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Efficacy and Synergistic Potential of Aqueous Plant Extracts Against Whiteflies, *Bemisia tabaci* Genn. in Chilli Plants

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ABSTRACT

The whitefly, *Bemisia tabaci,* causes serious yield losses by transmitting plant viruses and through aggressive feeding on the plant sap. Decades of overreliance on chemical insecticides for whitefly control have driven pest resistance and a detrimental impact on the environment, necessitating the development of sustainable alternatives. This study evaluates the potential of sustainable aqueous extracts against *B. tabaci* on chilli. Bioassays were performed using extracts produced from dried plant samples (10g) extracted using 100ml of distilled water at a 1:10 (w/v) ratio at room temperature. Adult mortality was recorded at 24 hours post-treatment and expressed as mean percentage ± SD. Statistical significance was determined by ANOVA and the Tukey HSD test. Lethal concentration (LC) values were derived via probit analysis. Among the extracts tested, the lowest LC50 value was shown by *A. indica* (63677.9ppm), *C. citratus* (65385.6ppm) and *P. acidus* (80449.5ppm). Synergistic combinations of these extracts were evaluated using LC25 and LC50 in a ratio of 1:1. Synergistic effects were observed in the combination of *A. indica* (LC25) with *P. acidus* (LC50) as well as *P. acidus* (LC25) with *C. citratus* (LC50). These findings highlight the potential of botanical extracts in mitigating whitefly infestation on chilli.

Keywords: *Bemisia tabaci*, efficacy, lethal concentration, plant extracts, synergism

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INTRODUCTION

The increasing popularity of international cuisines and spicy foods has driven the growing demand for chillies, from fresh to processed. Cultivating chilli (*Capsicum annuum* L.) crops can be a highly rewarding endeavour for farmers and home gardeners alike. Despite being known for their spiciness, chilli plants are also susceptible to pests. The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the primary pests of chilli. This pest's aggressive feeding behaviour and role as a vector for plant viruses disrupt plant physiology and productivity (Yadav et al., 2022). The ability of the whiteflies to rapidly populate within the plant intensifies the damage and contributes to significant yield losses due to the production of chilli fruits with low market viability (Kammara et al., 2023). The common approach to managing pests and diseases in chilli plant cultivation is the use of chemical pesticides. The underregulated and indiscriminate application of pesticides during the preharvest or postharvest stages often results in pesticide residues that pose risks to human health and the environment and hinder international trade (Tambo et al., 2024; Yami et al., 2025). These risks are exacerbated by the flooding of the market with illegal pesticides, often leading to the misuse of chemicals beyond recommended levels (Rahman, 2021). In the single year of 2020, agricultural activities in Malaysia consumed around 36 000 metric tons of pesticides, mostly organophosphates, pyrethroids, glyphosate, and 2,4-D-dimethylammonium (Malhotra & Aman, 2024). In addition, the level of understanding among Malaysian farmers regarding the environmental and human health risks of pesticides remains moderate, as reported in a study on pesticide usage awareness among farmers in Malaysia (Sabran & Abas, 2021). Many farmers apply these pesticides without proper protective measures, increasing their risk of exposure, and illegally use some organophosphates banned by the government, which eventually results in health problems (Hossain et al., 2010). Without safe management, awareness and sensibility on the pesticide usage by farmers, it can cause a detrimental impact not just on the environment but also on humans and other organisms, including the pest's natural enemy. Despite the growing emphasis in the academic literature on incorporating biopesticides into crop protection strategies (Chandler et al., 2011; Jalloh et al., 2026), chilli cultivation in Malaysia still relies heavily on synthetic insecticides. However, the emergence of resistance in whitefly biotypes has rendered many of the conventional approaches ineffective (Horowitz et al., 2020). These challenges highlight a need for plant-derived alternatives that demonstrate efficacy against whiteflies without causing adverse effects to the environment and end users. Furthermore, research into the potential synergy of these extracts may offer a way to leverage multiple bioactive compounds to overcome resistance in whiteflies. Therefore, the present study aims to evaluate the efficacy and synergistic effects of aqueous plant extracts against whiteflies in chilli plants.

