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Repellent activity of Salix alba bark extract and Guava oil-based Formulation against Fruit Fly, Bactrocera dorsalis (Diptera: Tephritidae) to protect Guava from infestation under laboratory and semi-field conditions.

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Abstract

Bactrocera dorsalis (DIPTERA: TEPHRITIDAE), the primary pest of guava (*Psidium guajava*), damages numerous horticulture fruits in tropical and subtropical regions. Use of insecticides adversely affects agro-ecosystem and has environmental consequences. The repellent activity of *Salix alba* (Willow) bark extract and a Guava oil-based formulation was investigated to protect guava fruit from infestation by the Fruit Fly, *B. dorsalis*. In laboratory and semi-field experiments, the efficacy of these natural repellents was assessed. *B. dorsalis* significantly deterred guava fruit treated with *S. alba* formulation as compared to the extract alone, which exhibited significant anti-feeding activity, and caused a decline in fecundity rate by 60% and larval mortality rate by 90%. When females were allowed to oviposit on guava fruits in laboratory and field after mating, these flies preferred fruits for oviposition that were not sprayed with *S. alba*-guava oil formulation. GC-MS analysis showed esters and terpenes as the main components for exhibiting repellent property in *S. alba*-based formulation. Hence, guava oil formulation of *S. alba* showed a complete protection of guava fruits against *B. dorsalis* in the laboratory and semi-field conditions to control infestation by *B. dorsalis*. Further research and field trials are warranted to optimize the application methods and assess the long-term effects on crop yield and overall fruit quality.

1. Introduction

In horticulture, guava is a well-known fruit crop, has a high nutritional content and is of great economic significance among rural farmers for income generation (Joseph and Priya 2011). Guava requires high investment for its protection against fruit pest as it requires the use of expensive fertilizers and the application of agrochemicals such as fungicides and insecticides for the pest control. The use of chemicals for pest control is worrisome not because of higher monetary investment but adverse environmental impact associated with their use. Amongst the 33.3–66.7% damage caused by fruit fly infestation, *B. dorsalis* (Tephritidae) is a major pest of guava causing documented losses of up to 100%, worldwide (Dejen and Hassen, 2018; Liu et al., 2014; Sarwar et al., 2014). Hence, this fruit fly species has been included in quarantine list of pest species according to the International Standards for Phytosanitary Measures (ISPM Measures 2018). *B. dorsalis* poses risks to a wide range of fruit crops across India, East Asia, and the Pacific (Clarke et al., 2005) due to its higher reproductive potential, short life cycle, broad host range, rapid dispersal ability and high competitiveness (Duyck et al., 2004; Ekesi et al., 2007; Vargas et al., 2007; Vayssières et al., 2015). Of the several pests that infest guava *B. dorsalis* causes the highest damage especially in rainy season (~ 100% damage) despite several management strategies (Cornelius et al., 2000; Damodaram et al., 2014).

In the recent years, the demand for natural medicines from medicinal plants has been increasing, which is an alternative to synthetic products (Braithwaite et al., 2014). Plant extracts can be used as an alternative for synthetic insecticides in terms of effectiveness and inexpensiveness (Singh S, 2001). The bark of various trees is a valuable source of bioactive compounds (polyphenolic) with broad biological activities. India, as a tropical country has a huge diversity in plants and many have not been tested for their pesticidal properties. Different herbal forms such as whole plant, powder, extracts, and essential oils are used to check insecticidal activities (e.g., as a fumigants, anti-feedents, egg reducing, and growth regulating agents) (Opender, 2004; Negahban and Moharramipour, 2007; Carvalho et al., 2018). The ancient literature well-recognises willow bark, *Salix alba* (Salicaceae) for its potential in numerous medicinal drugs, and health supplements in the Polish Pharmacopeia and the European monograph (Adnan et al., 2015; Alamgeer et al., 2018; Fortini et al., 2016; Hostanska et al., 2007). *S. alba* grows on moist soil and is common in cold and low-temperature regions of the Northern Hemisphere (Lauron-Moreau et al., 2015). Recently, the willow bark has been implicated as an alternative to aspirin for its potential in curing symptomatic indications of fever, infections, mild rheumatic complaints, headache, and chronic pain syndromes and extracts of bark have antioxidant abilities (Schmid et al., 2001). There are few studies on antifungal and antimicrobial activities (Choi et al., 2018; Javed et al., 2020) of *S. alba*, but little is known about its insecticidal activities. Since, there is limited information available regarding the effect of *S. alba* extracts on *B. dorsalis* under laboratory or field conditions, we aimed to explore the potential of *S. alba* extract as well as its formulation against *B. dorsalis*. The results of this study demonstrated the efficacy of *S. alba* extract and its oil-based formulation in altering the life history traits of *B. dorsalis* such as egg hatchability, and larval mortality. The study provided valuable insights into the use of *S. alba* bark extract and a Guava oil-based formulation as promising tools for fruit fly management in the agricultural sector. The environmentally friendly nature of these repellents makes them attractive alternatives to synthetic pesticides, promoting the adoption of sustainable pest control practices to safeguard Guava crops and preserve the ecosystem's balance.

