Agricultural Sciences Journal
Available online at http://asj.mnsuam.edu.pk/index.php
ISSN 2707-9716 Print
ISSN 2707-9724 Online
https://doi.org/10.56520/asj.v6i1.382

Research Article

DETERMINATION OF INSECTICIDAL PROPERTIES IN TOTAL SOLUBLE PROTEINS OF INDIGENOUS PLANTS AGAINST CROP PESTS

Muhammad Mahmood Ahmed[1*], Asma Akbar[2], Tuba Tariq[3], Sajjad Ali[4], Alishba Hadi[2], M. Shahid Rizwan[5], Qaiser Shakeel[5]

[1]*Department of Bioinformatics, IBBB, The Islamia University of Bahawalpur, Pakistan*
[2]*Department of Biotechnology, IBBB, The Islamia University of Bahawalpur, Pakistan*
[3]*Department of Biochemistry, IBBB, The Islamia University of Bahawalpur, Pakistan*
[4] *Department of Entomology, Faculty of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur*
[5]*Cholistan Institute of Desert studies (CIDS), The Islamia University of Bahawalpur*
**Corresponding author:* mahmoodahmad@iub.edu.pk

Abstract

Chemical pesticides used in crops have become a major concern due to their harmful effects on the environment and human health. This study aims to identify and characterize the insecticidal proteins in local plants and determine their efficacy against common crop pests. By analyzing the mode of action and toxicity of these proteins, this research could provide a sustainable and eco-friendly approach to pest management. The goal was to evaluate the insecticidal efficacy of various plant total soluble proteins against crop pests e.g. whitefly and mustard aphid. Two types of protein extracts were used, pellet protein (P) and supernatant protein (S). Aphid mortality data was collected after12, 24, and 48 hours of feeding. The results showed that Eucalyptus-P and Kikar-P had the highest mortality (9.67) on aphid followed by jamun-P, Sheesham-P, and jamun-S (9.33). While the Bakain-P had the lowest amount of mortality (6.33) on aphids after 12, 24 and 48 hours of feeding. Insect Bio-feeding assay on mustard whitefly was conducted and data collected after 12- and 24-hours treatment. The results showed that Eucalyptus-P and Sheesham-P exhibited the highest mortality (7.33) against whitefly followed by Jamun-P and Kikar-P (5.33) after 12 and 24 hours. Whereas Neem-S showed the lowest mortality at (2.67) on whitefly population. Overall, proteins from *Eucalyptus globulus*, *Acacia nilotica*, *Dalbergia sissoo* and *Syzygium cumini* showed the highest mortality on both pests. Overall, plant total soluble proteins demonstrated more insecticidal properties and potential compared to supernatant proteins which can be used as effective biopesticides against aphid and whitefly.

Keywords: *Total soluble proteins, local plants, crop pests, whitefly, mustard aphid, biopesticides.*
(Received: 17-Jan-2024 Accepted: 20-Apr-2024) Cite as: Ahmed. M. M., Akbar. A., Tariq. T., Sajjad. A., Hadi. A., Rizwan. M. S., Shakeel. S., 2024 Determination of insecticidal properties in total soluble proteins of indigenous plants against crop pests. Agric. Sci. J. 6(1): 58-70.

1. INTRODUCTION

Crop production is affected worldwide by insect pests. It is concerning that only mosaic viruses cause an annual global loss of around $17.7 billion (Oliveira et al., 2014). Numerous pests, such as bacteria, fungi, weeds, and insects, have a negative impact on agriculture, reducing yield and degrading product quality (Kumar, 2012). Synthetic chemicals are frequently utilized for insect control but their toxicity threatens the health of farmers, livestock, and food buyers. As a standard method for controlling field pests, pesticides have been extensively used (Desneux et al., 2007; Weisenburger, 1993). The continued use of synthetic chemicals has resulted in the development of resistance among agricultural pests, the accumulation of environmental toxins harmful to human health, and adverse effects on non-target arthropods (Biondi et al., 2012; Weisenburger, 1993). Most engineered pesticides include: synthetic substances that are unsafe for humans and pose threats to

environmental degradation (Abbassy, 2017; Bempah et al., 2011).

Exposure to agrochemical pesticides poses a significant danger to human health and the environment. According to the World Health Organization (WHO) and the UN Environment Program (UNEP), one in five million patients in developing countries is exposed to pesticide poisoning while working on plant cultivation (Jallow et al., 2017). Additionally, these chemicals target major groups of destructive insects. The use of biopesticides is also effective. Requires proper knowledge of biology, food habits, and life cycle of insects, and how biopesticides work. Botanical insecticides, medium modest, successful, protected, and simple to process and apply, can likewise be a good choice for pest control of farmers in developing countries (Ali et al., 2012; Belmain et al., 2001). However, combined use of biopesticides containing biological control specialists may not cause intense impacts but rather may cause subtle (e.g. physiological and behavioural) effects leading to population reduction growth of bio-control services (Biondi et al., 2015; Biondi et al., 2012). Various herbal alternatives can contribute to management strategies, Bioactive Ingredients or biopesticides are products from plants that contain many active substances (Isman, 2006). Historically, the properties and potentials of some plants and their secondary metabolites against various insects are known (Jaber et al. 2018) These have been tested against whiteflies with good results, but its critical evaluation is crucial especially in whitefly control (Ashfaq, 2019). It offers effective and bio-rational control alternatives (Guerra et al., 2020).

Plants like *Azadirachta indica*, *Eucalyptus*, *Acacia nilotica*, *Calotropis gigantean*, *Dalbergia sissoo*, *Albizia lebbeck*, *Melia azedarach*, *Moringa oleifera*, *Datura stramonium* and *Syzygium cumini* possess medicinal importance. They exhibit antimicrobial, antifungal, antimalarial, anti-ulcer, anti-hyperglycemic, and anti-inflammatory properties. These plants are known to relieve congestion, and muscle and joint pain, and may treat various conditions such as dry skin, diarrhea, somatic, and skin disease (Nasri & Rafieian-Kopaei, 2014). They are also rich in antioxidants, promote relaxation, and act as natural insect repellents. In this study, we extracted total soluble protein of 09 insect-repellent plant leaves. We checked the insecticidal efficiency of two protein fractions against crop pests to determine their potential as alternatives to chemical pesticides.

The main objectives of this study were to isolate total soluble and supernatant protein extracts from selected local plants and to determine the insecticidal properties of these proteins against common crop pests.

2. MATERIALS AND METHODS

2.1. Plant material used for the experiment

The experiment was conducted at Entomology lab of Islamia University Bahawalpur (IUB) Bahawalpur, Pakistan. The plants used in the study included *Azadirachta indica* (Neem), *Eucalyptus* (Sufaida), *Acacia nilotica* (kikar), *Calotropis gigantean* (Aak), *Dalbergia sissoo* (Sheesham), *Albizia lebbeck* (Shareen), *Melia azedarach* (Bakain), *Moringa oleifera* (Moringa), *Datura stramonium* (Thorn apple) and *Syzygium cumini* (Java Plum).

