⁻¹, respectively). All the leaf and bark fractions are more

superior than the positive control, Indo-neem (Azadirachtin 0.15% EC) after 72 and 96h (LC_{50} = 2642.32 and 1174.22 mg L⁻¹, respectively).

Residual toxicity of isolated compounds of *T. sebifera* against *A. craccivora*

The experimental results on residual toxicity of the isolated compounds against *A. craccivora* with respect to LC_{50} values are shown in Table 5. Among four compounds isolated from leaf fractions of *T. sebifera*, gallic acid was most effective (LC_{50} =1303.68 mg L⁻¹) against *A. craccivora* and was followed by shikimic acid and quercetin-3-O-glucoside (LC_{50} =1725.09-1855.93 mg L⁻¹) as compared to kaempferol-3-O-glucoside (LC_{50} =3762.69 mg L⁻¹). With respect to per cent mortality, gallic acid at 5000 mg L⁻¹ also reported 98% mortality ($F_{4,49}$ =135.79; p <0.0001) and was followed by shikimic acid, quercetin-3-O-glucoside and kaempferol-3-O-glucoside (88, 80 and 58%, respectively) ($F_{4,49}$ =129.71, 106.32 and 61.00; (***) P < 0.0001) (Fig. S14). All the tested compounds are not superior to the positive control, Indo-neem (Azadirachtin 0.15% EC) after 72h and 96h (LC_{50} =2642.32 and 1174.22 mg L⁻¹, respectively) except gallic acid after 72 h (LC_{50} = 2339.69 mg L⁻¹).

Table 5

Efficacy of compounds isolated from leaf fractions of *Triadica sebifera* against *Aphis craccivora*; Note: LC₅₀=Lethal concentration to kill 50% of test insect; Mean of five replications; n=300 insects per treatment; LC₅₀ was calculated for fractions showing > 50% mortality using Probit analysis

Compounds	LC ₅₀ (mg L ⁻¹)	Confidence limits (mg L ⁻¹)	Slope ± SE	Chi square	P value
Kaempferol-3-O-glucoside (72h)	4512.54	3455.14 – 6789.22	2.02 ± 0.31	0.94	0.82
Kaempferol-3-O-glucoside (96h)	3762.69	2924.95 – 5398.97	1.95 ± 0.28	1.36	0.72
Quercetin-3-Oglucoside (72h)	3068.62	2462.99 – 4093.71	2.09 ± 0.28	2.21	0.53
Quercetin-3-Oglucoside (96h)	1855.93	1550.48 – 2261.15	2.39 ± 0.27	2.60	0.46
Gallic acid (72h)	2339.69	1928.91 – 2933.01	2.24 ± 0.27	2.03	0.57
Gallic acid (96h)	1303.68	1118.34 – 1520.76	3.07 ± 0.32	1.44	0.70
Shikimic acid (72h)	2826.31	2320.31 – 3606.86	2.30 ± 0.29	1.68	0.64
Shikimic acid (96h)	1725.09	1447.64 – 2080.49	2.47 ± 0.27	0.23	0.97
Azadirachtin (72 h)	2642.32	2013.70 – 3816.64	1.53 ± 0.22	0.99	0.80
Azadirachtin (96 h)	1174.22	973.61 – 1416.60	2.28 ± 0.25	5.16	0.16

Detoxification enzyme activities of LEE, BEE and SO against *A. craccivora*

Detoxifying enzymes (GST and AChE) activities in *A. craccivora* fed with bean leaf discs treated with different concentrations of LEE, BEE and SO is presented in Fig. 1. Results showed that AChE activity of nymphs of *A. craccivora* indicated that all the concentrations of LEE, BEE and SO significantly inhibit the AChE activity ($F_{4,14}=631.00$ to 968.50 , $p<0.0001$) as compared to control. However, LEE, BEE and SO at 2% reported higher inhibition of AChE (0.19 ± 0.00 to 0.75 ± 0.30 mU/mg) and was followed by 1% (0.28 ± 0.20 to 1.45 ± 0.13 mU/mg). Among them, BEE 2% (0.19 ± 0.00 mU/mg) showed higher inhibition of AChE and was followed by SO and LEE (0.42 ± 0.03 and 0.75 ± 0.30 mU/mg, respectively) as compared to lower concentrations (0.25-0.5%) (Fig. 1a).

Similarly for GST assay, all the concentrations of LEE, BEE and SO significantly inhibit the GST activity ($F_{4,14}=31.79$ to 500.37 , $p<0.0001$) as compared to control. Among them, BEE at 2% (5.37 ± 0.50 mU/mg)

showed higher inhibition of GST and was followed by LEE and SO (9.41 ± 1.13 and 35.01 ± 0.63 mU/mg, respectively) as compared to lower concentrations (0.25-0.5%). However, LEE, BEE and SO at 2% reported higher inhibition of GST (5.37 ± 0.50 to 35.01 ± 0.63 mU/mg) and was followed by 1% (7.77 ± 0.11 to 41.26 ± 1.88 mU/mg) (Fig. 1b).

