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RESEARCH ARTICLE

Development and evaluation of emulsifiable concentrate formulation containing *Sophora alopecuroides* L. extract for the novel management of Asian citrus psyllid

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Abstract

Utilization of non-host plants semiochemicals to mediate insect behavior offers a promising opportunity for novel management of insect pests in field crops and fruits. Therefore, there is still a substantial opportunity for the development of natural prophylactic as an eco-friendly approach in the novel pest management programs. *Sophora alopecuroides* extract has been used as a natural pesticide in the control of agricultural and household pests, but the low persistence effect and rapid biodegradability limit its use on a wider scale in pest management programs. In this study, an emulsifiable concentrate formulation containing *S. alopecuroides* extract (SAE-EC) was developed with a simple procedure and evaluated for its ovicidal, antifeedant, and repellent effects against *Diaphorina citri* under laboratory and semi-field conditions. Our results indicated that SAE-EC at 15, 30, and 50 mg/mL concentrations provide complete protection against psyllids for a period of 96 h after application both under laboratory and semi-field conditions, while the aqueous methanolic extract of *S. alopecuroides* loses its persistence 48 h after application. Furthermore, the emulsifiable concentrate at 20 and 30 mg/mL concentrations, only 15.97% and 31.97% of eggs were able to hatch, and at similar concentrations, 72.86% and 85.5% of honeydew secretion were reduced as compared to the control. SAE-EC at 30 mg/mL concentration has not shown any phytotoxic symptoms on *Murraya paniculata* seedlings. Fourier transform infrared (FTIR) study revealed the presence of alkaloids in emulsifiable concentrate after 3 months of its preparation placed under ambient temperature. Furthermore, the particle size and polydispersity index (PDI) of the emulsifiable concentrate were also confirmed by dynamic light scattering (DLS). Our finding indicated that emulsifiable concentrate formulation prolongs the persistence of *S. alopecuroides* extract and enhances its efficacy both under laboratory and semi-field conditions. It has been concluded that the emulsifiable concentrate formulation containing *S. alopecuroides* extract might be developed as an eco-friendly novel prophylactic against citrus psyllid.

Keywords Botanical pesticides · Asian citrus psyllid · Emulsifiable concentrate · *Sophora alopecuroides* · Novel prophylactic

Introduction

The population of the world is increasing rapidly and food insecurity is one of the major concerns in developing countries (Handelman and Brynen 2019; McNabb 2019). The agricultural sector mainly relies on the use of synthetic pesticides to overcome productivity losses because of pests (Dey 2016). The massive use of synthetic pesticides in agricultural sectors to minimize production losses results in groundwater pollution, pesticide resistance, and adverse effects on humans and animals (Benelli et al. 2019; Dey 2016; Govindarajan et al. 2018; Hashem et al. 2018). Botanical pesticides have gained interest in recent decades as a possible alternative to synthetic pesticides (Isman 2017; Pavela and Benelli 2016). Plant extracts and essential oils are widely studied as an alternative to synthetic pesticides due to low mammalian toxicity, rapid biodegradability, and being relatively economical as compared to chemical pesticides (Benelli et al. 2019; Isman 2017; Isman et al. 2011; Pavela et al. 2019a). However, besides having toxic, repellent, antifeedant, and ovicidal effects of botanicals

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against various agricultural pests, botanical pesticides face various challenges and constraints with respect to use on a wider scale for pest management due to rapid biodegradability, volatility, and the lack of solubility in water (Isman 2015; Isman 2017; Pavela 2014). Therefore, it is necessary to develop appropriate formulations which enhance the persistence of such botanicals by minimizing the volatility of the bioactive components through controlled release methods (Damasceno et al. 2018; Pavela et al. 2019b). Various techniques are used to overcome these issues, including encapsulation, nanoemulsion, nanoparticle synthesis, and emulsifiable concentrate formulations to enhance the solubility, persistence, and efficacy (Ahmadi et al. 2018; Damasceno et al. 2018).

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is one of the important pests of citrus around the globe (Rizvi et al. 2019; Vanaclocha et al. 2018; Zheng et al. 2018), acting as a prominent vector of huanglongbing (HLB), a bacterial pathogen causing citrus greening disease (Gupta et al. 2017; Rizvi et al. 2018). Currently, there is no cure for HLB, so the main reliance is on the application of synthetic insecticides in the control of the psyllid to prevent the dispersal of citrus greening disease (Tian et al. 2018). Due to the massive use of chemical insecticides for the control of the citrus psyllid, it results in the development of resistance to various classes of insecticides (Chen et al. 2018; Pardo et al. 2018; Tian et al. 2018). Therefore, utilization of phytochemicals for the management of the citrus psyllid, which is relatively safer for the environment, is one of the possible alternatives to chemical insecticides. *Sophora alopecuroides* is mainly dominated by alkaloids and the main bioactive compounds are matrine, oxymatrine, aloperine, cytisine, sophocarpine, and sophoridine (Ma et al. 2018). In our previous study, *S. alopecuroides* L. alkaloids extract showed a strong behavioral effect against the citrus psyllid (Rizvi et al. 2019). Similarly, the plant extracts lose their persistence in a short period of time due to rapid biodegradability and volatility, which limit their use in pest management on a broad scale (Benelli and Pavela 2017; Eka et al. 2017; Pavela 2014).

Therefore, the current objective of this study is to formulate *S. alopecuroides* extract emulsifiable concentrate (SAE-EC) formulation and to determine its persistence against the citrus psyllid and the safety of emulsifiable concentrate against *M. paniculata* potted seedlings (phytotoxic effects). Furthermore, the ovicidal, antifeedant, and repellent effects of SAE-EC against the citrus psyllid under both laboratory and semi-field conditions were also assessed.

Materials and methods

Insects

The laboratory-reared population of citrus psyllid was used in this study. The psyllids were reared in a cage with *M. paniculata* seedlings under 27 ± 2 °C, 65% ± 5% RH in the key laboratory of natural pesticides and chemical biology South China Agricultural University Guangzhou, P.R. China.