2.1.1. Instruments and Chemicals required

Following instruments and glassware were used including micropipette (1000ul, 100ul, 10ul), SDS-PAGE apparatus, Nano-drop spectrophotometer, vortex machine, centrifuge machine, digital weight machine, nitrogen gas cylinder, PH meter, stirrer machine, beaker, reagents bottles, measuring cylinder, glass pipette, mortar and pestle, falcon tubes (50ml, 15ml), Eppendorf tubes, petri dishes, camel hair brush, and magnifying glass.

Chemicals include Acetone, Methanol, Ammonium acetate methanol, Sucrose, Urea, Thiourea, CHAPS, DTT, PMSF,

Biolyte, Trichloroacetate, liquid nitrogen, distal water, 10% SDS, isopropyl alcohol, acrylamide, Polyvinylpyrrolidone, tris buffer, bisacrylamide, 10% APS (ammonium persulfate), TEMED (tetramethyl ethylene diamine), acetic acid, glycerol, β-mercaptoethanol.

2.1.2. Treatments

Treatments were applied in different combinations and coded as presented in Table 1.

Table 1. Protein and supernatant protein detection of plant material.

Plants	Protein	Code	Supernatant	Code
Water	NA	T0	Buffer	T1
Shareen	Detected	T2	Not detected	NA
Eucalyptus	Detected	T3	Detected	T11
Jamun	Detected	T4	Detected	T12
kikar	Detected	T5	Detected	T13
Neem	Detected	T6	Detected	T14
Datura	Detected	T7	Not detected	NA
Sheesham	Detected	T8	Not detected	NA
Moringa	Detected	T9	Not detected	NA
Bakain	Detected	T10	Detected	T15
Aak	Not detected	NA	Detected	T16

2.2. Protein Extraction

2.2.1. Two-dimensional protein electrophoresis

Protein analysis and detection are common applications for two-dimensional gel electrophoresis (2D-Gel). Sodium Dodecyl Sulphate-Polyacrylamide Gel (SDS-PAGE) was used just to detect protein extracted from selected insect repellent plants. Distilled water is used to prepare all reagents, and stringent precautions are taken to avoid contamination, such as thoroughly cleaning all equipment with distilled water.

2.2.2. Reagent Preparation

Extraction 10% TCA/acetone (acetone): 50 g of TCA was dissolved in a small volume of acetone (500 ml). Then 1% DTT (dithiothreitol) was added and mixed well and stored at -20°C. Then 80% acetone with 400 mL volume was prepared. 0.5 g (0.1%) DTT was mixed in 100ml dH_2O and was stored at -20 °C. To prepare 80% methanol: 400 mL methanol, 100 mL dH_2O and 0.5 g (0.1%) DTT were mixed together and was stored at -20 °C. Next we prepare 100% methanol by adding 0.5 g DTT in 500ml of methanol,, and Stored at -20°C. we also, prepared 100% ammonium acetate in methanol (0.1 M NH4Ac + 0.1% DTT) by adding 3.85 g of NH4Ac in 500 ml methanol, followed by 0.5 g DTT, then mixed well, and stored at -20°C.

To prepare the SDS Extraction Buffer, we added following reagents: 0.1 M Tris-HCl, 2% SDS, 30% sucrose, 1% DTT (added before use), and 1 mmol PMSF (added before use).

For the preparation of 500 ml solution, 150 g of sucrose was added and dissolved completely in 300 mL of dH2O. Then 10 g SDS and 25 ml Tris-HCl were added solution (2M Tris-HCl pH 8.0) and finally the volume was increased up to 500ml by adding dH2O. Before using 1% DTT and 1 mmol PMSF (Phenylmethanesulfonylfluoride), stored at room temperature (SDS low-temperature precipitation). 174 mg of PMSF was dissolved in a low amount of isopropyl alcohol to prepare 100mM the PMSF stock solution, whose ultimate volume was 10 ml.

2.2.3. Lysis Buffer (50 ml)

Urea 7M (4.2 g/10 ml), Thiourea 2 M (1.52 g/10 ml), 4% CHAPS (0.4g/10ml), DTT 1% (0.1 g/10 ml), 0.2% (W/V) BioLite 50µL able (40%)/10 ml. For the preparation of 50 ml solution, 15 ml of dH_2O was used to dissolve 21 g of urea, 7.6 g of thiourea, and 2 g of CHAPS. Then dH_2O was added to bring the total volume up to 50 mL, then

thoroughly combine while reserving bubbles and dirt. Now dispense into 1ml tubes and store at -20℃. Do not freeze and thaw frequently. The rest is thrown away. Then, if necessary, take the tube from the refrigerator and leave it at room temperature to melt. To dissolve the protein, add 2 µL of 100 mmol PMSF to a tube, that contains lysis buffer, and another tube containing 10 µL Biolyte (ampholytes), 0.02 g DTT, and mix the lysis buffer well in the two tubes. When adjusting the concentration necessary, take another tube from the refrigerator add 0.01 g DTT and 5 µL of BioLite, and mix well.

2.2.4. 10% (w/v) SDS

10 g of high-purity SDS was added in a beaker and dissolved about 80 ml. Heated at 68 ℃, 100 mL was the end amount, which we kept at ambient temperature.

2.2.5. Protein Extraction

First, a medium-sized mortar was prepared along with the reagent's TCA/acetone, 80% Acetone, 100% acetone, 80% methanol, and 100% ammonium acetate. All reagents were cooled to -20°C before use. Then, the sample was grounded to a very fine powder in liquid nitrogen (1.5-4 g), and the powder was transferred to a 50ml tube and add 5% - 10% PVP (Polyvinylpyrrolidone) to the tube. The centrifuge was pre-cooled to 4°C. Next, 30 ml of pre-cooled TCA/acetone was added to the tube, and the mixture was vigorously shaken for 15 min at 4°C followed by 1 hour at 20°C. Then, 15 ml SDS extraction buffer, 15 ml tri-saturated solution, 1% DTT, and 1% PMSF (150 µl 100 mmol PMSF) were added at room temperature and the mixture was whisked for 1 hr.

Afterward, the mixture was centrifuged for 30 minutes at room temperature, and the upper layer of phenol was taken and placed on ice. Pre-cooled 0.1M ammonium acetate methanol was added to the upper phenol layer at a volume five times that of the upper phenol layer. The mixture was shaken properly and stored overnight at -20°C. The supernatant was discarded, and 30 ml of 80% methanol was then centrifuged for 15 minutes at 4°C and 10,000 rpm to collect the supernatant in a fresh tube with a pipette. The pellet was left behind.