Discussion

In the current study, residual toxicity of leaf/bark extracts, seed oil, fractions, isolated compounds and its combinations (extracts with seed oil); synergistic activity of leaf/ bark extract with seed oil against *A. craccivora* is discussed. All the extracts/fractions/compounds/seed oil of *T. sebifera* showed toxicity against nymphs of *A. craccivora*. In the case of leaf extracts, the LEE 80% was found most effective ($LC_{50} = 6756.42$ mg L⁻¹) and was followed by LME 80% as compared to other extracts after 96 h of treatment. Our results agree with the findings of Ahmed et al.¹⁷ who reported, the ethanolic leaf extract of *Citrullus colocynthis* against *Brevicornye brassicae* ($LC_{50} = 10400$ mg L⁻¹) after 72 h but not superior to our studies. Similarly, leaf ethanolic extract of *Ricinus communis* was more promising ($LC_{50} = 553$ mg L⁻¹; 58.6% mortality) against *Myzus persicae*¹⁸. In other studies, leaf methanolic extracts of *Prosopis juliflora* at 1% also showed 86.6% mortality against *A. craccivora*¹⁹. Similarly, among the bark extracts, BEE 80% ($LC_{50} = 5115.98$ mg L⁻¹) was found to be more effective and was followed by BEE 100% as compared to others after 96 h of treatments. The present study also confirms the findings of the previous report, where bark methanol extract of *Prosopis juliflora* at 1% showed 93.3% mortality against *A. craccivora*¹⁹.

The SO of *T. sebifera* in the current study was found to be more effective after 72 h and 96 h of treatment ($LC_{50} = 2504.59$ and 850.94 mg L⁻¹, respectively) than leaf and bark extracts. Our results are agreed with the findings of Fenigstein et al.²⁰, who reported seed oil of *Ricinus communis* (1.5%) showed 75% mortality of *Bemisia tabaci* as compared to the present study where seed oil of *T. sebifera* recorded higher mortality against aphids at a lower concentration. In a similar study, neem seed oil 2% also showed a 71% reduction in the population of *Lipaphis erysimi*²¹. The insecticidal activity of SO of *T. sebifera* in the current study may be due to the presence of unsaturated fatty acids (oleic, linoleic and palmitoleic acid).

The residual toxicity of the combination of SO with 80% LEE/BEE of *T. sebifera* against *A. craccivora* showed significant synergistic interaction under laboratory conditions. Among the combinations, BEE + SO (1:1 ratio) was found to be more effective ($LC_{50} = 144.26$ mg L⁻¹) against *A. craccivora* and was followed by SO+LEE at 1:1 ratio, LEE+SO+BEE at {(1+1):2} ratio, LEE +BEE at 1:3 ratio as compared to other mixtures 96 h after treatment under laboratory conditions. Similarly, under plant growth chamber, the binary mixtures of SO+LEE ($LC_{50} = 223.82$ mg L⁻¹) were more effective against *A. craccivora* and was followed by SO+BEE as compared to LEE, SO, and BEE. Based on FEI values, SO+BEE showed synergistic interaction.

Among the among leaf fractions, *n*-hexane fraction ($LC_{50}=196.61 \text{ mg L}^{-1}$) was the most promising against *A. craccivora* as compared to other fractions. However, among the bark fractions, ethyl acetate fraction was most effective ($LC_{50}=813.45 \text{ mg L}^{-1}$) against *A. craccivora*. The efficacy of *n*-hexane fraction of leaf against *A. craccivora* may be due to the presence of metabolites like *n*-hexadecanoic acid, galaxolide, ethyl phthalate, octadecanoic acid, ethyl ester, etc. Similarly, *n*-hexane fraction of bark, the predominant metabolites are galaxolide followed by ethyl phthalate and 1-octadecene. It is also confirmed that the efficacy of tested fractions against the *A. craccivora* also may be due to the presence of flavonoids (kaempferol-3-O-glucoside, quercetin-3-O-glucoside) and phenolic acids (gallic acid and shikimic acid). In the current study, *n*-hexane leaf fraction showed strong efficacy against *A. craccivora* as compared to *n*-hexane fraction of *Ageratum houstonianum* and *Eupatorium adenophorum* ($LC_{50}=2881-2590 \text{ mg L}^{-1}$) after 96 h²²⁻²³. Present results also confirm the findings of other reports where leaf *n*-hexane fraction of *Ricinus communis* showed 92-96% mortality against *Sipha flava* and *Melanaphis sacchari*²⁴⁻²⁵. Similarly, ethyl acetate leaf fraction in the current study also reported promising ($LC_{50}=367.75 \text{ mg L}^{-1}$) than ethyl acetate fraction of *Trillium govanianum* ($LC_{50}=2186.3 \text{ mg L}^{-1}$) against *A. craccivora* after 96 h²⁶.