2. Materials and Methods

2.1. Collection and processing of *S. alba* bark

The bark from the *S. alba* plant was collected from Chandigarh university campus (Punjab), in polythene bags to the laboratory, where it was dried at room temperature (25°C) for 7 days. Approximately 1 kg of bark was collected spread on the filter paper for drying and further grounded into a fine powder using a Cryo-mill (RETSCH). The powdered samples were then stored in a clean glass container until further analysis. Ten grams of finely powdered bark was used for extract preparation. The extraction was done in a series of solvents namely hexane, ethanol, methanol, and water each for 48 h at 25°C. Later the extracts were pooled together. The filtrate was concentrated using a rotary vacuum evaporator (PERFIT, India) under reduced pressure (370 m bar) at 40°C to form a semi-solid extract, and the sample was stored at 4°C for further analysis.

2.2. Preparation of guava oil-based formulation

A *S. alba extract* (SAE) formulation was prepared using a method of simple mixing described by Wiwattanapatapee et al. (2009). The semi-solid extract was oven-dried and ground into a fine powder. The dried SAE (10 g) was then diluted in 10 ml of distilled water and blended with soap nut (5%) and, coconut oil or guava oil (5%) drop-by-drop; The resulting mixture was kept over a magnetic stirrer (200 rpm, 40–50°C) for 30 minutes, resulting in an emulsion that constituted (10% w/v) of SAE. A control was also prepared without SAE. For foliar application, the formulation was further diluted with (50%) distilled water (DW) to form 20:80 SAE-oil formulation.

2.3. Physio-chemical characteristics

2.3.1. pH

Using a calibrated pH meter (METTLER TOLEDO), the pH of formulated samples (1% aqueous solution) was measured at 25 ± 1°C.

2.3.2. Thermal and cold stability of emulsion

To evaluate the thermal stability of emulsions, 5 ml of formulation were taken in a test tube. To make the volume up to 10 ml, hard water was added to the sample at a rate of 2–5 ml/min, at a temperature of 32°C, with continuous stirring. The contents were then immediately transferred to a 50 ml graduated cylinder immediately, and kept undisturbed for 1 h at 32°C to check the formation of any creamy layer at the top and deposition of sediment at the bottom (CIPAC MT 36.3, 2003). Similarly, cold stability of the formed emulsions were checked by taking a volume of 5 ml of formulated sample in a test tube, and placing it in the ice-cold water maintained at 10°C for 1 h with stirring at short intervals. The formation of any turbidity or oily layer, or both, was checked during this time.

2.3.3. Storage stability test

Collaborative International Pesticides Analytical Council (CIPAC) method (No. 46.3, 2000) was conducted to test storage stability of the formulations. The formulations were kept at three different temperatures (4°C, 25°C, and 54 ± 2°C) for 14 days. After storage, the formulations were visually checked for any colour changes or separation of layers for the emulsion stability. Three-replicated sets were tested for storage stability test.

2.3.4. Rearing of *B. dorsalis*

The cultures of fruit flies, *B. dorsalis* were reared on an artificial diet inside a cage (90 cm ×45 cm ×45 cm) at room temperature. The adults were provided with an artificial diet containing a mixture of sugar (Dextrose-L) and protein hydrolysate (Protinex, Pfizer Ltd.) in a ratio of 1:1 in a petri dish, and allowed to lay eggs on guava placed inside the cage.

2.4. Bio-efficacy testing

2.4.1. Settling behaviour of *B. dorsalis*

The settling behaviour of *B. dorsalis* adults on guava treated with SAE and its formulation (10% w/v), corresponding to the dosages 10 mg/ml, while most stable formulation of SAE was tested in choice experiment under 25 ± 1°C, 70% relative humidity

(RH). A total of 100 flies of both sexes were placed in a cage, each containing 6 guavas including one untreated guava. The numbers of flies were counted after 24, 48, and 96 hours of release.

2.4.2. Feeding behavior

The feeding activity of adult *B. doraslis* was assessed through the amount of material excreted by the adults confined to guava sprinkled with the aqueous SAE and its formulation. Later, the treated fruits were placed in a jar containing 10 adult *B. dorsalis* and covered with muslin cloth lined with 60 mm Whattman filter paper. After 48 hours, the filter papers were removed and immersed in ninhydrin solution (1% w/v) prepared in aqueous acetone for 3 minutes. The excreted material present on filter papers reacted with ninhydrin, forming dark-purple spots (Tiwari et al., 2011). The number of purple spots on each filter paper was counted to assess feeding activity.

2.4.3. Oviposition preference assay

Females flies of *B. dorsalis* were allowed to lay eggs on guava treated with SAE and its formulation along with one untreated guava (n = 5). Seven-day-old mated females (20 per cage) were released in five oviposition chambers and allowed to lay eggs on treated and untreated guava fruit. The number of punctures after the 24 hours, and the number of eggs laid per puncture were recorded under Nikon stereomicroscope (Japan). To calculate oviposition preference index (OPI), the number of eggs on each guava treated and non- treated was counted using formulae:

$$\mathrm{OI} = \frac{(\mathrm{Numberofeggslaidonexperimentalfood} - \mathrm{Numberofeggslaidoncontrolfood})}{(\mathrm{Numberoftotaleggslaid})}$$

2.4.4. Effect of *S. alba* treatment on development of *B. dorsalis*

S. alba extract and its formulation treated and untreated guava were placed in a plastic jar with sand. Eggs (n = 100) of *B. dorsalis* were placed in treated and untreated fruit (n = 5) with a brush at 27 ± 1°C, 70 ± 2% RH for 17 days and checked for larval development and the mortality rate of *B. dorsalis*, second instar larvae were taken, and SAE (0.5, 1, 1.5 ml) and its formulation (1, 2, 3 ml) were mixed separately with 40 ml media kept in sterile petri-dish. Media without treatment was considered as a control. The mortality of larvae and adult was recorded at each concentration after treatment. The monitoring of mortality and larval development was done for 19 days (time needed to finish development).