Next, 30 ml of pre-chilled methanol and 30 mL of pre-chilled acetone were added to both the supernatant and pallet tubes. The pallet was dried for 10-15 minutes on ice under vacuum, and the precipitate was dispersed with a pipette. The dried pallet was dissolved in 500 µl of lysis buffer at room temperature for more than 1 hour, and 500 µl of lysis buffer was also added to the supernatant (Figure 1).

2.2.6. Quantification of the detected proteins

After protein extraction, the next step involved protein detection, which was

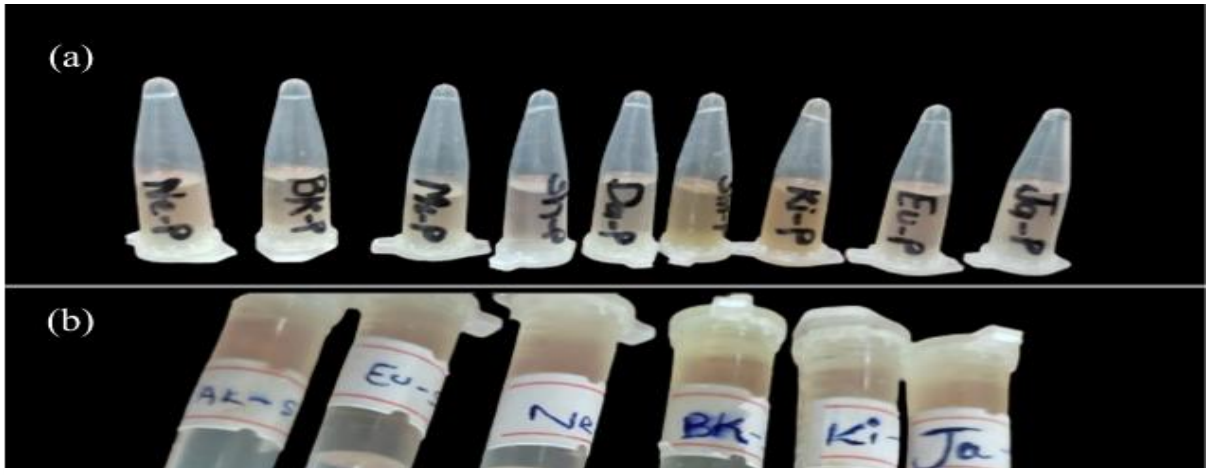

Figure 1. Extraction of total soluble and supernatant protein from different plant species (a) total soluble proteins extracted from different insect repellent plants (b) supernatant protein extracted from insect repellent plants.

accomplished using (SDS-PAGE). In this procedure, proteins moved through a polyacrylamide gel were exposed to an electric field, first denatured by SDS and then sorted according to their molecular weights (Roy & Kumar, 2014). Thereafter nanodrop was used to determine quantitative values of selected proteins (Vennapusa et al., 2020).

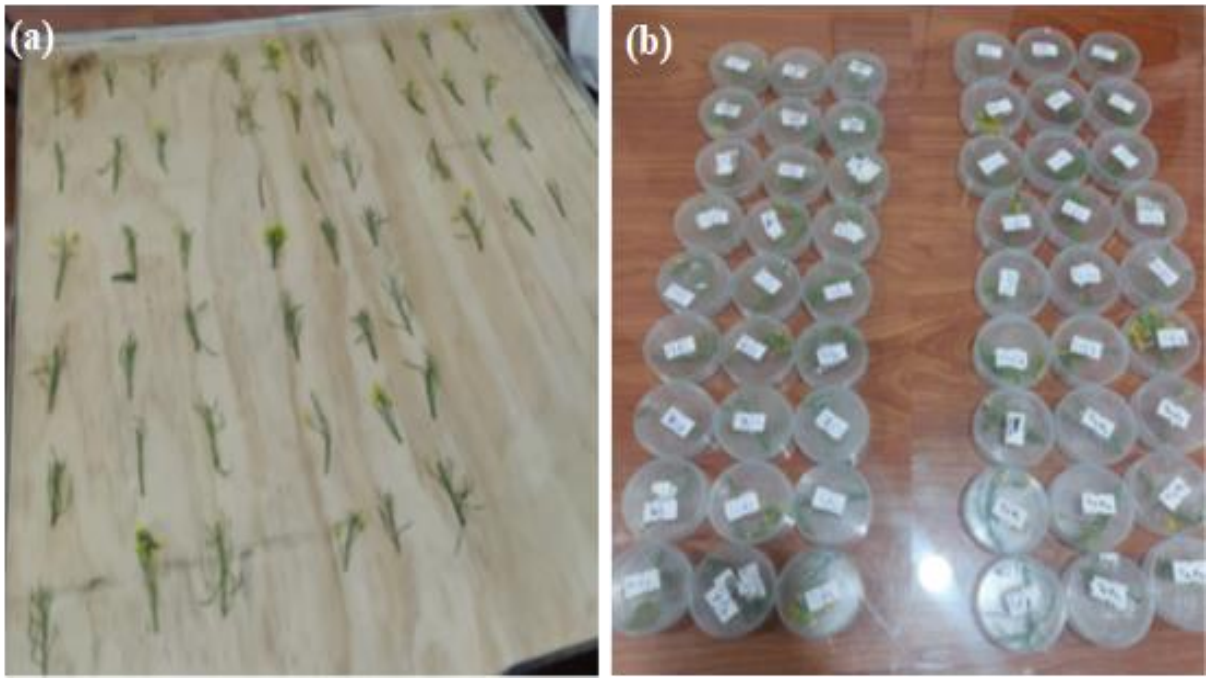

Figure 2. Experimental setup for insect feeding study (a) Incubation of protein and supernatant with insect feed (b) Aphid feeding on prepared feed

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

GC-MS was used to identify the metabolites and compounds available in selected plant extracts.

2.3. Insecticidal Bioassays

2.3.1. Whitefly bioassay

B. tabaci culture was maintained on cotton seedlings under the controlled lab conditions i.e. 26 °C temperature an 80% humidity. Cotton plants with a high density of nymphs and pupae were chosen, adults were eliminated, and bioassays were conducted in 30 ml specimen containers. Filter-sterilized (0.22 m) fake diets (with or without total soluble pellet protein/supernatant protein) were used. The interior surface of the tube cap was stretched with a 100 µl diet sandwiched between two pieces of UV-sterilized parafilm (DIAB et al., 2006). To prevent sample degradation and microbial contamination, the diet was replaced every other day. To compare the survival of adult whiteflies, diet A5 was compared against a diet comprising 5% yeast extract and 30% sucrose. In each bioassay tube, 10 adults were placed, and the experiment was carried out in triplicate. Bioassays were conducted for two intervals of time, and mortality was calculated at the end of 12 and 24 hours by numbering the dead insects at the bottom of the tube (Figure 2).