Plant material and extraction procedure

The leaves and flowers of *S. alopecuroides* were collected from Gamba Skardu Baltistan, Pakistan, in August 2018. The plant species were identified by Dr. Sajid Sultan National Agriculture Research Council (NARC) Islamabad, Pakistan, and voucher specimen was submitted in the NARC herbarium. The plant material was extracted twice for 2–4 h with 70% aqueous methanol under room temperature and concentrated under *vacou*. The chemical analysis of the *S. aleopocridius* extract was previously reported (Ma et al. 2018; Rizvi et al. 2019).

Preparation of emulsifiable concentrate (SAE-EC)

An emulsifiable concentrate formulation of *S. alopecuroides* extract (SAE) was prepared by following the method of Wiwattanapatapee et al. (2009) using a simple mixing procedure. The semisolid extract was oven-dried and ground into a fine powder. Then the dried SAE (10 g) was diluted in 10 mL of 70% aqueous methanol and mixed with Tween 80 (30%) and Span 80 (20%) using a mortar and pestle and placed over a magnetic stirrer for 30 min at room temperature; the resultant emulsion contains (10% *w*/*v*) of SAE. Then, 0.05 g of butylated hydroxytoluene (BHT) and soybean oil (30%) was slightly added and mixed to give a homogeneous concentrate mixture. An emulsifiable concentrate without SAE was also prepared and used as a control. The emulsifiable concentrate was diluted in distilled water for foliar application.

Settling behavior of ACP

The settling behavior of adult psyllids toward *M. paniculata* potted seedlings treated with different concentrations of SAE-EC, i.e., 0.1, 0.5, 1, 2, and 3% *w*/*v*, corresponding to the dosages of 1, 5, 10, 20, and 30 mg/mL, respectively, was observed in a choice experiment under the following laboratory conditions: 26 ± 2 °C, 65% ± 5% RH. A mixed population of 50 adult psyllids was released into the cages (60 cm × 30 cm × 60 cm), each cage with six *M. paniculata* potted seedlings and one from each treatment including the control. The seedlings were sprayed with 1 mL of desired concentrations of SAE-EC and allowed to dry. The numbers of psyllids per seedlings were recorded after 24, 48, and 96 h after release. An aqueous methanolic extract of guava was used as a positive control.

Feeding behavior of ACP

The feeding activity of adult psyllids was evaluated through the amount of honeydew excreted by the adults confined to *M. paniculata* leaf disc dipped in SAE-EC solution. Briefly, 3 mL of 1.5% of agar solution was added to 60-mm diameter plastic disposable Petri dishes in order to form a solidified bed. The leaf disc, with an average size of 5.65 ± 0.1 cm (± SE) in length, was dipped in desired concentrations of SAE-EC and allowed to dry. Ten CO_2-anesthetized adult psyllids were released in each Petri dish and capped with a lid lined with 60 mm Whatman filter paper. Finally, the Petri dishes were wrapped with lab Parafilm and turned upside down. Forty-eight hours after release, the filter papers were removed and immersed in 1% of *w*/*v* ninhydrin solution (Sigma-Aldrich) in aqueous acetone for 3 min. The honeydew present on filter papers reacted with ninhydrin, whereby forming dark-purple spots (Tiwari et al. 2012). The feeding activity was assessed by counting the total number of purple spots on each filter paper disc under a stereomicroscope.

Ovicidal activity

The ovicidal activities of SAE-EC were evaluated by confining *M. paniculata* seedlings containing eggs. Briefly, a mixed population (eight males and eight females) of adult psyllids were released into gauze nets for a period of 5 days. After 5 days, the psyllids were removed and the total numbers of eggs were counted under a stereomicroscope. Then, the seedlings were sprayed using a hand sprayer with the desired concentrations of SAE-EC (approximately 1 mL per seedling) and allowed to dry at room temperature. Later, the seedlings were again confined with gauze nets. The numbers of hatched nymphs were counted until all of the eggs had hatched (14 days from the eggs being laid). All of the laboratory conditions were similar to the settling bioassay. The eggs' mortality was calculated by using the formula given below.

$$\text{Egg mortality } (\%) = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs laid}} \times 100 \quad (1)$$

Evaluations of *S. alopecuroides* extract emulsifiable concentrate repellent effect under semi-field conditions

The repellent effect of SAE-EC under semi-field conditions was assessed by following the procedure of Hall et al. (2018). Briefly, *M. paniculata* potted seedlings grown in a greenhouse were used for this bioassay. Each potted seedling was pruned in such a way that it should contain both old and new leaves. The potted seedlings were sprayed with 5, 7.5, 15, 30, and 50 mg/mL of SAE-EC with a manual handheld sprayer (approximately 1 mL per seedling), with five potted seedlings from each treatment, and allowed to dry at room temperature. The control seedlings were sprayed with the emulsifiable concentrate without SAE. Methanolic extract of *P. guajava* and DEET were used as a positive control. Later, the dried potted seedlings were placed under semi-field conditions (24.27 ± 3.65 °C and 78.77 ± 6.65 RH) inside of the greenhouse. The seedlings were indiscriminately placed 200 cm apart from each other within a row and in each row, the placement of seedlings was not the same. Initially, mixed populations of 5000 adult psyllids were released at ~ 1:00 p.m. The psyllids were free to move within the greenhouse and to land on the sprayed seedlings. After 48 h at ~ 10:30 p.m., the number of adult psyllids on stems, mature leaves, and flush shoots were counted. Around 1000 additional psyllids were released in the greenhouse after 94 and 144 h respectively. The total numbers of adults per plant (stems, mature leaves, and new foliage) were counted after 12, 24, 48, 72, 96, 120, 144, and 168 h, respectively. The phytotoxicity of SAE-EC and aqueous methanolic extracts of SAE, *P. guajava* and DEET to the mature leaves and flush shoots was also determined. For phytotoxic measurement, a damage rating scale (0–5) was used. The average necrosis percentage per leaf per plant was rated as follows: "−" (0% for no considerable visible injury), slight "+" (1–25%), medium "++" (25–50%), and high "+++" (> 50%). The number of eggs laid per potted seedling was also assessed by excising the flush shoots, and eggs were counted under a stereomicroscope.