2.4.5. Ovipunctures assay

Number of punctures on guava fruit were analysed between the SAE, the formulation treated and the control experiments when 20 females (6–7 days old) were allowed to lay eggs and feed guava fruit (n = 4) per cage. The experiment was repeated at least five times under laboratory conditions.

2.4.6. Egg hatchability and phytotoxicity ratings assay

Eggs hatched by 20 *B. dorsalis* females were analysed at different time intervals (12, 24, 48, 72, 96, 120) on the guava fruit treated with SAE, its formulation and a non-treated fruit per cage. Phytotoxicity testing were performed by treating mature leaves and flush with SAE and its formulation under semi-field conditions in the laboratory.

2.4.7. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was used to identify chemical compounds present in *S. alba* extract and its formulation. The patterns of the mass spectra fragmentation and their retention indices were compared with the ones stored in the computer library to identify the chemical components found in the plant extracts (Qadir et al., 2020).

GC-MS analysis of the willow bark extract of *S. alba* and the bio-formulation was performed using the equipment Thermo Scientific TSQ 8000, model: TRACE 1300 GC with EI ion source programmable to 350°C. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was 250°C and the oven temperature was programmed as follows: 60°C for 15 min, then gradually increased to 280°C at 3 min. The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results obtained have been tabulated.

2.6. Data analysis

The toxicological parameters were calculated according to Finney's mathematical methods (J. & Finney, 1948). All of the statistical tests were conducted by using Statistica 7 software; *P* values of less than 0.05 were considered significant. The results of sexual and oviposition behavior tests, results were analyzed statistically using descriptive metric methods, and then an analysis of variance (ANOVA) was performed on XLSTAT. Contingency x2 tests were performed for both the control and the group fed with mix diet (diet with *S. alba*). All experiments were repeated at least twice in replicates of minimum five to demonstrate reproducibility of results. Field experiments were repeated sequentially in multiple locations in the two growing seasons of guava.

3. Results

3.1. Development of SAE formulation

Two different formulations of the *S. alba* extract were prepared (Table 1), but the formulation consisting of 5% guava oil was found effective. An emulsifier (5% soap nut) was found to be suitable for the development of formulations from the SAE. Therefore, the formulation (10% w/v) of SAE was successfully developed with the addition of only 5% emulsifier blends.

Table 1
Formulation of *Salix alba* extract (SAE) and its formulation with varying concentration of bark powder, soap nut and oil along with physio-chemical tests.

Formulation	Concentration of SAE (%)	Concentration of Soap nut (%)	Concentration of oil (%)		Physical appearance	Cold test	Storage stability (14 days)		
			Coconut oil	Guava oil			4°C	25°C	54°C
F1	10	5	5	-	Light brown	Phase separation	un stable	stable	stable
F2	10	5	-	5	Light brown to yellow	No phase separation	stable	stable	stable

3.2. Physio-chemical properties

The prepared formulations appeared as light brown to yellow in colour, which may be due to variation in colour of the bark extracts of *S. alba* (brown). Among all the formulations (F1, F2), F2 was found stable up to 1 hour at 10°C without any phase separation (as revealed by no formation of turbidity or oily layer or both) and qualified for the cold test. The pH values of the developed formulations were found to be acidic (4.63–5.85), slightly lower than pure crude extract (6.5–7.5). Further, the storage capability of all the formulations were found stable at 4°C, 25°C, and 54°C after incubation for 14 days and passed all the tests (Table 1). The formulation remained uniform and homogenous without any precipitate formation as observed in conventional formulations.

3.3. Settling behavior of *Bactrocera dorsalis*

The settling behaviour of *B. dorsalis* adults differs significantly between SAE, formulation treated and the control groups after 24, 48, and 96 hours ($F = 96.87$; $df = 4, 24$; $P = 0.005$, $F = 95$; $df = 4, 24$; $P = 0.005$, and $F = 95$; $df = 4, 24$; $P = 0.005$) after release. SAE formulation showed minimal settling behaviour of flies compared to the SAE alone and the control groups. This settling behaviour of *B. dorsalis* determines the repellent effect of both SAE and its formulation under semi-field conditions. However, there were significant differences in settling behavior of *B. dorsalis* adults across different time intervals [Figure 1 (A)].

3.4. Feeding behaviour

The SAE formulation exhibited a maximum anti-feedant activity ($P < 0.005$**) against *B. dorsalis* [Fig. 2 (A)] in comparison to the SAE alone and the control group flies as shown by the minimal purple spots in filter paper. However, the use of SAE alone caused a reduction in the amount of honeydew drops formed by the ninhydrin reaction [Fig. 2 (B.)] ($P < 0.05$*).

3.5. Oviposition Preference Index (OPI)

To check egg-laying preference, we allowed the female flies to lay eggs on both treated (SAE and formulation), and non-treated guava fruit. *B. dorsalis* showed a negative preference for guava treated with *S. alba* formulation, with an oviposition index (OI) of -0.97 as compared to the control and SAE treated guava [Fig. 1(B)], indicating repellent effect of *S. alba* formulation.

3.6. Effect on mortality and development of *B. dorsalis*

The SAE and its formulation showed a high larval mortality activity (Fig. 3). Almost all larvae (99.9%) died after 15 days of SAE and the formulation treatment (Table 2). No significant differences were observed among different concentrations of SAE. However, mortality rate differed significantly with different exposure times ($P = 0.002$***, $P = 0.001$***, $P = 0.001$***).