2.3.2. Aphid Bioassay

Mustard aphid, *Lipaphis erysimi*, was collected from the oil field crop of the Islamia University Bahawalpur, research area. The 2nd instar nymphs were selected and collected in petri dishes for bioassay. Take fresh insecticidal free leaves of mustard and dip in protein solution for 3 to 5 sec after air dry put these leaves in a petri dish. In each bioassay petri dish, 10 second instar nymphs were placed. The experiment was carried out in triplicate, and bioassays were conducted three times apart. Mortality was calculated after each interval by numbering the dead insects in each petri dish.

3. Results

The purpose of the current research was to examine the insecticidal efficiency of 10 different plant total soluble protein by bioassay on two crop insect Aphid and whitefly. After carefully analyzing the obtained data, it is revealed that all 10 plants total soluble protein have an insecticidal property which affect the insect and caused their death.

3.1.

3.2. Protein Detection test

Figure 3 showed protein bands obtained from Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) carried out by protein detection test (Figure 3).

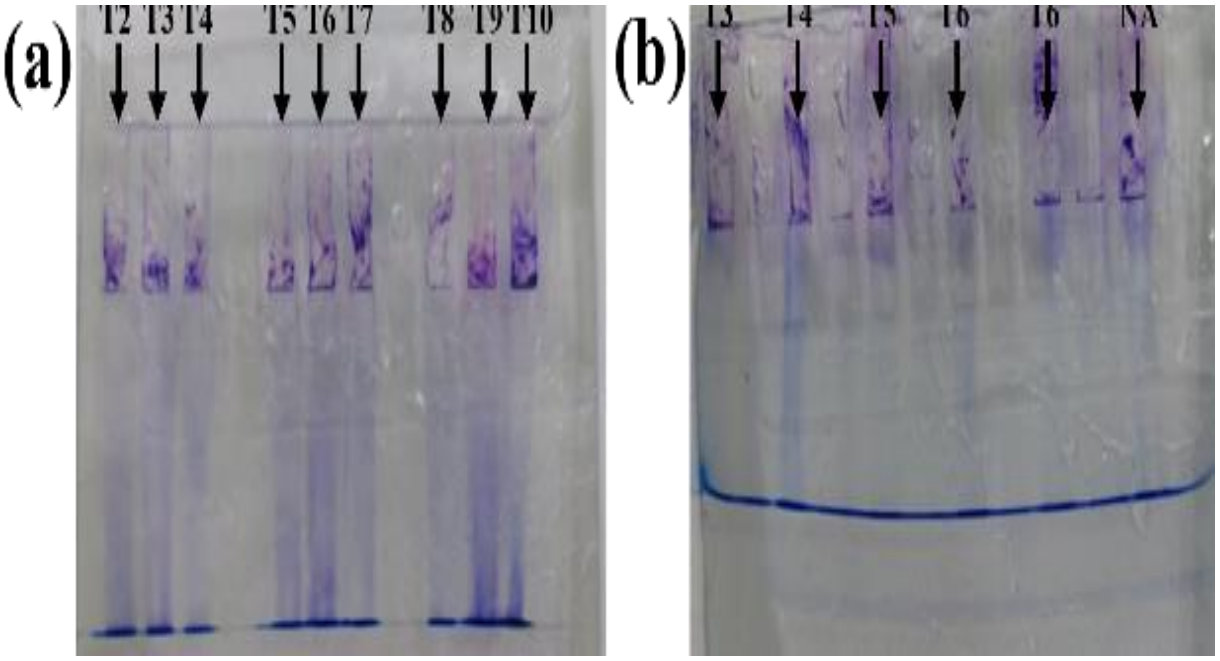

Figure 3. Gel electrophoresis (a) before and after staining of gel for different pellet total soluble protein (b) presenting 6 different total soluble supernatant proteins

3.3. Quantitative analysis of proteins

Quantitative protein detection is conducted using Nanodrop spectrophotometers and the analysis showed the concentration of total soluble protein in 9 plants and total supernatant protein in 6 plants out of 10, this purity detection is based on 260 and 280 absorbance (Figure 4). However, due to the difference in absorbance values, data for the other plants were excluded.

3.4. Insecticidal Bioassay

The results obtained from the bioassay conducted to evaluate the insecticidal efficacy of 10 different plants against Aphid and Whitefly insects revealed significant variations in mortality rates.

3.4.1. Total mortality rate of the Aphid

The analysis of variance (ANOVA) for total mortality data of aphids after three-time intervals (12h, 24h and 48h) following treatment with various plant total soluble proteins. The ANOVA showed a significant difference in mortality rates among treatments (F = 11.76, $P \leq 0.0001$), indicating that the treatments had a notable effect on aphid mortality. Post hoc tests can further elucidate which specific treatments led to significant differences in mortality rates (Table 2).

3.4.2. Total mortality rate of the Whitefly

The total mortality rate of whiteflies following two intervals (12 and 24 hours) of treatment with different plant total soluble proteins is shown in Table 3 as the outcome of an analysis of variance (ANOVA). The source of variance among errors as well as between treatments is investigated by the ANOVA. It reveals a significant difference in mortality rates between treatments (F = 2.55, P = 0.0129), indicating that the type of plant protein treatment has an impact on whitefly mortality. The mean square error (MS) provides insight into the variability