Characterization of emulsifiable concentrate formulation

Fourier transform infrared analysis

To confirm the presence of alkaloids in the SAE-EC after 3 months of its preparation, it was analyzed separately by Fourier transform infrared (FTIR) spectroscopy using potassium bromide (KBr) as a pellet and was recorded by ABB FTLA 2000–100 (Quebec, Canada) at a resolution limit of 16 cm–1. The SAE-EC was dissolved in distilled water and centrifuged (Eppendorf 5804R, Eppendorf International) at 14,000×*g* for 20 min at 20 °C, and the resultant pellets were freeze-dried and used for FTIR analysis.

Dynamic light scattering measurements

Dynamic light scattering (DLS) was applied to measure the average particle size and polydispersity index (PDI) on a Zetasizer Nano ZES (Malvern, UK). Each sample was examined in triplicate at 25 °C at a scattering angle of 90°. Double-distilled water was used as a reference for the dispersing medium. The results are presented as the mean average particle size, and each sample was replicated three times.

Physical properties of *S. alopecuroides* extract emulsifiable concentrate

The physical properties of SAE-EC (including viscosity, pH, and particle size) were determined by dispersing the emulsion in double-distilled water by stirring with a magnetic stirrer at 500 rpm for 10 min in order to complete the emulsification processes at room temperature (26 ± 2 °C). Briefly, 1.5 g of SAE-EC was diluted in 25 mL of distilled water to obtain 1:20 dilutions. The pH of the emulsion was measured by an Ohaus portable pH meter (Ohaus Corporation, USA). The viscosity of the emulsion was determined by using an Ndj-2 Rotational Viscometer, China (250 rpm).

Statistical analysis

Statistical analysis of the ovicidal activity data was conducted using probit analysis (SPSS 17.0) to determine the 50 and 90% lethal concentration. All of the statistical tests were conducted by using SPSS 17.0; P values of less than 0.05 were considered significant. For the SAE-EC deterrence against citrus psyllids under semi-field conditions, numbers of adult psyllids on stems and mature leaves and of adults on flush observed each day were subjected to repeated measured analyses of variance and numbers of eggs on flush were subjected to simple analyses of variance. PROC GLIMMIX (SAS Institute, 2010) was used for these analyses because the count data did not follow the normal distribution (based on the Shapiro–Wilk test). Mean comparisons among repellents were made by least squares means. The damage rating data were analyzed using the F-approximation of the Friedman test (Ipe 1987) and the associated rank sum multiple comparison test with PROC GLM. The procedure was used after ranking the data within each replication from lowest to highest value using PROC RANK (SAS Institute, 2010). All statistical analyses were conducted at the 0.05 level of significance.

Results

Effect of SAE-EC on settling behavior of ACP

The settling behavior of ACP adults was not significantly different among the various concentrations of SAE-EC tested and the control 24 h ($F = 18.98$; df = 4, 24; $P = 0.243$) after release. However, significant differences were observed 48 h ($F = 66$; df = 4, 24; $P = 0.005$) and 96 h ($F = 86$; df = 4, 24; $P = 0.001$) after release (Fig. 1). However, there was no considerable repellent effect of 30 mg/mL methanolic extract of *P. guajava* observed 48 h after application used as a positive control.

Effect of SAE-EC on ACP feeding

A concentration-dependent antifeedant effect of SAE-EC on the feeding activity of ACP was observed. With the exception of 1 mg/mL of SAE-EC tested, all of the treatments of 5, 10, 20, and 30 mg/mL significantly reduced the amount of honeydew excretion as compared to the control ($F = 84.47$; df = 4, 24; $P < 0.0001$). Furthermore, there was a reduction of 86% and 92% of honeydew excretion by SAE-EC at 20 and 30 mg/mL concentrations (Fig. 2). However, the antifeedant activity of SAE-EC was low as compared to cyantraniliprole, which caused an 80% reduction in honeydew droplet secretion by ACP at 0.1 μg/mL (Tiwari and Stelinski 2013).

Ovicidal bioassay

The mean percentage of eggs laid per potted seedling ranged from 23 to 42%. The hatching percentage of eggs from the control plant was 93.70 ± 1.37. There were significant differences ($F = 92.78$; df = 5, 29; $P < 0.0003$) in egg-hatching inhibition among the treatments. However, 1 mg/mL did not significantly affect egg hatching. After 14 days of exposure to SAE-EC, egg hatching was inhibited by 84.03, 68.03, 54.23, and 35.12% at 30, 15, 7.5, and 5 mg/mL, respectively. The LC_{50} concentration required to inhibit 50% of an egg from hatching was 9.01 mg/mL. However, the ovicidal activity of SAE-EC was low as compared to acetamiprid with LC_{50} value of 0.0028 mg/mL (Table 1).