METHODOLOGY

Culturing *B. tabaci*

Colonies of the whiteflies, *B. tabaci,* utilised in this study, were from a field collection in an infested commercial greenhouse located in Kampung Ampang Baru, Ipoh, Perak (4° 35' 18.55282"-101° 8' 15.63179"). Chemical management consisting of weekly applications of the neonicotinoid insecticide Imidacloprid was utilised in the greenhouse. Collection of the pest sample was carried out in the morning during the inactive period of whiteflies (Iqbal et al., 2022). Leaves containing adults and nymphs were picked carefully and confined in an insect-proof cage. The whiteflies colonies were then maintained in insect-proof cage on untreated chilli plant of 4 months old, prior to the bioassay to establish a culture. This acclimation period eliminates any acute residual effects from prior field exposure. The insect cage was constructed from a clear plastic container measuring 62cm × 33cm × 36cm (length × width × height) with a grid-like lid. The opening of the container was secured with fine mesh fabric, preventing the escape of the whiteflies while ensuring ventilation. The lid has a rectangular opening, 13cm × 11cm (length × width), allowing access for monitoring and collection of the insect without fully removing the lid. If the existing chilli plant deteriorates or shows signs of imminent death, a new chilli plant is introduced in the cage to maintain the infestation. Illumination was provided through an LED light with a diameter of 5.2cm, operating voltage ≤36V. The LED light has an adjustable clip that allows it to be affixed to the top of the container. The colonies in the rearing cage were maintained throughout the experiment period at 27-29 °C and 78 ± 10% relative humidity.

Molecular Identification of an Adult Whitefly Sample

The sample of wild strain of adult whitefly, *B. tabaci*, collected was subjected to a molecular identification process. The whitefly specimen is submitted to the Centre for Insect Systematics (UKM), Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM). DNA from the adult *B. tabaci* was extracted using NucleoSpin DNA Insect Kit (Macherey-Nagel, Germany). The universal primers, forward primer LCO1490 (5'GGTACAACAAATCATAAAGATATTGG-3') and reverse primer HCO2198 (5'TAAACTTCAGGGTGACCAAAAAATCA-3') were utilised for Polymerase Chain Reaction (PCR) amplification (Folmer et al., 1994). PCR reactions were set up in a final reaction volume of 25 µL utilising a Techne Prime Thermocycler. The reaction mixture consisted of 12.5 uL Mastermix, 1 uL of each primer, 5 uL ddH_2O and 5 uL of DNA template. The PCR thermal cycling conditions were optimised for hemipteran targets, with initial denaturation for 30s at 95 °C, annealing for 1min at 47°C - 52 °C, extension for 30s at 72 °C, and a final extension for 10min at 72 °C. The PCR products were then electrophoresed at 100V on a 1.5% agarose gel for 20 min. Bands were visualised under

UV light using an Accuris Instruments transilluminator to confirm successful amplification of the target fragment (Syahrawati et al., 2025). Sequencing analysis was performed, and all obtained sequences were edited using Sequencher version 4.1.4, and sequence similarity was compared with those in the GenBank database using the BLASTn program of the National Centre for Biotechnology Information (NCBI).

Plant Sample Collection and Preparation of Aqueous Plant Extract

Seven locally available plants were sourced either from a local grocery store or collected from nature (Table 1). The plant species were chosen based on the following selection criteria: possessing insecticidal activities based on past literature search, ease of local availability, and accessibility. The plants collected were identified through structural characteristics and anatomical features. Further validation of the plant species was evaluated against the taxonomic standards of the Royal Botanic Garden, Kew, electronic data repository (Plants of the World Online [POWO], 2026). The plant parts used were washed thoroughly with tap water to remove dirt, insects and foreign elements before being left to air-dry at room temperature under shade. The air-dried plants were then pulverised using a Waring laboratory blender. The ground plant materials were immersed in distilled water at a 1:10 (w: v) solid to solvent ratio for 24 hours at room temperature to obtain an aqueous extract of each plant (Osmić et al., 2019). All the extracts obtained were filtered by using Whatman No.1 filter paper to obtain a homogenous extract that was used immediately for the bioassay. The efficacy of the extracts was assayed across a concentration range from 25,000 ppm to 100,000 ppm to establish the lethal concentration values (Wallace et al., 2021).