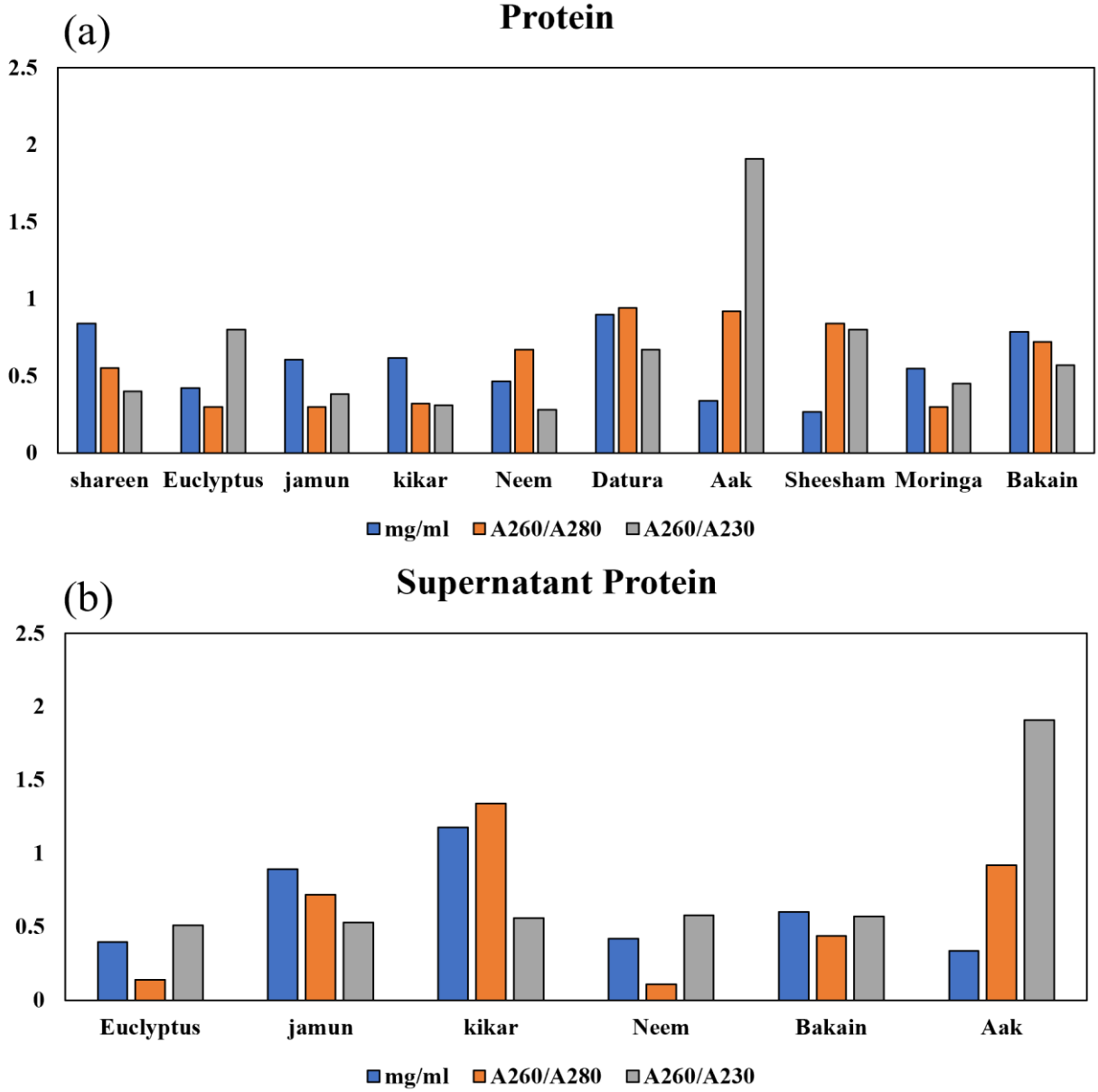

Figure 4. Quantitative protein detection using Nanodrop spectrophotometers (a) Nanodrop detected total soluble protein (b) Nanodrop detected total supernatant soluble protein of 6 plants.

within treatments, while the critical value (CV) helps assess the significance of the treatment effects.

3.4.3. Total soluble protein on aphid's mortality rate of the Aphid

The potential of various proteins as biopesticides by measuring the mortality rates of aphids after treatment was investigated. Figure 5 (a) shows the mortality result of 9 different total soluble proteins and 6 different supernatant proteins after 12 hrs. The total soluble protein of Euclyptus-P, sheesham-P and kikar-S showed the highest mortality rate of aphid, but Bakain-P and Euclyptus-S showed the zero mortality. The mean value of T3, T8, and T13 were same 2.67 or 26.7% with comparison to T10 and T11 their mean values are 0.00 (0%) respectively. Figure 5 (b) showed the aphid's mortality rate of 9 different total soluble protein and 6 different supernatant protein after 24 hrs. The mortality rate of aphid after treatment with T6 the mean

Table 2. Analysis of Variance Table for total Mortality data after three intervals of times (12h, 24h, 48h) (Standard error, Critical value and mean value of Aphid data)

Source	*DF*	*SS*	*MS*	*F*	*P*
Treatment	16	361.647	22.6029	11.76	0.0000
Error	34	65.333	65.333	1.9216	
Total	50	426.980			
Grand Mean	7.3137	***CV***	18.95%		

Table 3. Analysis of Variance Table for Total Mortality and Mean value of Whitefly data were calculated after two-time intervals (12h and 24h)

Source	*DF*	*SS*	*MS*	*F*	*P*
Treatment	15	109.917	7.32778	2.55	0.0129
Error	32	92.000	2.87500		
Total	47	201.917			
Grand Mean	4.0417	***CV***	14.95%		

value was 5.00 or 50%, T9 was 4.33 (43.3%), T3, T4 and T12 was 4.00 (40%) and T8 was 2.00 (20%) at 24 hrs. Thus, T6 showed the highest mortality and T9, T3, T4 and T12 showed the moderate mortality rate and T8 showed the lowest mortality rate respectively.

Figure 5 (c) showed the aphid mortality rate of 9 different total soluble protein and 6 different supernatant protein after 48 hrs. The mortality rate of aphid after treatment with T7, T8, T12, T15 and T16 the mean value was 4.67 or 46.7% and T13 was 2.00 (20%). Thus T7, T8, T12, T15, and T16 showed the highest mortality rate and T13 showed the lowest mortality rate after 48 hrs respectively. Figure 5 (d) showed the aphid total mortality rate of 9 different total soluble protein and 6 different supernatant protein after 3 intervals of times. The total mortality rate of aphid after treatment with T3, T5 the mean value was 9.67 or 96.7% and T4, T8 and T12 was 9.33 (93.3%), T8 was 8.67 (86.7%), T7, T15, and T16 was 8.33 (83.3%), T13 was 8.00 (80%), T9 was 7.67 (76.7%), T14 was 7.00 (70%), T2 6.67 (66.7%) and T10, T11 were 6.33 (63.3%). Thus, T3 and T5 showed the highest mortality rate and T10, T11 showed the lowest mortality rate respectively. The mortality rate of aphid after treatment with T12 the mean value was 9.33 or 93.3% and T11 was 6.33 or 63.3%. Thus, T12 showed highest mortality rate and T11 showed lowest mortality rate after total intervals of time.

3.4.4. Total soluble proteins (TSP) on the mortality of Whitefly

The efficacy of different proteins produced by plants to regulate whitefly populations across varying periods is shown in Figure 6. Figure 6 (a) showed the whitefly mortality rate of 9 different total soluble protein and 6 different supernatant protein after 12 hrs. The mortality rate of whitefly after treatment with T3 and T8 the mean value was 3.00 or 30%, T2, T11 and T14 was 1.00 or 10%. Thus, T3 and T8 showed the highest mortality rate at 12 hr respectively. Figure 6 (b) showed the whitefly mortality rate of 9 different total soluble protein and 6 different supernatant protein after 24 hrs. The mortality rate of whitefly after treatment with T3 and T8 the mean value was 4.33 or 43.3% percentage, T14 and T15 was 1.67 or 16.7%. Thus, T3 and T8 showed the highest mortality rate and T14 and T15 showed the lowest mortality rate at 24 hr respectively. The mortality rate of whitefly after treatment with T13 the mean value was 4.33 or 43.3% and T10, T11, T12, T15 and T16 was 3.00 (30%) to 3.33(33.3%) and T14 was 2.67 (26.7%). Thus, showed the highest mortality rate and T10, T11, T12, T15, T16 showed a moderate mortality rate but T14 showed the lowest mortality rate after 2 intervals of time.