Table 2
Days dependent insecticidal effects of different concentrations *S.alba* extract (SAE) and its formulation on *B. dorsalis* larval mortality.

Exposure of SAE (in days)	**Treatment**					
	***S. alba* extract (ml)**			**Formulation (ml)**		
	0.5	**1**	**1.5**	**1**	**2**	**3**
1 days	0%	0%	0%	10%	20%	54%
5 days	0%	0%	0%	15%	35%	75%
10 days	**9.03%**	**14.65%**	**15.34%**	**38.5%**	**43%**	**90%**
15 days	35%	50%	65%	85%	75%	100%
Fobs	9.96	10.46	10.39	25.96	39.66	75.30
P value	0.05*	0.05*	0.005**	0.05*	0.005**	0.001***

3.7. Ovipunctures and assay

Number of punctures on guava fruit differed significantly between the SAE, the formulation treated and the control experiments ($t = 11.49$, df = 4, $P = 0.0001$). The highest number of punctures was recorded on untreated guava fruit [Fig. 1 (C)].

3.8. Egg hatchability assay

In the control experiment, a large percentage of eggs (90 ± 1.34) were hatched. There was a significant difference in egg hatching among the treatments ($F = 91.78$; df = 2; $P < 0.0001$). The formulation caused around 100% egg mortality, while non-phytotoxic to mature leaves. SAE alone caused slight reduction in egg hatchability as compared to the control, while no phyto-toxicity was observed against mature leaves and flush (Table 3).

Table 3
Egg hatchability and phytotoxicity ratings against SAE and its formulation under different time exposure.

Treatments	Hours						Number of eggs	Phytotoxicity ratings
	12	24	48	72	96	120		MatureFlush
								leaves
control	11.5 ± 0.6^{a}	15.75 ± 1.4^{a}	31.75 ± 0.7^{a}	47.5 ± 1.6^{a}	42.25 ± 0.7^{a}	59.65 ± 1.0^{a}	87.7 ± 6.5^{a}	
SAE	0.0 ± 0.0^{b}	5.0 ± 0.5^{b}	12.0 ± 2.5^{b}	21.40 ± 2.4^{b}	35.0 ± 2.8^{b}	34.71 ± 0.2^{b}	87.11 ± 8.4^{b}	–
Formulation	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{b}	-+

Phytotoxicity ratings B–“(0–1%),” slight B+ “(1–25%)” 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. Post - hoc comparisons are shown as letters.

3.9. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Tables 4 and 5 indicate active compounds present in SAE and its formulation using GC-MS analyses, with their retention time (RT), and relative abundance (peak area values obtained at the different injections of the same compound) in the sample formulation. In SAE, a total of 15 peaks with 75 different compounds were identified, most abundant were aromatic hydrocarbons (23.47%), propionates (10.12–7.66%), and esters (8.32–6.83%). GC-MS analysis of *S. alba* formulation showed a total of 35 peaks with 174 different compounds, most abundant were monoterpenes (11.12%), diethyl phthalate (13.51%), isopulegol terpenoid (7.90%), acyclic monoterpeniods (5.75–2.04%) and esters (13.51%). The GC-MS chromatograms obtained for SAE and its formulation are shown in Figs. 4 and 5, respectively.

Table 4
Most Abundant Volatiles analyzed during GS-MS within the SAE.

Compounds from GC-MS	Retention Time (min)	Relative Abundance (%)
Anthracene, Phenanthrene, Benz[a]azulene	11.73	23.47
Octatriacontyl pentafluoropropionate	23.77	10.12
Oleic acid, 3-(octadecyloxy)propyl ester	24.24	8.32
Octatriacontyl pentafluoropropionate	24.18	7.66
	24.09	7.24
Diethyl Phthalate, Phthalic acid, ethyl pentadecyl ester	9.79	6.83
Octadecane	24.35	6.42
Eicosanoic acid, Pentadecanoic acid	12.90	4.39

Table 5
Most Abundant Volatiles analyzed during GS-MS within the formulation.

Compounds from GC-MS	Retention Time (min)	Relative Abundance (%)
L-à-Terpineol	6.88	11.12
Diethyl Phthalate	9.58	13.51
Isopulegol	7.12	7.90
trans-Linalool oxide (furanoid)	5.78	6.21
cis-Linaloloxide	5.92	5.75
Linalyl acetate	6.01	4.10
8-hydroxymenthol	7.83	2.04
Octasiloxane, Heptasiloxane, Tetrasiloxane	13.09	2.40
Cyclodecasiloxane, Octasiloxane,	23.98	12.56
2-Furanmethanol	6.67	1.96
Phthalic acid, Ethyl isoporpyl ester	9.58	13.51
1-Monolinoleoylglycerol trimethylsilyl ether, 9,12,15-Octadecatrienoic acid	23.60	11.23
Carbonyl sulfide	3.44	0.64

4. Discussion

The present study investigated the repellent activity of *S alba* (white willow) bark extract and a guava oil-based formulation against the Tephritidae family fruit fly species, *B. dorsalis* with the aim of protecting guava fruits from infestation in both laboratory and semi-field settings. The laboratory experiments demonstrated that both SAE and its formulation exhibited significant repellent activity against *B. dorsalis* as compared to the untreated control. The observed repellent effect could be attributed to the presence of active compounds in the SAE and the unique chemical composition of guava oil (Julkunen-Tiitto, 1989; Zhang et al., 2022).