Table 1
List of plant samples screened for potential biopesticide activity against adult whitefly, B. tabaci

Scientific Name	Common Name	Family	Plant Parts Used	Source
Allium cepa	Onion	Amaryllidaceae	Bulb	Store bought[1]
Azadirachta indica	Neem	Meliaceae	Leaves	Collected[2]
Cymbopogon citratus	Lemongrass	Poacea	Leaves	Store bought[1]
Murraya koenigii	Curry leaves	Rutaceae	Leaves	Collected[3]
Ocimum sanctum	Holy basil	Lamiaceae	Leaves	Collected[2]
Phyllanthus acidus	Star gooseberry	Phyllanthaceae	Leaves	Collected[3]
Plectranthus amboinicus	Indian borage	Lamiaceae	Leaves	Collected[2]

Note. [1]- Segi Fresh Klebang, Chemor, Perak; [2]- 4° 36' 42" North, 101° 6' 48" East; [3]- 4° 41' 30" North, 101° 6' 26" East

Bioassay



Figure 1. The bioassay arena was utilised to evaluate the *B. tabaci* adult mortality

The adult mortality bioassay was conducted in accordance with the IRAC Susceptibility Test method number 008-2009 with slight modifications (Insecticide Resistance Action Committee [IRAC], 2009). Clear plastic cups of 80mm, 70mm, and 50mm (height: top diameter; base diameter) with a 250ml volume capacity, were used as the bioassay arena. The opening of the plastic cup was covered with a circular piece of fine netting material and closed with the plastic lid of a 60mm × 15mm (diameter × depth) petri dish with a circular hole of 30mm diameter. A 1-inch steel nail was used to pierce a hole in the bottom of the plastic cup. An uncontaminated young chilli leaf with a petiole approximately 25mm was then dipped in the tested extracts for 30 sec by holding the leaf by the petiole. The leaf was left to air dry for 5 minutes, and the petiole of the test leaf was passed through the hole in the bottom of the plastic cup. The petiole was then wrapped with wet cotton wool and aluminium foil to maintain turgidity of the leaves throughout the experiment (Figure 1). Ten individual whiteflies per replicate were evaluated. Distilled water represented the negative control. Commercially available organic pesticide, Baba Mr Ganick®, Aphid Buster with major active ingredient plant alkaloid, Faboideae, was used as a positive control. The percentage mortality was determined at 24 hours. A whitefly adult was considered dead if it did not respond to probing with a fine paintbrush. The 24-hour mortality data were corrected for control mortality by applying Abbott's formula (Piepho et al., 2024). To satisfy the assumptions of the One-Way Analysis of Variance (ANOVA) test, an arcsine square root transformation was performed. The assumption of normality and homogeneity of variance were confirmed using the Shapiro-Wilk test and Levene's test, respectively.

Synergistic Assay

Based on the probit analysis and findings from the bioassay, extracts with the lowest LC25 and LC50 values were selected for evaluation of their synergistic potential. Binary mixtures were prepared by using the sublethal, LC25 and lethal, LC50 concentrations of the aqueous plant extracts. The combinations of *A. indica*, *P. acidus* and *C. citratus* were made by mixing the extract in a ratio of 1:1 at the specific lethal doses. Individual extracts at LC25 and LC50 and 12 binary mixtures were assayed for lethal effects using the leaf

dip bioassay. Four replicates of each combination were used for the bioassay. Distilled water represented the negative control. The percentage mortality of adult whitefly was determined at 24 hours. Whitefly, without any detectable movement, even upon probing with a soft paintbrush, was classified as dead (Bandyopadhyay et al., 2014; Kulawardhana et al., 2018; Kumrungsee et al., 2022). All bioassays were conducted in the laboratory at 28 ± 2°C and 50 ± 10% relative humidity.