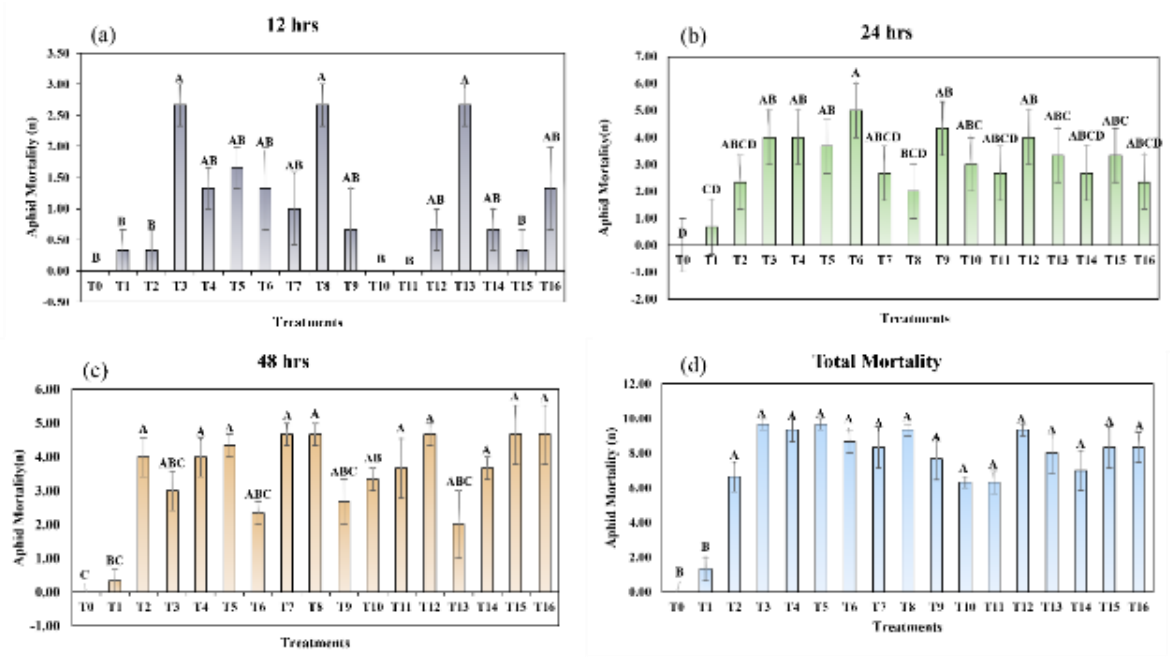

Figure 5. Effect of insecticidal peptides extracted from insect repellent plants on aphid (a) Effect of 10 different insecticidal total soluble protein on aphid's mortality rate in 12hrs (b) Effect of 10 different insecticidal total soluble protein on aphid's mortality rate in 24 hrs (c) Effects of 10 different insecticidal total soluble protein on aphid's mortality rate in 48 hrs (d) Effects of 10 different total soluble protein on aphid by total mortality rate

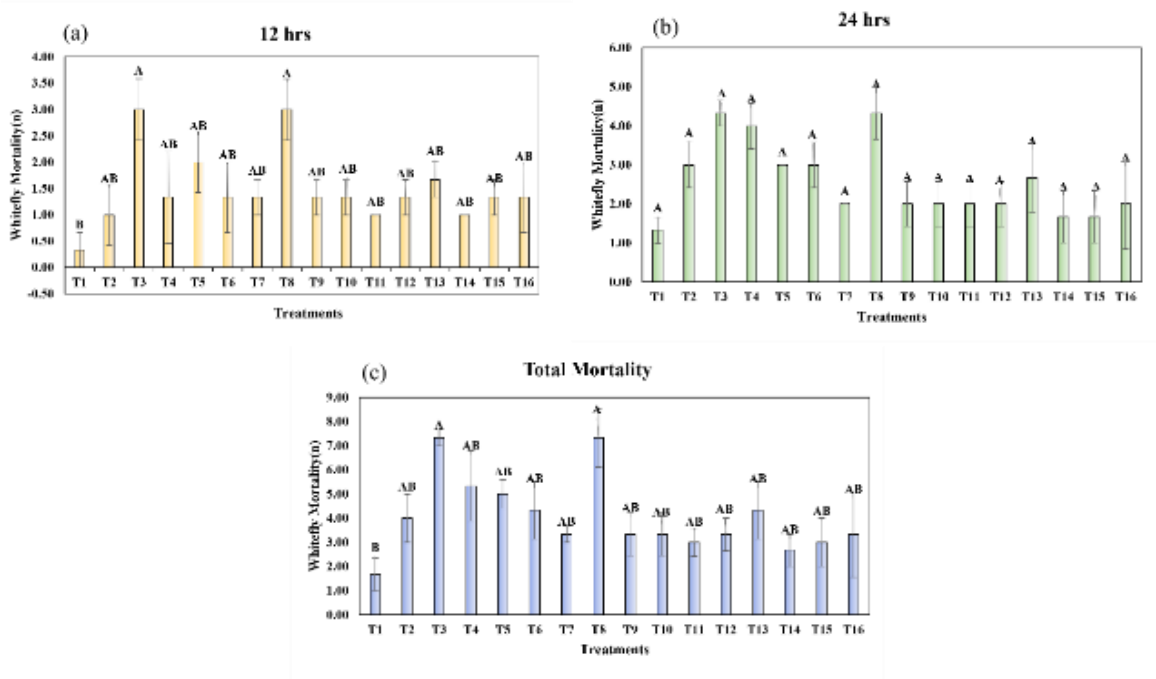

Figure 6. Effect of insecticidal peptides extracted from insect repellent plants on whitefly (a) Effect of 10 different insecticidal total soluble protein on Whitefly's mortality rate in 12 hrs (b) Effect of 10 different insecticidal total soluble protein on Whitefly's mortality rate in 24 hrs (c) Effect of 10 different insecticidal total soluble protein on Whitefly's total mortality rate