The use of synthetic chemical pesticides for pest management causes hazardous effect on the humans and the environment. The development of resistance in insects against these chemical pesticides has increased interest in development and use of botanical insecticides (Benelli et al., 2019; Simon et al., 2009). Natural plant extracts are commonly used in pest management due to their insecticidal activity against various life history traits of the pest. While plant extracts are effective in pest control, it is it is challenging to maintain the stability of the botanical extracts and preserve the active metabolites present in the extract under field conditions (Borges et al., 2018). Factors such as temperature, humidity, and rainfall can influence the longevity of the repellents (Katarzyna et al., 2021). Understanding these dynamics is crucial for devising appropriate application strategies, ensuring long-lasting protection against fruit fly infestation. The laboratory experiments shed light on the durability and persistence of the repellent effect of SAE and formulation under natural environmental conditions. Among the various formulations we tested, F2 formulation, which constitutes *S. alba* bark extract, was found to be stable when blended with guava oil rather than coconut oil, which served as the biodegradable and cost-effective surfactant blend. The F2 formulation passed the quality characteristics recommended by CIPAC and IS specifications (BIS 1997), suggesting its effectiveness in controlling *B. dorsalis* under field conditions too.

The repellent effect of SAE, when emulsified with guava oil had significant results against *B. dorasalis* as compared to SAE alone. The adults of *B. dorsalis* showed a strong preference for settling on the untreated guava as compared to the treated guava. Our results were in accordance with the previous studies (Ranganath, H Suryanarayana, MA Veenakumari, 1997; Rishi Kumar, Singh R, 2003; Shah et al., 2017; Sharma et al., 2016), where ovi-position deterrent effect was observed due to plant extract against fruit flies. This study highlighted direct and delayed toxicological effects of SAE and its formulation. The SAE formulation showed higher insecticidal activity in terms of larval mortality reduced egg hatchability and fecundity in *B. dorsalis*. It is worth emphasizing the repellent effect of SAE-oil based formulation, as this can be used to control the infestation by inhibiting the production of eggs by fruit flies of economic significance. The presence of around 34 phenolic compounds as already reported in the literature, attributes for its potent insecticidal activity (Piątczak et al., 2020). In previous studies, spraying of plant extract (*Azadirachta indica*) on the leaves of fruit plants has shown promising results in pest control (Silva et al., 2012). In the present study, SAE formulation showed no phytotoxicity, Therefore, we recommend formulation treatment on leaves of guava as an alternative for handling pest fruit fly *B. dorsalis.*

The repellent effect observed in the laboratory setting was successfully translated to the field, indicating the potential practical application of SAE-based repellents in large-scale guava production. The field trials confirmed the efficacy of the SAE and guava oil-based formulation in preventing fruit fly infestation in guava orchards. The effectiveness of the *S. alba*-based repellents was evident in their ability to deter fruit flies from approaching and laying eggs on guava fruits as observed in settling behaviour and ovi-position preference of *B. dorsalis*; however, SAE-oil formulation showed significant results in the repelling the flies towards guava fruit as compared to the SAE alone. The presence of chemical compounds in plant extracts is primarily responsible for their biological properties such as, anti-feedant and insecticidal properties. The mode of action of the repellents is linked to their active phytochemicals, such as alkaloids, terpenoids, phenolics, proteinase inhibitors, and growth regulators, which work by emitting volatiles that interfere with the sensory receptors of fruit flies, leading to their avoidance of treated surfaces (Maia and Moore, 2011). The present study was designed to obtain the bioactive compounds present in SAE and SAE-Guava oil formulation using GC-MS. In SAE and its formulation, a total of 75 and 174 different types of bioactive compounds have been identified, respectively. Both the samples were found to contain different types of important chemical constituents, which were fatty acid esters, terpenes, acyclic hydrocarbons, and their oxygenated products which were consistent with the previous findings of Qadir et al., 2020). Esters, found in both the SAE and it's formulation, are the volatile organic compound that exists naturally in many plants emitting floral fragrance (Copping and Duke, 2007), and is attributable for the repellent activity as previously reviewed by Nesterkina et al. (2018). Behavioural studies as done by Feng et al. (2018) demonstrated that esters and some of its naturally occurring and synthetic analogues have the potential in controlling insects at different life stages. In our study, menthol present in the SAE formulation, is well-known for its agro-biological and repellent activity against mosquitos and other agricultural pests (Kalemba and Synowiec, 2020; Samarasekera et al., 2008).

The repellent formulation developed in this study offers several advantages over conventional chemical pesticides. Chemical pesticides have been the primary method of fruit fly control, but their prolonged use raises concerns about environmental pollution, residues in the fruits, and the development of pesticide-resistant fruit fly populations (Veltri et al., 1994). In contrast, the *S. alba* bark extract and guava oil-based formulation are environment friendly, biodegradable, and pose minimal risk to non-target organisms, including beneficial insects and human health. However, further research is warranted to optimize the formulation, assess the long-term effectiveness, and evaluate its impact on non-target organisms and soil health. Additionally, efforts should be made to understand the economic feasibility of large-scale production and application of the repellents. Collaboration between researchers, farmers, and policymakers is essential to ensure successful integration of natural repellents into fruit fly management programs and enhance overall fruit production and quality.