Data Analysis

All bioassays were done in quadruplicate, and the values were shown as the mean of adult whitefly mortality in percentage values along with their standard deviations (± SD). A one-way ANOVA test was performed to determine the mean difference of adult whitefly mortality in percentage between the treatment groups. Mean separation was performed using the Tukey HSD test. The adult whitefly mortality rate at different concentrations of the aqueous plant extracts was subjected to probit analysis to determine the lethal concentrations that caused 50% (LC50) and 95% (LC95) mortality. The data is log-transformed in order to satisfy the assumption of probit analysis. The analysis was performed using Statistical Package for the Social Sciences (IBM SPSS) version 27. The rate of mortality for adult whitefly in individual extracts was determined at LC25 and LC50. The percentage of mortalities due to the binary mixtures was obtained and compared to the expected mortalities based on the method proposed by Colby (1967). The expected efficacy was calculated using the formula as in Equation 1:

$$\% E = A + B - (AB/100) \quad [1]$$

Where A and B are the mortality values for the individual extracts.

The efficacies of the combinations were determined to be antagonistic, additive or synergistic by X^2 comparisons as in Equation 2:

$$X^2 = (O_m - E)^2 / E \quad [2]$$

Where O_m *is* the observed mortality due to binary mixtures, E is the expected mortality, X^2 with df= 1 and $\alpha = 0.05$ is 3.841. X^2 values > 3.841 with observed mortality greater than expected mortality represent a synergistic effect, X^2 values > 3.841 with observed mortality lower than the expected mortality constitute an antagonistic effect of the plant extracts mixtures, while X^2 values < 3.841 depict additive effects.

RESULTS AND DISCUSSION

Molecular Analysis of *B. tabaci*

Molecular characterisation in this study revealed that the whiteflies collected from the field in Kampung Ampang Baru, Ipoh, Perak have 100% sequence similarity with the *Bemisia tabaci* isolate WF-NP with Gen Bank Accession number OR646686 (Table 2). The isolation source is indicated as a leaf from a field in Coimbatore, Tamil Nadu, India, in the host plant Bhendi in the Tamil native language, or Okra (*Abelmoschus esculentus*). The insect sample in this study also shows 99% sequence similarity with the *Bemisia tabaci* isolate TNAU01 in host plant Okra, *Bemisia tabaci* isolate Coimbatore, *Bemisia tabaci* isolate Salem_Brinjal, and *Bemisia tabaci* isolate Asia 1 New Delhi Brinjal (Table 2) in host plant brinjal (*Solanum melongena*), respectively.

Table 2
Comparison of the generated sequence of B. tabaci*, isolated in this study from an infested vegetation area in Kampung Ampang Baru, Ipoh, Perak, with* B. tabaci *isolates available at NCBI*

Isolate Name	The Most Similar Sequences in Genbank	
	Gen Bank Accession Number	Sequence Similarity (%)
Bemisia tabaci isolate WF-NP	OR646686.1	100
*Bemisia tabac*i isolate TNAU01	MN911178.1	99.84
Bemisia tabaci isolate Coimbatore	MW488192.1	99.84
Bemisia tabaci isolate Salem_Brinjal	MW488189.1	99.84
Bemisia tabaci isolate Asia 1 New Delhi Brinjal	OQ706117.1	99.70

B. tabaci is a cryptic species complex with 46 cryptic species that includes Africa, Asia I, Asia II 1-12, Asia 1-India, Asia III, Asia IV, Asia V, Australia, Australia/Indonesia, China1-7, Indian Ocean, Ru, Middle East Asia Minor I-II (MEAM), Mediterranean (MED), MEAM K, New World 1-2. Japan 1-2, Uganda, Italy 1, and Sub-Saharan Africa 1-5 (Rehman et al., 2021). Although the interspecific differences may be subtle, the different cryptic species of *B. tabaci* also differ in terms of insecticide resistance, host preference, parasitoids associated with endosymbiont faunas and virus transmissions (MacLeod et al., 2022).

The findings of this study are consistent with prior research that reported the largest number of sequences presented from Malaysia were the Asia 1 species, which is also predominantly distributed in other countries of South East Asia (Mahmood et al., 2022). An earlier study also established an introductory map of *B. tabaci* species in Malaysia that shows recovery of the Asia-1 species in 9 out of 10 states sampled (Shadmany et

al., 2019). Another research has provided evidence for significant levels of resistance of Asia- 1 whiteflies against selected organophosphate, pyrethroid and neonicotinoid insecticides in cotton growing regions in India. The study strongly suggests that large-scale organophosphate insecticide usage by the Indian farmers, long-term exposure of *B. tabaci* to the insecticide, regular outbreak of crop diseases in endemic areas and mandatory seed dressing treatment resulted in increased resistance to the chemical insecticides (Naveen et al., 2017). Meanwhile, in a study conducted in Malaysia on a wild strain of adult *B. tabaci* belonging to the Asia 1 biotype, a high level of resistance against diafenthiuron was detected (Shadmany et al., 2015). The insecticide resistance often led farmers to increase the application frequency, often with higher doses, which further intensifies the resistance. Pest control failure due to insecticide resistance not only poses a severe threat to the crop's productivity but also reduces the quality of the produce.