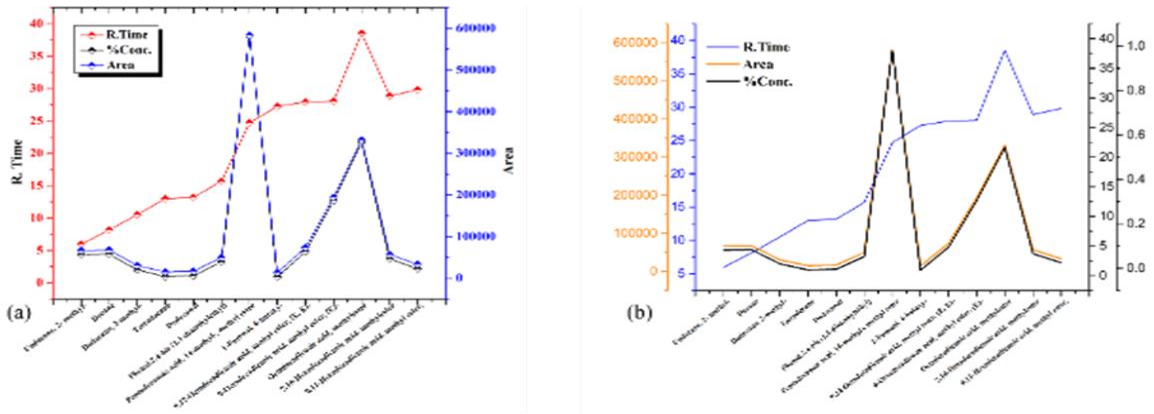

Figure 5. (a) Quantitative result graph of GC Mass of Eucalyptus extract (b) Quantitative result graph of GC Mass of Sheesham extract

3.5. GC-MS spectrometry

3.5.1. Insecticidal compounds detected from proteins

Further, the plant proteins were subjected to GC-MS analysis, results shown in Figure 7 (a, b, c) in the form of a graph that represents the concentration, surface area, and retention time for each component. The components were Dodecane, Decane, Oxalic acid, 2-ethyl hexyl ester, Hexadecane, Methyl tetradecanoate, Pentadecanoic acid,14-methyl-, methyl ester, 1-Pentanol, 3-methyl-2-propyl-,9-12-Octadecadienoic acid, methyl ester, (E, E)-, 6-Octadecadienoic acid, methyl ester, (Z)-, Octadecadienoic acid, methyl ester. Heat map of characteristics metabolites GC Mass A. indica also shown in Figure 7 (a and b). Before 10.0 min, only two compounds were detected: 2-methyl-undecane and dodecane Figure 7 (a). Between 10.0 and 20.0 min, however, dodecane, 2-methyl-, tetradecane, dodecanal, phenol,2,4-bis (1,1-dimethylethyl), Methyl ester of 1-butanol-4-butoxy-14-methyl pentadecanoic acid. Retention times between 20.0 and 30.0 min were optimal for the detection of the methyl esters 9,12-octadecadienoic acid, (E, E)-methyl ester, 9-octadecadienoic acid, (E)-methyl ester, Octadecadienoic acid, methyl ester, and 7,10-hexadecadienoic acid, methyl ester.

4. Discussion

In this research, we utilized two protein fractions: pellet proteins and supernatant proteins extracted from selected plants. The extracted proteins were then applied on two insect species, whitefly, and aphid, using a bioassay method. It is almost always the case that the untreated control treatment has a larger population than the treated treatment. The examined plant species included *A. indica*, *Eucalyptus globulus*, *Vachellia nilotica*, *C. gigantean*, *D. sissoo*, *A. lebbeck*, *M. azedarach*, *M. oleifera*, *D. stramonium*, and *S. cumini*, successfully and efficiently managed to suppress the sucking pest of cotton and mustard crop at all levels. According to previous researches, the evaluated plant leaf extracts demonstrated their efficacy as insecticides against the cotton, mustard and other crops' sucking pest complex (Adebayo, 2003). According to the total mortality rate of aphid after 12hr, 24hr and 48hr, the greatest amount of aphid decrease was observed with the mean value of Eucalyptus-P (9.67) or 96.7%, kikar-P (9.67) or 96.7%, jamun-P (9.33) or 93.3%, sheesham-P (9.33) or 9.33% and jamun-S (9.33) or 93.3% whereas the smallest amount of aphid decrease was observed with shareen-P (6.67) or 66.7%, bakain-P (6.33) or 63% and Eucalyptus-S (6.33) or 63.3% respectively.

Similarly, the maximum amount of whitefly was observed with the mean value of Eucalyptus-P (7.33) or 73.3% and Sheesham-P (7.33) or 73.3% whereas the minimum amount of whitefly was observed with Neem-S (2.67) or 26.7% respectively.

Although the research only partially supported (Ali, 1987) findings, which showed that some Culex and Anopheles species were susceptible to the insecticidal effects of *A. nilotica* fruits (Garad) water extract. *A. nilotica* acetone extracts were found to be poisonous to larvae of *Aedes aegypti* and *Culex quinquefaciatus*, according to (Chaubal et al., 2005). Similar outcomes were recently attained by (Zaitoun et al., 2012), who demonstrated the acute (212.1 ppm) and chronic (144.2 ppm) effects of A. nilotica acetone extract against Culex pipienis, which resulted in 93.33 % larval mortality as well as a reduction in egg hatchability and suppression of adult emergence.

According to (Mouna et al., 2021) the effectiveness of the plant Eucalyptus aqueous extracts were found to have an 85% repellency rate at 50% concentration against aphid, which makes them the most efficient. According to (Ansari et al., 2000) under laboratory circumstances, tests were done to determine the larvicidal, growth-inhibiting, and repellent effects of *D. sissoo* oil against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. When 1 ml of oil was applied to human participants' exposed body parts, it also demonstrated a potent repulsive effect. For 8 to 11 hours, they had insect protection. Sissoo oil provided protection (91.62%) that was similar to that of commercial Mylol oil (93.81.2%), which contains di-butyl and dimethyl phthalates.

The antifeedant and larvicidal ability of *Phyllanthus emblica* and *Syzygium cumini* against *Plutella xylostella* was also evaluated. *Phyllanthus emblica* methanolic leaf extract at 3% concentration and 48 hours of exposure absorbed 24.64% of the leaf area. In contrast, *Syzygium cumini* ethanolic leaf extract revealed that 39.26% of the leaf was eaten after 48 hours of exposure. After the insect consumed these leaves, the mortality rate of the insect was noted to determine the larvicidal action. After 48 hours of exposure, a 3% methanolic leaf concentration of *Phyllanthus embolic* had an almost 90% death rate. *Syzygium cumini*, on the other hand, demonstrated mortality at higher concentrations and at maximal exposure of about 50%. It was reported that *Syzygium cumini* leaf extract depicted better insecticidal activity against *Plutella xylostella* (Minj et al., 2017). These findings substantiate our results and emphasize further investigation on insecticidal properties of these plants.

5. Conclusion & future perspectives

The primary methods of controlling pests (whitefly and mustard aphid) have been fading away because of their deteriorated effects on environment and human health. This study develops understanding about usefulness of ecofriendly plant based insecticides which could prove valuable to combat both insect pests loses and environmental degradation. The total soluble and supernatant proteins of selected insect repellent plants were used in bio feed assay against whitefly and aphid.

The highest mortality of aphid was showed by Eucalyptus-P and sheesham-P 96%, kikar-P and jamun-P, jamun-S 93% and the highest whitefly mortality percentage was showed by Eucalyptus-p and sheesham-P are 73%. Thus, it is proved that soluble protein is more effective on aphid and whitefly mortality rate compared to supernatant protein. GC-MS analysis of these plant extract revealed compounds which could have potential to paralyze or kill insects by targeting particular receptors in their bodies.