5. Conclusions

The findings of this study are significant as *B. dorsalis* infestation poses a severe threat to guava production and quality, worldwide. As demonstrated in the present study, identifying natural repellent alternatives such as plant-based extracts and their formulations, farmers can adopt eco-friendly approaches to manage fruit fly infestation, reducing reliance on synthetic chemical pesticides. We conclude that SAE can be effective in controlling the infestation of *B. dorsalis*, if it is timely sprayed on guava plants. SAE acts as an effective repellent both for feeding and for ovipositing of *B. dorsalis*. Hence, SAE will not only prevent egg-laying but will also reduce use of insecticidal sprays, which ultimately will minimize health and environmental hazards.

Declarations

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Authors contribution

Seema Ramniwas: Data collection, data analysis, and draft preparation. Tanveer Bilal: Material preparation. Aanchal Sharma: first draft preparation and data analysis.

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Figures

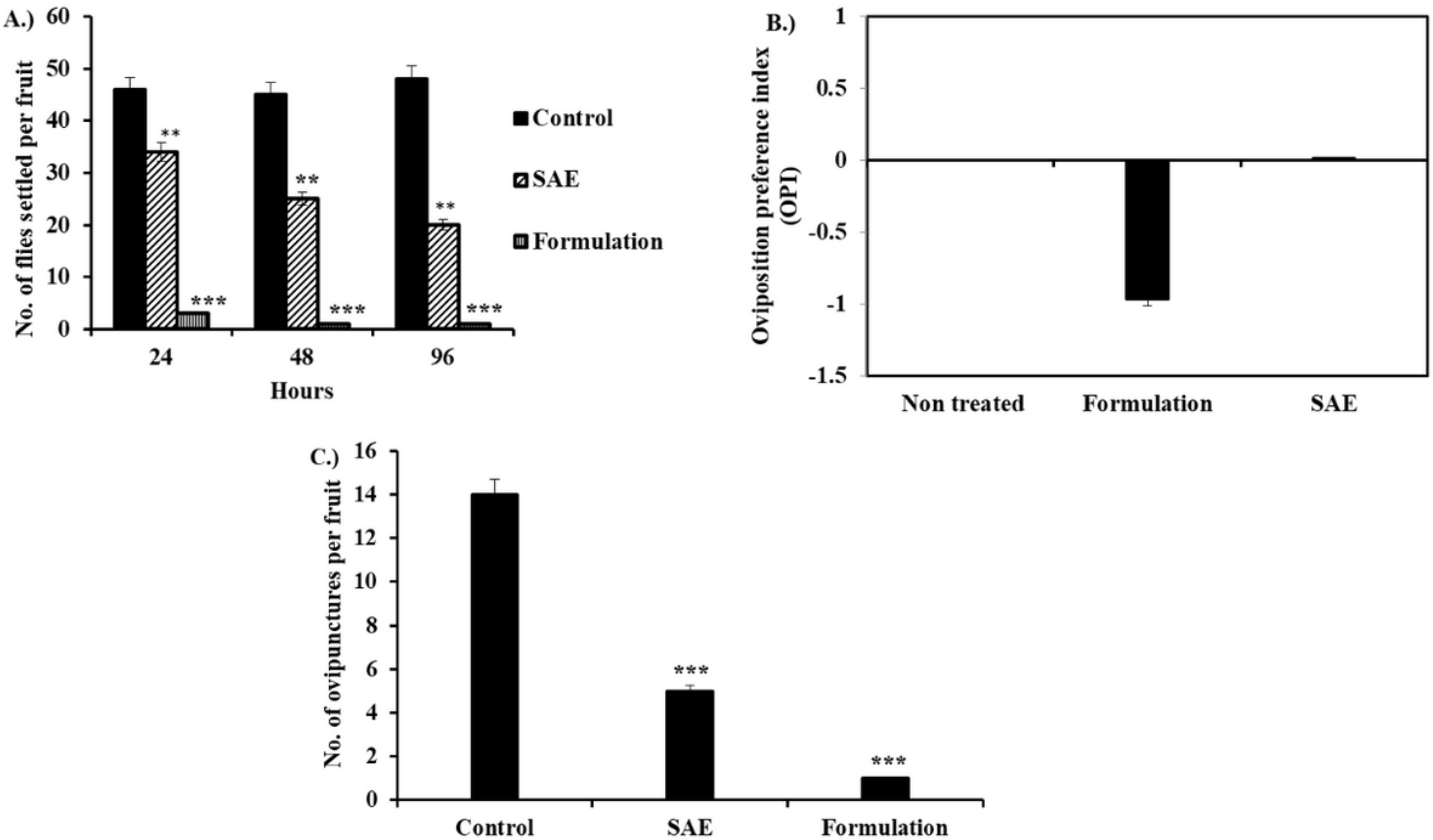

Figure 1

A) Effect of SAE and its formulation on settling behavior of *B. dorsalis* on guava fruits. B) Oviposition preference index (OI) of *B. dorsalis* on guava fruit treated with *S. alba* extract (SAE), and its formualtion in comparison with non-treated guava fruit as a control. C) Number of ovipunctures observed on guava fruit when treated with *S. alba* extract (SAE) and its formulation as compared to the control.