Efficacy of Aqueous Plant Extracts Against Adult Whitefly, *B. tabaci*

All the aqueous extracts investigated in this study exhibited insecticidal activity against the adult whitefly. The percentage mortality of adult whitefly resulting from the tested extracts bioassayed against adult whitefly at different concentrations is presented in Table 3.

Table 3
Efficacy of different extracts against adult whitefly B. tabaci *24 hours post-treatment*

Treatment	Mean ± SD [95% CI] [a]
Allium cepa	73.68 ± 13.59 [52.06, 95.31]
Azadiracta indica	76.32 ± 10.08 [60.28, 92.35]
Cymbopogon citratus	89.47 ± 14.89 [65.79, 100.00]
Murraya koenigii	55.26 ± 15.79 [30.14, 80.39]
Ocimum sanctum	65.79 ± 10.08 [49.75, 81.83]
Organic pesticide	84.21 ± 13.59 [62.59, 100.00]
Phyllanthus acidus	86.84 ± 10.08 [70.81, 100.00]
Plectranthus amboinicus	57.89 ± 28.51 [12.54, 100.00]

Note. [a] One-way ANOVA was conducted on mortality data following an Abbott correction and an arcsine square root transformation. Shapiro-Wilk $W = 0.94$; $p = .094$; Levene's test $F = 0.42$; $p = .879$; ANOVA $F = 2.10$; $p = .083$

At the highest concentration, all the extracts produced more than 50% mortality of adult whitefly; nevertheless, chilli leaves treated with the aqueous extracts of *C. citratus* exhibited the highest percentage mean mortality (89.47%), followed by *P. acidus* (86.84%) and *A. indica* (76.32%) (Figure 2).

The lethal concentrations that cause 25%, 50% and 95% mortality of *B. tabaci* adults exposed to the treatments are shown in Table 4. Lowest LC25 values were given by *A. indica* (29530.11ppm), *C. citratus* (39445.68ppm), *P. amboinicus* (43299.98ppm) and *P. acidus* (52271.03ppm). Meanwhile, the lowest LC50 value was presented by the aqueous extract of *A. indica* (63678.0ppm), followed by *C. citratus* (65385.6ppm) and *P. acidus* (80449.5ppm). This indicates the extracts have high insecticidal activity against the whiteflies at lower concentrations. Lowest LC95 values were obtained from the aqueous extracts *C. citratus* (224246.2ppm), followed by the extracts *P. acidus (*230245.9ppm)*, M. koenigii* (250182.0ppm) and *O. sanctum* (261826.8ppm) (Table 4). Among the seven plant extracts, *C. citratus* and *P. acidus* were the most toxic to whiteflies after contact at high concentrations. Determination of the lethal concentrations is important for formulating an effective biopesticide as well as recommending an effective concentration to end users for targeted pest control while preventing excessive application. This ensures long-term efficacy of the biopesticides and minimises any risk of resistance development.