6. Conflict of Interest

All contributed authors have no conflict of interest.

7. REFERENCES

Abbassy, M. S. J. J. E. A. T. (2017). Farmer's knowledge, attitudes and practices, and their exposure to pesticide residues after application on the vegetable and fruit crops. Case study: north of delta, Egypt. 7(5), 1-6.

Adebayo, T. J. L. A. U. o. T., Ogbomoso, Nigeria. (2003). Efficacy of mixture

formulations of synthesis and botanical insecticides in the control of insect pests of okra and cowpea.

Ali, A., Ahmad, F., Biondi, A., Wang, Y., & Desneux, N. J. J. o. P. S. (2012). Potential for using Datura alba leaf extracts against two major stored grain pests, the khapra beetle Trogoderma granarium and the rice weevil Sitophillus oryzae. 85, 359-366.

Ali, O. (1987). Larvicidal activity of some plant extracts against three of mosquitoes M. Sc. Thesis, Faculty of Science, University of Khartoum].

Ansari, M., Razdan, R., Tandon, M., & Vasudevan, P. (2000). Larvicidal and repellent actions of Dalbergia sissoo Roxb.(F. Leguminosae) oil against mosquitoes. Bioresource Technology, 73(3), 207-211.

Ashfaq, N. (2019). Insecticidal efficiency of native plant extracts against whitefly (Bemasia tabaci) on cotton crop. Pure and Applied Biology, 8(1). https://doi.org/10.19045/bspab.2019.80014

Belmain, S., Neal, G., Ray, D., Golob, P. J. F., & toxicology, c. (2001). Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. 39(3), 287-291.

Bempah, C. K., Donkor, A. K. J. E. m., & assessment. (2011). Pesticide residues in fruits at the market level in Accra Metropolis, Ghana, a preliminary study. 175, 551-561.

Biondi, A., Campolo, O., Desneux, N., Siscaro, G., Palmeri, V., & Zappalà, L. J. C. (2015). Life stage-dependent susceptibility of Aphytis melinus DeBach (Hymenoptera: Aphelinidae) to two pesticides commonly used in citrus orchards. 128, 142-147.

Biondi, A., Mommaerts, V., Smagghe, G., Vinuela, E., Zappala, L., & Desneux, N. J. P. m. s. (2012). The non-target impact of spinosyns on beneficial arthropods. 68(12), 1523-1536.

Boulos, I., Jabbour, J., Khoury, S., Mikhael, N., Tishkova, V., Candoni, N., Ghadieh, H. E., Veesler, S., Bassim, Y., & Azar, S. (2023). Exploring the World of Membrane Proteins: Techniques and Methods for Understanding Structure, Function, and Dynamics. Molecules, 28(20), 7176.

Chaubal, R., Pawar, P. V., Hebbalkar, G. D., Tungikar, V. B., Puranik, V. G., Deshpande, V. H., Deshpande, N. R. J. C., & biodiversity. (2005). Larvicidal Activity of Acacia nilotica Extracts and Isolation of D-Pinitol–A Bioactive Carbohydrate. 2(5), 684-688.

Cooper, W. R., Dillwith, J. W., & Puterka, G. J. (2010). Salivary proteins of Russian wheat aphid (Hemiptera: Aphididae). Environmental entomology, 39(1), 223-231.

Desneux, N., Decourtye, A., & Delpuech, J.-M. J. A. R. E. (2007). The sublethal effects of pesticides on beneficial arthropods. 52, 81-106.

DIAB, H. S. E.-D. T., Abdallah, M. D., ElTantawy, M. A. E. H., & Belal, M. H. (2006). Studies on Whitefly Bemisia tabaci (Genn.) Susceptibility to Pesticides CAIRO UNIVERSITY].

Guerra, J. G. O., Chávez, E. C., María, Y., Fuentes, O., Flores, J. L., Uribe, L. A. A., & Juárez, A. H. J. J. E. Z. S. (2020). Insecticidal activity of plant extracts against whitefly nymphs Bemisia tabaci (Hemiptera: Aleyrodidae) in laboratory. 8, 595-599.

Isman, M. B. J. A. R. E. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. 51, 45-66.

Jallow, M. F., Awadh, D. G., Albaho, M. S., Devi, V. Y., Thomas, B. M. J. I. j. o. e. r., & health, p. (2017). Pesticide knowledge and safety practices among farm workers in Kuwait: results of a survey. 14(4), 340.

Kumar, S. J. J. B. B. (2012). Biopesticides: a need for food and environmental safety. 3(4), 1-3.

Minj, N., Calistus, J., & Sri, S. (2017). Larvicidal and anti-feedant activity of Phyllanthus emblica and Syzygium cumini extracts on the diamondback moth: Plutella xylostella. Int. J. Biol. Res, 2, 97-100.

Mouna, M., Khadra, B., Assia, B., & Salim, L. (2021). Insecticidal effect of two aqueous extracts from the leaves of Salvia officinalis and Eucalyptus camaldulensis against Aphis fabae. Journal of Entomological Research, 45(2), 268-279.

Nasri, H., & Rafieian-Kopaei, M. (2014). Medicinal plants and antioxidants: why they are not always beneficial? Iranian journal of public health, 43(2), 255.

Oliveira, C., Auad, A., Mendes, S., & Frizzas, M. J. C. P. (2014). Crop losses and the economic impact of insect pests on Brazilian agriculture. 56, 50-54.

Roy, S., & Kumar, V. (2014). A practical approach on SDS PAGE for separation of protein. International Journal of Science and Research, 3(8), 955-960.

Sani, I., Ismail, S. I., Abdullah, S., Jalinas, J., Jamian, S., & Saad, N. (2020). A Review of the Biology and Control of Whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae), with Special Reference to Biological Control Using Entomopathogenic Fungi. Insects, 11(9). https://doi.org/10.3390/insects11090619

Singh, D. K. (2007). Toxicology of insecticides. In.

Vandermoten, S., Harmel, N., Mazzucchelli, G., De Pauw, E., Haubruge, E., & Francis, F. (2014). Comparative analyses of salivary proteins from three aphid species. Insect Molecular Biology, 23(1), 67-77.

Vennapusa, A. R., Somayanda, I. M., Doherty, C. J., & Jagadish, S. K. (2020). A universal method for high-quality RNA extraction from plant tissues rich in starch, proteins and fiber. Scientific reports, 10(1), 16887.

Weisenburger, D. D. J. H. p. (1993). Human health effects of agrichemical use. 24(6), 571-576.

Zaitoun, A., Madkour, M., Shamy, M. J. J. o. E. S., & Technology. (2012). Effect of three plants extracts on some bacterial strains and Culex pipiens L. stages. 5(1), 54-63.