Evaluations of SAE-EC deterrence against citrus psyllids under semi-field conditions

The SAE-EC was applied at 5, 7.5, 15, 30, and 50 mg/mL under semi-field conditions at South China Agriculture University, Guangzhou. Significantly, all of the concentrations deterred infestations during the period of 168 h after application. However, the SAE-EC at 15, 30, and 50 mg/mL concentrations provided complete protection against psyllids for a period of 96 h after application. Regarding phytotoxicity, all of the concentrations tested caused no visible phytotoxicity to the mature leaves. The emulsifiable concentrate at 50 mg/mL caused slight phytotoxicity (1–25%) to immature leaves. However, 50 mg/mL (SAE methanolic extract) caused slight phytotoxicity (1–25%) to mature leaves and medium phytotoxicity (25–50%) to immature leaves (Table 2). Similarly, due to the strong deterrence activity, fewer adults were settled on treated seedlings with 5, 7.5, 15, 30, and 50 mg/mL and no eggs were laid. In contrast, relatively large numbers of psyllids and eggs were laid on control seedlings. However, 50 mg/mL aqueous methanolic solution of SAE lost its persistence 48 h after application. Furthermore, methanolic extract of *P. guajava* and DEET, at

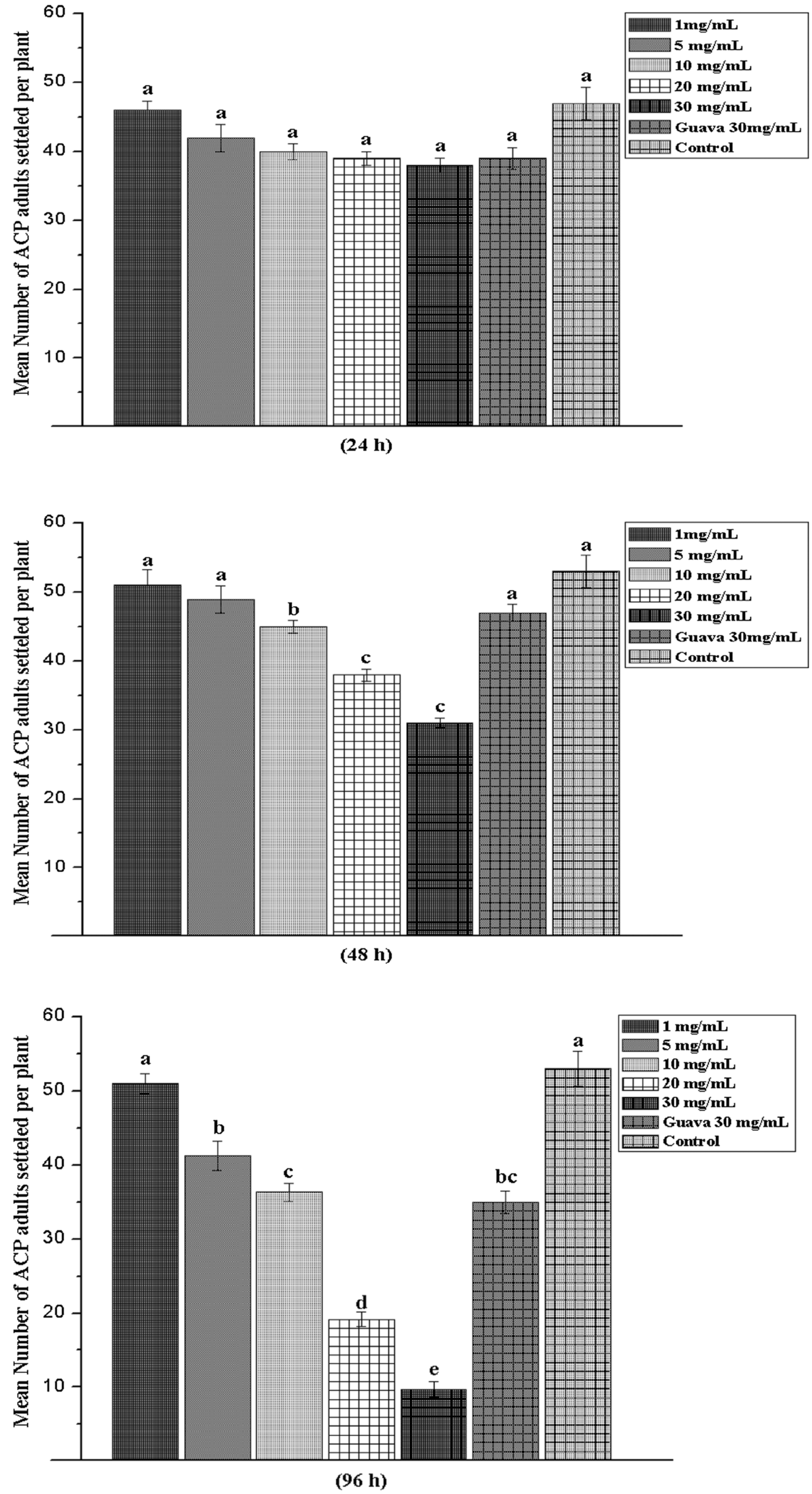

Fig. 1 Settling preference of ACP adults on *M. paniculata* seedlings treated with various concentrations of SAE-EC 24, 48, and 96 h after release of adults. Bars within a panel not labeled by the same letter are significantly different from one another according to Tukey's test ($P < 0.05$). 30 mg/mL methanolic extract of *P. guajava* was used positive control

a concentration of 30 mg/mL, which were used as a positive control, did not show any considerable significant repellent effects on psyllids; rather, they showed medium phytotoxicity to both mature and immature leaves (25–50%).

FTIR study

The FTIR spectra were taken for the characterization of the presence of alkaloids in SAE-EC after 3 months of its

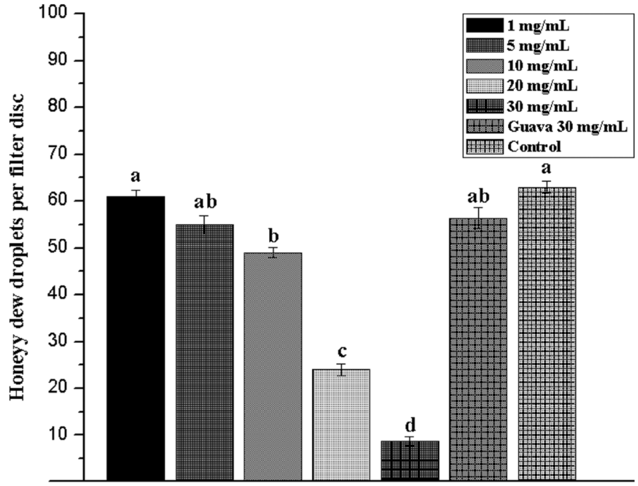

Fig. 2 Effect of SAE-EC on ACP adult feeding as measured by the number of honeydew droplets produced. Citrus leaf discs treated by various concentrations of SAE-EC or 20% acetone (as control) were exposed to 10 ACP adults. Bars not labeled by the same letter are significantly different from one another according to Tukey's test ($P < 0.05$)