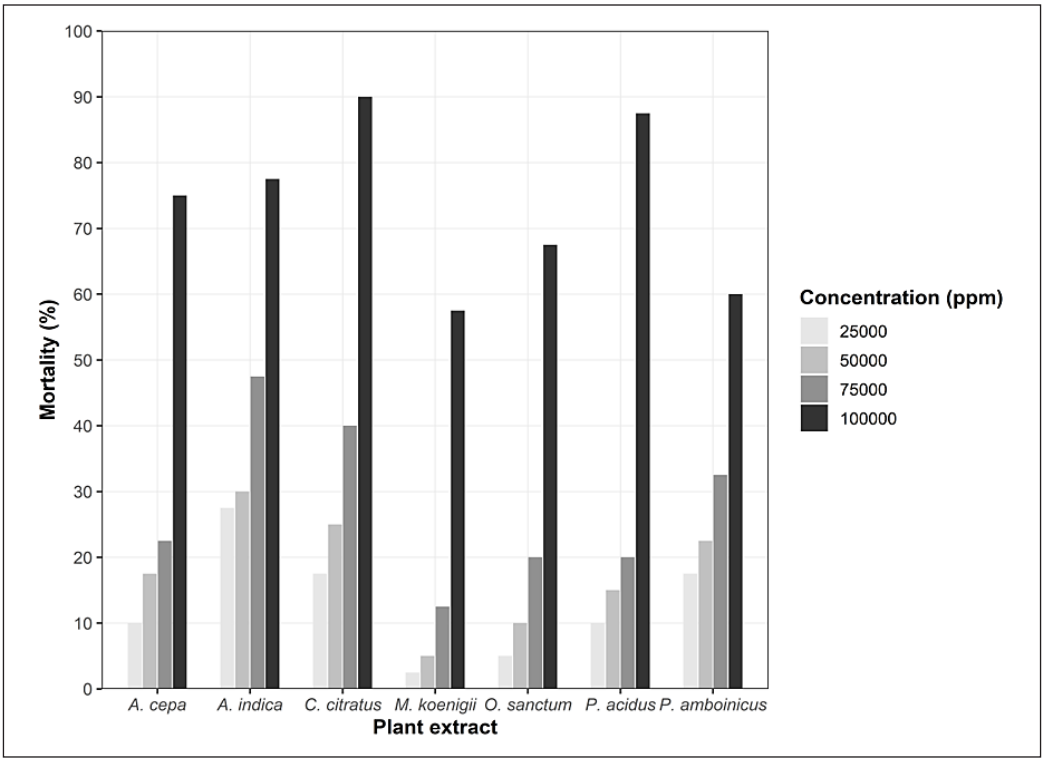

Figure 2. Percentage mortality of *B. tabaci* exposed to aqueous extracts at different concentrations(ppm) at 24 hours post-treatment

Table 4
Probit analysis of B. tabaci *exposed to selected aqueous extracts at different concentrations produced at 24 hours of extraction*

Plant Extract	n	Slope ± SE	LC25 [95% CI]	LC50 [95% CI]	LC95 [95% CI]	χ^2	df	p-value
Allium cepa	160	3.03 ± 0.60	52600.5 [43632.9, 63411.2]	87815.2 [71750.4, 107476.9]	306454.8 [164449.4, 571084.8]	28.45	14	0.012[b]
Azadiracta indica	160	2.02 ± 0.48	29530.1 [20114.0, 43354.3]	63677.9 [50350.8, 80532.5]	414791.2 [161938.2, 1062452.6]	12.32	14	0.581[a]
Cymbopogon citratus	160	3.07 ± 0.54	39445.7 [31784.6, 48953.4]	65385.6 [55676.4, 76788.1]	224247.3 [139443.7, 360624.5]	26.54	14	0.022[b]
Murraya koenigii	160	4.42 ± 1.01	74802.3 [65488.7, 85440.4]	106266.9 [87344.4, 129288.9]	250180.3 [145125.5, 431283.4]	46.75	14	< .001[b]
Ocimum sanctum	160	3.75 ± 0.76	63144.3 [54281.0, 73454.8]	95491.0 [79386.7, 114862.2]	261827.7 [153367.8, 446989.0]	22.92	14	0.062[a]
Phyllantus acidus	160	3.60 ± 0.65	52271.0 [44258.1, 61734.8]	80449.4 [68782.5, 94095.4]	230246.0 [145714.8, 363814.8]	56.31	14	< .001[b]
Plecranthus amboinicus	160	1.86 ± 0.50	43300.0 [31695.4, 59153.3]	99900.0 [68881.2, 144887.2]	767343.1 [193645.6, 3040686.0]	16.11	14	0.307[a]

Note. n = total number of whiteflies; $\chi 2$ = Pearson goodness-of-fit chi-square. Control mortality was corrected using the Abbott Formula, Slope = change in mortality probit per unit change in log10(concentration)
[a] $P > 0.05$ = the model is fit,
[b] $P < 0.05$= significant heterogeneity, Heterogeneity factor was used in the calculation of 95% CI