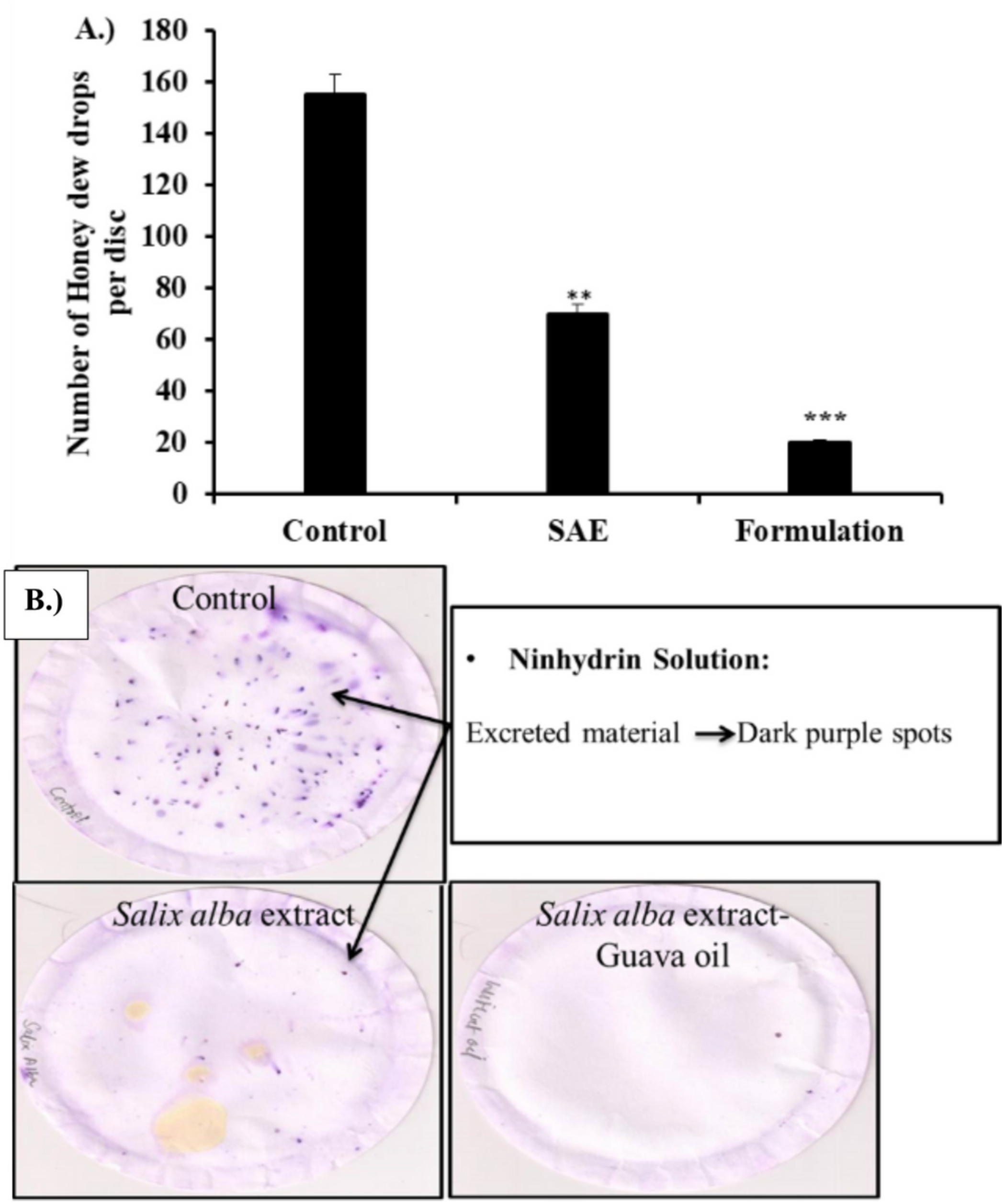

Figure 2

A) Effect of SAE and formualtion on *B. dorsalis* adult feeding as measured by the number of honeydew droplets produced when filter paper discs treated with 10% SAE and *S. alba*-Guava oil formulation exposed to 10 fruit fly adults. Different letters indicate significant differences ($P \leq 0.05$) as analysed Tukey's test ($P < 0.05$). B) Filter paper discs representing honey dew drops when treated with *S. alba* extract, *S. alba*-Guava oil bio-formulation and non-treated control.

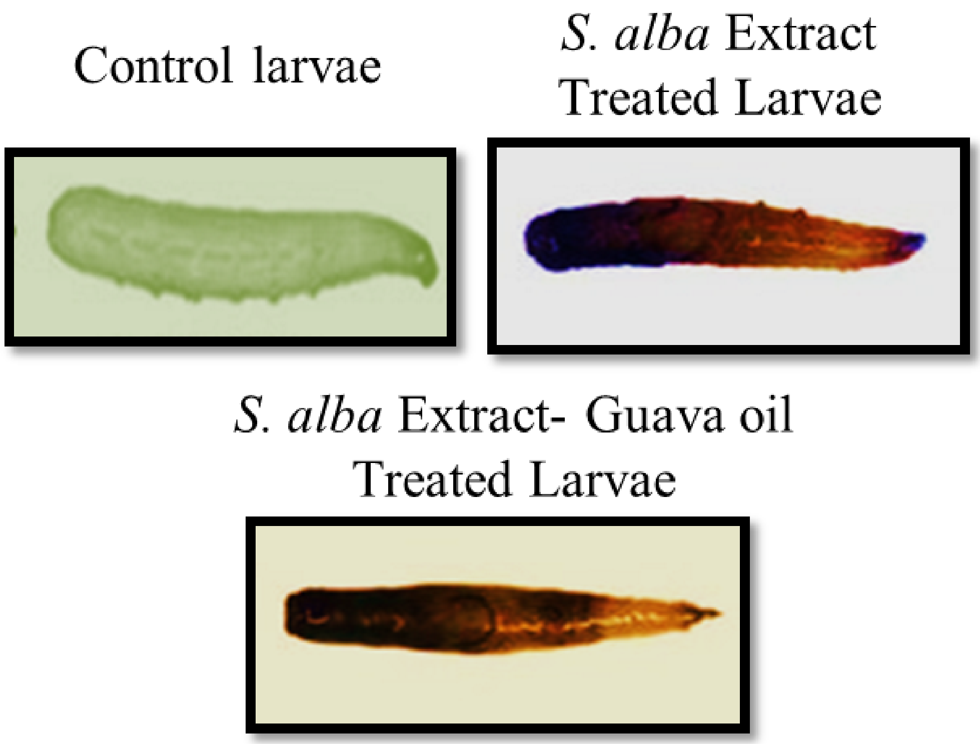

Figure 3

Impact of *S. alba* extract and formulation on the larvae of *B. dorsalis*.