preparation and are presented in Fig. 3. The FTIR spectra showed peaks which may be assignable to O-H, N-H stretches (3366.67 cm^{-1}), and N-H bending (1611.43 cm^{-1}). Similarly, as for all of the heterocyclic compounds, the N-H stretching vibration occurred in the region of 3500–3000 cm^{-1} (Krishnakumar et al. 1997; Raja et al. 2013; Ramasamy 2015). The FTIR band that appeared at 3366.67 cm^{-1} of the title compound has been assigned to N-H stretching modes of vibrations. Meanwhile, the carbonyl of a methyl ester (1642.30 cm^{-1}) and C-C stretches 1461.15 cm^{-1} and 1514.18 cm^{-1}. These observations suggested the presence of alkaloids in SAE-EC. Similarly, analytical techniques like TLC, FTIR, and HPLC are used for the qualitative and quantitative determination of bioactive compounds in the extract. However, in our study, we use FTIR analysis for rapid determination of the presence of alkaloids in the emulsifiable concentrate formulation; our results are similar to the findings of Altameme (2018), Malviya and Dwivedi (2019), and Sivapriya and John (2019) which determines bio-active alkaloids in the extract of *Frankenia hirsuta*, *Cinnamomum zeylanicum*, and *Ailanthus altissima* by using FTIR analysis.

Particle size and physical properties of SAE-EC

The mean particle size distribution profile is presented in (Fig. 4a, b). The mean diameter of emulsifiable concentrate without SAE was recorded at 256.9 ± 3.86 nm and the SAE-SC at 263.9 ± 2.27 nm. However, the DLS trace of emulsifiable concentrate without SAE showed a bimodal size distribution, with the minor fraction of the population having a mean diameter over 1 μm. For these reasons, emulsifiable concentrate without SAE showed a PDI value of 0.44 ± 0.008, while SAE-SC of 0.35 ± 0.03, respectively. The PDI data revealed that SAE-SC showed a narrow size distribution as compared to the emulsifiable concentrate without SAE. The emulsifiable concentrate, when diluted with water (1:20), rapidly provided a stable light-green-colored emulsion, while emulsifiable concentrate without SAE possessed white-colored emulsion in water (Fig. 5). A very little creamy layer was developed after 24 h of dilution; however, the layer was easily dispersed under mild shaking with hands. The pH value (5.97) of the aqueous emulsion obtained from the emulsifiable concentrate was lower than that (6.32) from the corresponding aqueous control emulsifiable concentrate. The viscosity (4.29 cP) of the SAE-EC was slightly higher than that (3.38 cP) of the control.

Discussion

The development of resistance against traditional pesticides and their hazardous effect on the environment and humans'

Table 1 Ovicidal activity of SAE-EC against Asian citrus psyllid eggs

Treatment	% Eggs unhatched	LC_{50} (95% CL) mg/mL	LC_{90} (95% CL) mg/mL	Slope ± SE	X^2 (df)	*P* value
1 mg/mL	12.23 ± 2.54	9.01 (12.20–18.54)	24.55 (45.34–64.32)	1.09 ± 0.12	4.89 (3)	0.33
5 mg/mL	35.12 ± 2.23					
10 mg/mL	54.23 ± 1.54					
20 mg/mL	68.03 ± 1.34					
30 mg/mL	84.03 ± 1.34					
Control	6.30 ± 1.01					
Acetamiprid[d]	95.12 ± 0.28	0.0028 (0.012–0.026)	0.025 (0.24–0.48)	1.31 ± 0.231	3.92 (3)	0.97

[a] *M. paniculata* potted plant containing eggs were sprayed with 1 mL of each treatment, plants were sprayed to runoff

[b] 50% lethal concentration

[c] $\chi 2$ chi square, df degrees of freedom

[d] Acetamiprid used as a positive control for comparison purpose

Table 2 Comparative deterrence against citrus psyllid and phytotoxicity of SAE-EC diluted in distilled water applied to outdoor *M. paniculate* potted seedlings 5, 7.5, 15, 30, and 50 mg/mL foliar sprays

Treatments	Mean number of adults per plant (Stems, mature leaves and new foliage) (hours)								Number of eggs	Phytotoxicity ratings	
	12*	24	48*	72	96*	120	144*	168		Mature leaves	Flush
Control	8.5 ± 0.86a	11.75 ± 1.37a	21.75 ± 0.85a	27.5 ± 1.70a	32.25 ± 0.87a	42.75 ± 1.03a	47.5 ± 1.55a	57.55 ± 0.86a	27.7 ± 65a	–	–
5 mg/mL	0.0 ± 0.0b	0.0 ± 0.0b	2.0 ± 0.40b	4.0 ± 0.41b	5.5 ± 0.86b	7.5 ± 0.64b	21.5 ± 0.64b	27.0 ± 1.08b	15.5 ± 12b	–	–
7.5 mg/mL	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	2.75 ± 0.47c	4.0 ± 0.41c	8.95 ± 0.47b	10.5 ± 0.64c	25.5 ± 0.64b	1.71 ± 0.23c	–	–
15 mg/mL	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	1.75 ± 0.26d	4.98 ± 0.63d	6.50 ± 0.28b	9.75 ± 0.48d	17.35 ± 0.47c	0.0 ± 0.0d	–	–
30 mg/mL	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0e	4.54 ± 0.28d	5.20 ± 0.40bc	8.25 ± 0.40d	14.75 ± 0.48 cd	0.0 ± 0.0d	–	–
50 mg/mL	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0	0.0 ± 0.0e	0.0 ± 0.0e	4.75 ± 0.47c	5.0 ± 0.40e	13.50 ± 0.29d	0.0 ± 0.0d	–	+
50 mg/mL (SAE methanolic extract)	0.0 ± 0.0b	0.0 ± 0.0b	9.25 ± 0.47d	24 ± 1.08a	32.5 ± 1.04a	36.25 ± 1.25d	43.25 ± 1.11a	55.75 ± 1.25a	17.4 ± 4.5b	+	++
DEET (30 mg/mL)	0.0 ± 0.0b	2.71 ± 2.22c	12.98 ± 0.52e	26.33 ± 1.02a	30.27 ± 1.16a	34.32 ± 1.87d	40.36 ± 2.76a	54.12 ± 2.76a	18.67 ± 1.98b	++	++
Psidium guajava (30 mg/mL)	0.0 ± 0.0b	3.97 ± 2.22d	19.5 ± 0.65f	25.55 ± 1.31a	30.67 ± 1.16a	36.24 ± 1.65d	44.87 ± 2.12a	54.33 ± 1.76a	19.12 ± 1.51b	++	++