In order to facilitate the adoption of alternative pest control methods by farmers, it is necessary that their preparation and extraction processes be easy and viable (Nascimento et al., 2022). The plants used for the preparation of extracts in this study are commonly found and cultivated locally. This local availability ensures a consistent supply and increases their feasibility for application in small-scale farming. The selected plants also represent a diverse range of phytochemical compounds that have known insecticidal activity. The aqueous extraction carried out in this study using water as a solvent is a simple and sustainable extraction method without application of high heat or ultrasonic waves that can degrade bioactive compounds such as phenols and thermolabile compounds such as flavonoids (Mungwari et al., 2024). Water is considered the greenest extraction solvent as it is non-toxic to human health and the environment.

The recovery of phytochemicals from plant materials is influenced by many factors, including the solubility of the compounds in the extraction solvent, the choice of solvent, the degree of polymerisation of the bioactive compound, the interaction of the active compound with other plant constituents, as well as the extraction technique and duration (Yannick Stéphane et al., 2021). Water is a highly polar solvent with the ability to extract a wide range of polar substances, while also being low-cost, non-toxic and non-flammable (Abubakar & Haque, 2020). A previous study has reported that extraction using water resulted in a higher yield of extract compared to methanol (Singh et al., 2017).

Past literature has also revealed the efficacy of aqueous plant extracts against other phytophagous insects. The aqueous extracts (10% w/v, i.e., 100g of plant powder per litre of water) of ten pesticidal plant species, *A. indica, Ocimum basilicum, Nicotiana tabacum, C. citratus, Tephrosia vogelii, Aloe vera, and Lantana camara. Trichillia emetica, Vernonia amygdalina* and *Lippia javanica* were assessed for their contact toxicity and antifeedant properties against the fall armyworm, *Spodoptera frugiperda*. The study demonstrates that out of the ten screened species, the four most promising for efficacy were shown by the extracts of *L. javanica, O. basilicum, C. citratus,* and *A. indica* (Phambala et al., 2020). Another study reported that 10% aqueous extract of unpeeled neem seed sprayed on a papaya plant significantly stops the feeding of aphid, *Aphis nerii* and showed significantly higher mortality compared to the control (Hernández-Castro et al., 2005).

The bioassay in this study revealed the lowest lethal concentration value, expressed by *A. indica, C. citratus* and *P. acidus. A. indica* is widely recognised for its biopesticidal activity stemming from a range of bioactive compounds, including limonoids such as azadirachtin, salannin, meliantriol, and nimbin. However, the extraction of limonoids into aqueous solvents such as water is limited, the mortality of the adult whitefly in this study is most likely triggered by water-soluble bioactive constituents such as saponins, phenolic compounds, tannins, flavonoids and cardiac glycosides present in neem (Kumar et al.,

2018). These compounds are also known to induce strong antifeedant activity and inhibit insect growth (Agbo et al., 2019; Juma et al., 2022). A past study reported that neem-derived saponins exhibited strong insecticidal activity by inhibiting acetylcholinesterase (AChE) in the insect pests' nervous system. Inhibition of AChE is responsible for antifeedant behaviours, repellence and mortality in insect pests (Sami et al., 2018).

The primary bioactive constituent largely attributed to the pesticidal activity of *C. citratus* is the monoterpene, citral, composed of two isomers: geranial and neral, along with other compounds such as myrcene, limonene and linalool (Faria & Barbosa, 2024; Pinto et al., 2015). Although these major compounds are predominantly lipophilic with minimal aqueous solubility in water, the presence of these compounds, even at low concentrations, is known to exert significant insecticidal activity due to their high intrinsic toxicity to insects (Kobenan et al., 2021). Phytochemical analysis in previous studies has revealed the presence of water-soluble secondary metabolites: tannins, saponins, reducing sugars, glycosides, flavonoids, and other phenolic compounds in the *C. citratus* (Alzobaay & Kadhim, 2018; Unuigbe et al., 2019). Saponins are natural surfactants that can act as a natural emulsifying agent (Akbari et al., 2023; Kregiel et al., 2017), which may facilitate the dispersion of hydrophobic compounds like terpenes to form microemulsions that contribute to the insecticidal activity of the lemongrass extract. *P. acidus* is not only used for its edible fruit but also for a wide spectrum of diseases (Tan et al., 2020); however, its insecticidal activity has not been conclusively established and still remains an area of ongoing investigation. Previous studies have characterised the bioactive compounds found in *P. acidus*, including cyclohexene-1-(2-propenyl), jatrorrhizine, ethoxyquin, and isocorilagin. These bioactive substances are known to exert insecticidal activities by disrupting the midgut, interfering with the neurotransmission and enzymatic activity, ultimately causing death in insect pests (Divekar et al., 2022; Gope & Rawani, 2025).