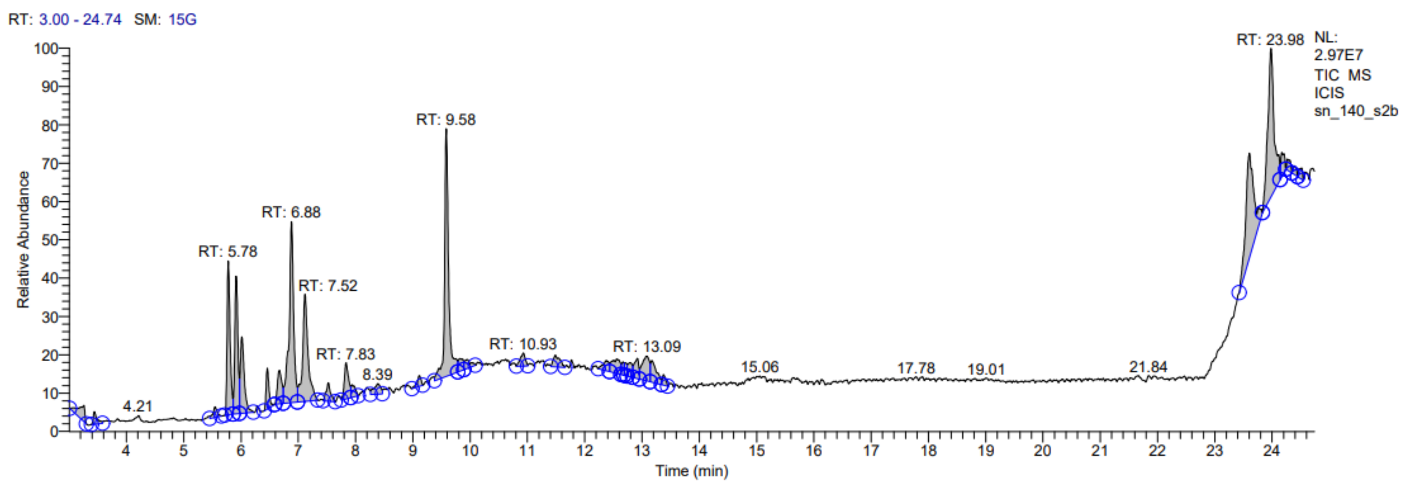

Figure 4

GC-MS chromatogram of of *Salix alba*-Guava formulation.

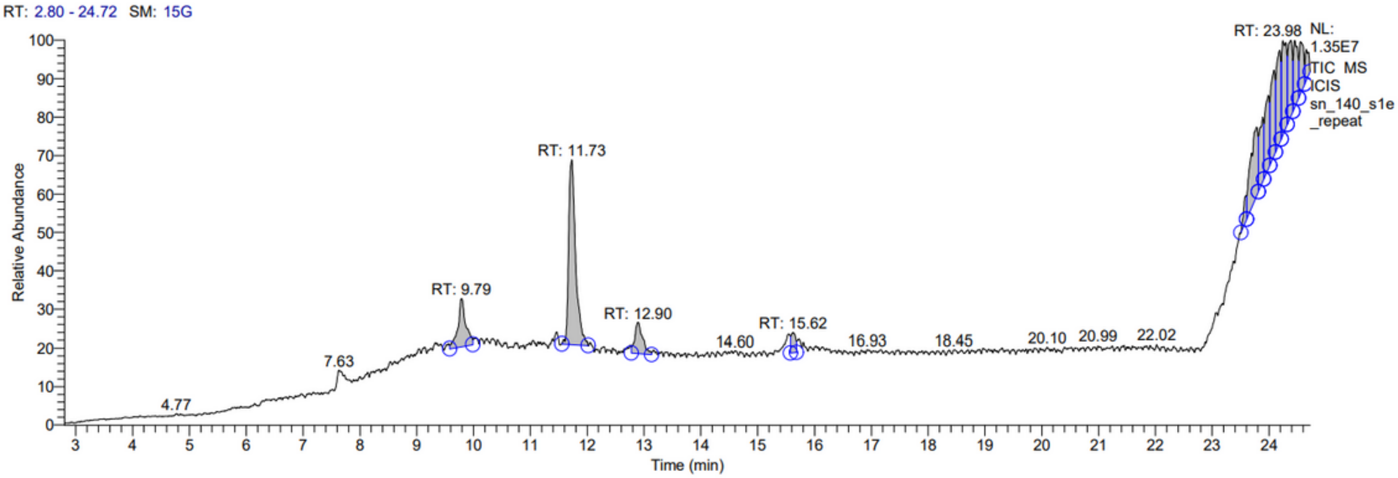

Figure 5

GC-MS chromatogram of methanolic extract of *Salix alba.*

Supplementary Files

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