*Represent the new release of citrus psyllid population in the cage after multiple days to maintain psyllid population

Phytotoxicity ratings "–" (0–1%), slight "+" (1–25%), medium "++" (25–50%), and high "+++" (> 50%)

For each treatment, mean numbers or percentages or ratings in the same column followed by the same letter are not significantly different ($P > 0.05$), least squares means. For 5, 7.5, 15, and 30 mg/mL concentrations 8 treatment and 72 error degrees of freedom and for 50 mg/mL concentrations 8 treatment and 36 error degrees of freedom

DEET (30 mg/mL) and aqueous methanolic extract of *P. guajava* were used as positive control by following the paper published by Hall et al. (2018), and control emulsifiable concentrate without SAE

botanical pesticides has been gaining more attention in pest management programs (Benelli et al. 2019; Koul 2019). Plant secondary metabolites play an integral role in phytophagous insects finding and locating a host (Birkett et al. 2004; Bruce and Pickett 2011). The secondary metabolites released by the plant act as a track line for herbivores (Cui et al. 2018; Heil and Bueno 2007). Masking or alteration in plant secondary metabolites, either by genetic engineering or by treating with non-host plant semiochemicals, results in the avoidance of a potential host (Ben-Issa et al. 2017; Zhang and Schlyter 2004). Regarding the repellent effect of SAE-EC against citrus psyllids, the results indicated that there was a concentration-dependent effect. The adults showed a strong preference for settling on the control seedlings as compared to the treated seedlings. ACP settling was not significantly reduced 24 h after release as compared to the control. However, after 48 and 96 h of release, only a few adults were observed on the treated plant as compared to the control. Previous studies have revealed that non-host plants' secondary metabolites alter the host-finding behavior of citrus psyllids (Hall et al. 2008; Rizvi et al. 2019; Silva et al. 2016; Zaka et al. 2010). The transmission of HLB from infected to uninfected plants is mainly attributed to the nymphs and adults of psyllids via feeding because the pathogen can only grow inside of the body of the eukaryotic host (Lopes et al. 2007; Silva et al. 2016). Therefore, keeping psyllids away from the citrus plants minimizes the chance of bacterial transmission within the citrus groves. Here, we found that SAE-EC greatly reduced the feeding activity of ACP, measured as the number of purple spots on the treated leaf disc. SAE-EC at 20 and 30 mg/mL reduced 72.86 and 85.5% of honeydew secretion as compared to the control. Further examination should be ensured by using EPG (electrical penetration graph) technology to obtain more precise information concerning the antifeedant activity of SAE-EC.

The management of citrus psyllids is mainly reliant upon the use of synthetic insecticides of various classes (Tian et al. 2018). Usually, these insecticides are used against psyllids at the adult stage (Tiwari et al. 2011). Very limited literature is available regarding the use of chemicals that target the eggs and nymphs of psyllids. The results of the current investigation showed that *M. paniculata* potted seedlings treated with SAE-EC produced concentration-dependent ovicidal activities. Only 31.7% and 15.9% of eggs were able to hatch into adults when confined with the dry residue of SAE-EC at 20 and 30 mg/mL, while in the control and 1 mg/mL concentration, the percentages of unhatched eggs were 6.30 and 12.23% respectively. Similarly, the lethal concentration of SAE-EC required to kill 50% eggs was 9.01 mg/mL, which was as low as compared to acetamiprid with LC_{50} value of 0.0028 mg/mL with edge of being environmentally friendly as compared to the synthetic insecticides. Our current investigation revealed that SAE-EC produced ovicidal activity