The residual toxicity of the isolated compounds against *A. craccivora* showed gallic acid was most effective ($LC_{50}=1303.68 \text{ mg L}^{-1}$) against *A. craccivora* and was followed by shikimic acid and quercetin-3-O-glucoside as compared to kaempferol-3-O-glucoside. The present results on the efficacy of gallic acid confirmed with that of Punia et al.²⁷ who reported the gallic acid at 3125 ppm incorporated diet fed to the larvae of *S. litura* showed 70% larval mortality, adult emergence, and delayed developmental period. In the current study, quercetin and kaempferol also showed toxicity against aphid and these results also confirmed with findings of earlier studies where, kaempferol and quercetin from methanolic extract of *Buddle jalbiflora* Hemsl., showed efficacy against *P. xylostella* ($LC_{50}=1.454 \text{ mg mL}^{-1}$), *Mythimna separata* (74-77% mortality) and antifeedant against *P. xylostella* ($AFC_{50}=1.019 \text{ mg mL}^{-1}$)²⁸.

Detoxification enzymes, such as GST, cytochrome P450 monooxygenases, and carboxyl/cholinesterases, are well known for helping plant-feeding insects to maintain physiological roles by detoxifying xenobiotic substances²⁹⁻³⁰. Pesticides and poisonous secondary metabolites from host plants are examples of xenobiotic substances³⁰. The plant extracts/fractions/essential oils have a variety of modes of action against insects, including neurotoxicity, insect growth/digestive enzyme inhibition, and inhibition GST and cytochrome P450 monooxygenase³¹. The AChE activities in *A. craccivora* fed with bean leaf discs treated with different concentrations of LEE, BEE and SO showed significant inhibition as compared to control aphid. Present results confirmed with the findings of Dolma et al.³² who reported *Tagetes minuta* oil showed inhibitory effect on AChE in *P. xylostella*. In a similar study, essential oils and synthesized nano-emulsion from *Basilicumocimum* L., *Cuminum cyminum* L., *Origanum marjorana* L. and *Matricaria chamomilla* L. showed decreased activity of AChE in *A. craccivora*³³. Similarly for the GST assay, all the concentrations of LEE, BEE and SO significantly inhibit the GST activity as compared to control. Our results conform with the findings of Phankaenet al.,³⁴ who reported caffeine from *Coffea arabica* extract

inhibits GST and carboxylesterase (CarE) in *Tribolium castaneum*. Similarly, our previous study also showed that *Tagetes minuta* oil inhibits GST in *P. xylostella*³².

The current study concludes that extract from leaf and bark, fractions and isolated compounds of *T. sebifera* can be used for the control of *A. craccivora*. The insecticidal activity might be due to the presence of individual or combination action of extracts and seed oil of *T. sebifera*. Based on the literature survey, the efficacy of *T. sebifera* extracts/fractions/ isolated compounds and seed oil against *A. craccivora* as well as the chemical composition of seed oil and *n*-hexane fractions had not previously been reported earlier. Thus, this study is unique and executed for the first time to examine the leaf, bark extracts and seed oil of *T. sebifera* against *A. craccivora*.

Conclusions

In the present study, among leaf/bark extracts, BEE 80% and 100% were found to be most effective followed by BME 50% as compared to LEE and LME 80%. The seed oil was found more effective than leaf and bark extracts. Among combinations, BEE+SO (1:1 ratio) was most effective against *A. craccivora* followed by SO+LEE at 1:1 ratio, LEE+SO+BEE at (1+1):2 ratios and LEE+BEE at 1:3 ratio. All the combinations/blends showed synergistic activity to *A. craccivora* but LEE + BEE at 1:3, 3:1, and 1:1 ratio showed the most significant synergistic interaction. Among leaf/bark fractions, the *n*-hexane leaf fraction was most effective against aphids. Among pure compounds, gallic acid was found most effective. LEE, BEE and SO significantly inhibit the AChE and GST activity in *A. craccivora*. Based on our findings, the leaf extracts/seed oil can be recommended for the control of aphids in crop plants under greenhouse/field conditions.

Declarations

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Declaration of Competing Interest

All authors of this manuscript declare that have no conflict of interest.

Author contributions

S.K.D., Investigation, data curation, formal analysis, validation, roles/writing - original draft, P.P.S., formal analysis, S.G.E.R., Conceptualization, supervision, funding acquisition, formal analysis, writing -editing.

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Figures

Fig.1a

Fig.1b

Figure 1

Detoxification enzyme activities: AChE (1a) and GST inhibition (1b) in *Aphis craccivora* treated with leaf extract, bark extract and seed oil of *Triadica sebifera*; Bars represents standard error (±SE) of three replications.

Triadica sebifera
(Leaf and bark: 1.0 kg)

Figure 2

Schematic representation of extraction and fractionation of *Triadica sebifera*

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