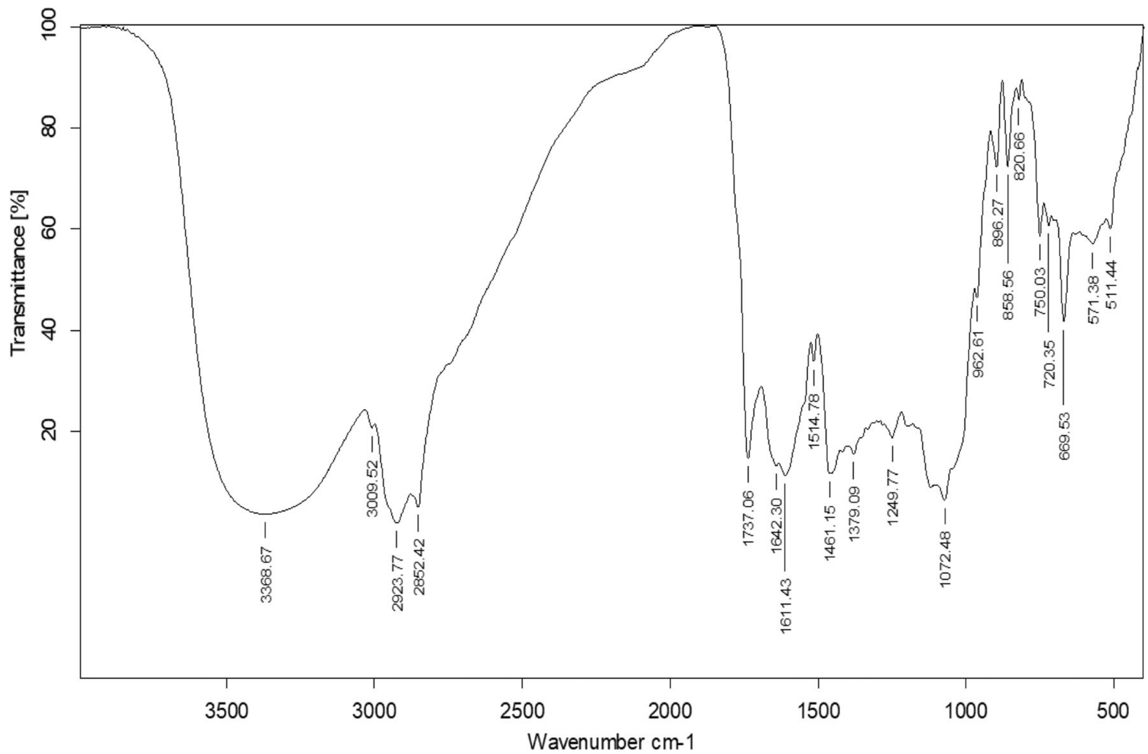

Fig. 3 FTIR absorption spectra of *S alopecuroides* extract emulsifiable concentrate pellets

against psyllids, as alkaloids are the main constituents in *S. alopecuroides* (Ma et al. 2018). Regarding the possible mode of action of alkaloids, they have multitarget mechanisms of action, including cytoplasmic membrane disruption, respiratory inhibition, and the inhibition of cell division (Cushnie et al. 2014; Erdemoglu et al. 2007; Marutescu et al. 2017), as well as the binding of muscarinic acetylcholine receptors (Andersson et al. 2008; Wink et al. 1998). This results in the modulation of respiration, the heart rate, and the central nervous system (Chowański et al. 2016). The possible mode of entry in living cells is due to the hydrophobic nature, which helps alkaloids to easily diffuse into living cells or into the surrounding area of target molecules (Wink et al. 1998).

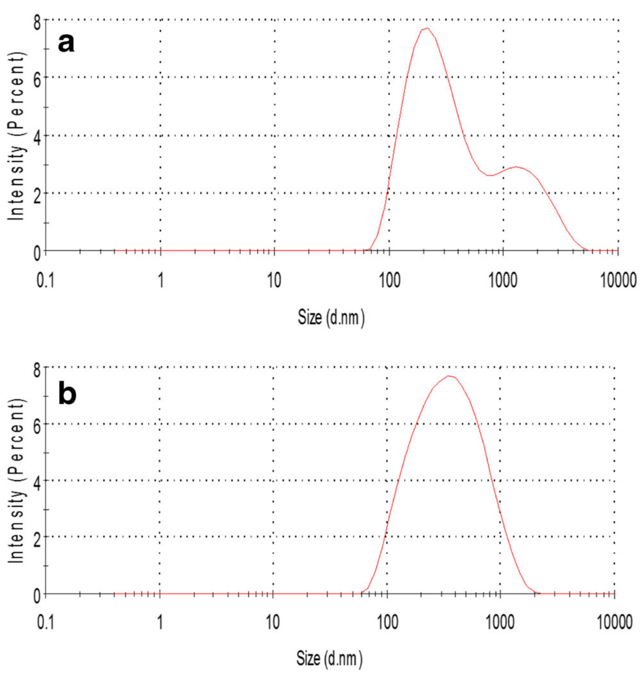

Fig. 4 The size distribution by intensity of emulsifiable concentrate in aqueous solution. **a** emulsifiable concentrate without SAE, **b** emulsifiable concentrate with SAE

The emulsifiable concentrate formulation is easily biodegradable and is relatively safe for the environment and mammals (Knowles 2008; Oka et al. 2006; Wiwattanapatapee et al. 2009). Similarly, the emulsifiable concentrate formulation has low persistence and easily biodegradable, as compared to the synthetic insecticides, SAE-EC is easily soluble in water because it just smeared on the plant surface, and make act as repellent against insects, however, there is no report that the plant extracts enter inside the plants and cause physiological changes. The ingredients used in SAE-EC are natural and safe for humans and the environment which makes the SAE-EC a possible novel alternative to the synthetic insecticides. The emulsifiable concentrate containing *S. alopecuroides* extract under both laboratory and semi-field conditions significantly deter citrus psyllids. Moreover, the formulation did not cause phytotoxic symptoms upon *M. paniculata* seedlings' mature

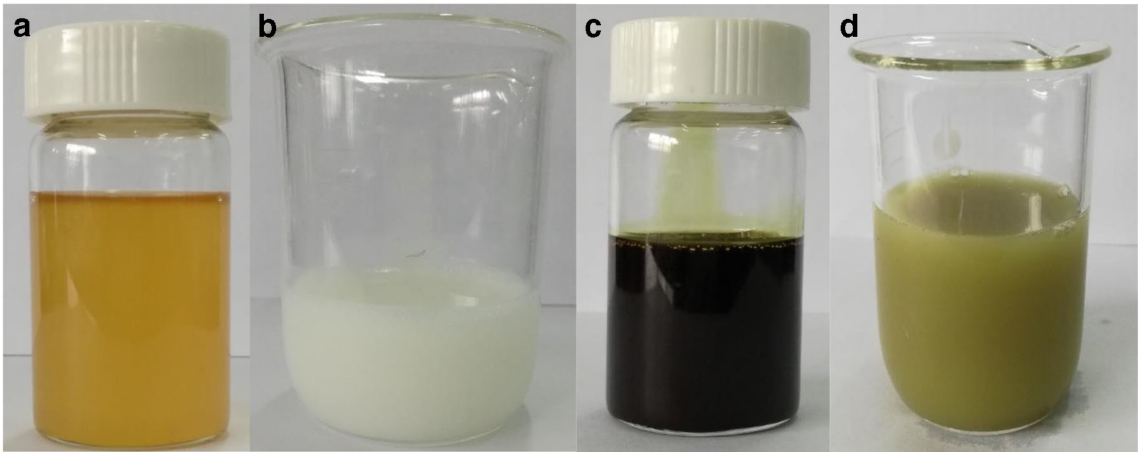

Fig. 5 Appearance of emulsifiable concentrate: **a** emulsifiable concentrate aqueous emulsion without *S. alopecuroides* extract, **b** emulsifiable concentrate diluted in 50 mg/mL distilled water, **c** *S. alopecuroides* extract emulsifiable concentrate aqueous emulsion, **d** *S. alopecuroides* extract emulsifiable concentrate at 50 mg/mL in distilled water

leaves, while minor phytotoxic symptoms (1–25%) appeared on immature leaves treated with 50 mg/mL of SAE-EC. Furthermore, the aqueous methanolic solution of SAE lost its persistence 48 h after application, while emulsifiable concentrate containing *S. alopecuroides* extract prolonged its persistence up to 168 h after application under semi-field conditions. The results of the current study revealed that the emulsifiable concentrate at 30 mg/mL did not show any phytotoxic symptom upon the *M. paniculata* seedlings. The formulations were significantly successful in deterring the psyllid populations by over 80% compared to the control with a high population. Similarly, the emulsifiable concentrate formulation did not cause the death of the males and females of parasitoid *Eriborus argenteopilosus* and did not cause any phytotoxic symptoms upon the broccoli leaves under field conditions (Eka et al. 2017). Furthermore, the results indicated that the persistence of SAE was prolonged when the extract was formulated as an emulsifiable concentrate. Further research should focus more on appropriate formulations, i.e., nano emulsions, cyclodextrins, and chitosan-based formulations, for better results.

Regarding the stability of alkaloids in the emulsifiable concentrate, the FTIR findings proved the presence of alkanes, alkyl halide, amine, aldehyde, and alkane, showing major peaks at 820.66, 858.56, 8966.27, 962.21, 1072.48, 1249.77, 1737.06, 2852.42, 2923.77, and 3368.67 cm^{-1} in the emulsifiable concentrate (Altameme 2018; Malviya and Dwivedi 2019), after 3 months of placing the emulsifiable concentrate in room temperature. Similarly, a very strong absorption band appearing in this region suggested the presence of alkaloids (Pavia et al. 2008; Raja et al. 2013; Singh et al. 2017), as alkaloids are the main bioactive constituents in *S. alopecuroides* (Ma et al. 2018). Our findings are in accordance with Altameme (2018), Malviya and Dwivedi (2019) and Sivapriya and John (2019), as they determine the presence of bio-active alkaloids in the extract of *Frankenia aucheri*, *Cinnamomum zeylanicum*, and *Ailanthus excels by* using FTIR analysis.

Similarly, SAE-EC has an excellent repellent effect against citrus psyllids with minimal phytotoxicity to *M. paniculata* seedlings. Our current investigation revealed that our emulsifiable concentrate showed stable aqueous emulsion after 1:20 dilution. The SAE-EC prolonged the repellent effect against psyllids under semi-field conditions, suggesting that the formulations would have the best efficacy up to 96 h after spraying, with minimal phytotoxicity to the *M. paniculata* seedlings. This may be due to the composition of soybean oil; for example, it contains long-chain polyunsaturated fatty acids and emulsifiers added in the emulsion, i.e., Tween and Span 80 (Wang et al. 2006), that enhance the adhesion ability of the emulsifiable concentrate on the leaves' surface. Mostly botanical pesticides due to their rapid degradation and volatility lost their persistence in a short period of time (Ahmadi et al. 2018). A similar observation was reported by Wiwattanapatapee et al. (2009), where the Derris emulsifiable concentrate was more efficient than the Derris extract solution against *Spodoptera litura*. Similarly, the particle size in emulsions is one of the key factors affecting their stability and efficacy. A reduction of the particle size usually precedes more stable emulsion formations and uniform spreading of active ingredients on the plant leaves' surface (Charman et al. 1992). Therefore, the SAE-EC with a 263.9-nm particle size and a 0.35 PDI was selected for the current bioassay with considerable repellent, ovicidal, and antifeedant effects against citrus psyllids. Moreover, the efficacy of SAE-EC can be improved through the repetitive application or through the addition of synergists in order to increase the effectiveness of SAE.

Conclusions

Generally, botanical insecticides are considered safe and they are suitable for use in restaurants, hospitals, schools, and homes (Isman et al. 2011). Botanicals exert lethal and sublethal effects on a wide range of insect pests and have the potential for the development of new safer insecticides (Benelli et al. 2019). Our current investigation concluded that SAE-EC showed a repellent effect against citrus psyllids under both laboratory and semi-field conditions. It has been concluded that the emulsifiable concentrate formulation prolonged the persistence of *S. alopecuroides* extract. The botanical extracts have gain interest in recent decades due to having minimal toxicity on the environment and less hazardous against mammals. The FTIR data indicated the stability of alkaloids in emulsifiable concentrate 3 months after preparation, which showed slow degradation of alkaloids in the emulsion. Based on the findings of the current investigation, the SAE-EC formulation is a possible novel alternative to the synthetic insecticide against citrus psyllids. Further trials should be conducted in open-field conditions and with respect to their impact on natural enemies.

Author contributions Conceived, designed, and performed the experiments: Syed Arif Hussain Rizvi. Zeng Xinnian gives the direction of research and reviewed the manuscript; Siquan Ling and Feng Xie help in analyzing the instrumental analysis.

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