Synergistic Interaction of Selected Individual Extracts at Sublethal and Lethal Concentrations

Individual extracts of *A. indica*, *P. acidus* and *C. citratus* at 25% and 50% sublethal and lethal concentrations were evaluated against the adult whitefly using the leaf-dip bioassay. Application of the binary extract of *P. acidus* at LC25 and *C. citratus* at LC50 and *A. indica* at LC25 and *P. acidus* at LC50 shows a synergistic effect with X^2 values > 3.841. Meanwhile, the following combination of *A. indica* and *P. acidus* at LC25:LC25, *A. indica* and *C. citratus* at LC25:LC25, *A. indica* and *C. citratus* at LC50:LC50, *A. indica* and *P. acidus* at LC50:LC25, *P. acidus* and *C. citratus* at LC50:LC25, *A. indica* and *C. citratus* at LC50:LC25 and *A. indica* and *C. citratus* at LC25:LC50 showed an additive effect with X^2 values < 3.841. A combination of the

extract *P. acidus* and *C. citratus* at LC25:LC25, *A. indica* and *P. acidus* at LC50:LC50 and *P. acidus* and *C. citratus* at LC50:LC50 reveals antagonistic effects with observed mortality values lower than the expected mortality (Table 4).

The binary extract of *A. indica* and *P. acidus* shows an additive effect at sublethal concentration, LC25; however, when the concentration of both extracts increased to LC50, the interaction becomes antagonistic. The mixtures of *A. indica* and *C. citratus* give an additive effect at both LC25 and LC50. The research findings show that a higher concentration of *A. indica* than *P. acidus* and *C. citratus* exhibits an additive effect. Increased concentration of *C. citratus* than *A. indica* also shows an additive effect. Nevertheless, when the concentration of *P. acidus* is higher than *that of A. indica, it causes a* synergistic effect. A similar concentration of *P. acidus* combined with *C. citratus* gives an antagonistic effect at both LC25 and LC50. Higher concentration of *P. acidus* than *C. citratus* gives an additive effect, but when the concentration of *C. citratus* increases, it induces a synergistic effect (Table 5).

A combination of extracts provides a broader spectrum of insecticidal activity with its multicomponent nature, delaying any potential resistance development (Selvamuthukumaran et al., 2024). The varied mechanism of action of the bioactive compounds in the extracts, which includes repellence, antifeedant, insecticidal and growth regulators, could act additively or synergistically (Dougoud et al., 2019). The synergistic or additive effects between extracts by which the lethal effects increase and the concentration of individual extracts can be reduced without affecting the efficacy can be exploited further in the development of a cost-effective biopesticide. Past research has also reported the effectiveness of combining extracts in managing pests. Earlier research found that the mixture of a common weed, *Datura stramonium* and neem, *A. indica* extracts was able to suppress the population of whiteflies, *B. tabaci,* on tomato, *Solanum lycopersicum* (Maina et al., 2025).

Prior study also reported synergistic interaction between the combined extracts of cinnamon, *Cinnamomum zeylanicum,* with *C. citratus* that exhibited high acaricidal activity compared to individual treatment against the larvae of *Rhipicephalus (Boophilus) microplus* (Singh et al., 2019). Synergistic effect between herbaceous shrub, Indian catmint, *Anisomeles malabarica* and *P. emblica* caused statistically significant larvicidal activity against the malaria vector, *Anopheles stephensi* (Jayakumar et al., 2014). In the current study, the synergistic effect of the mixture of *P. acidus* and *C. citratus* and *A. indica* and *P. acidus* at a specific lethal concentration resulted in higher mortality of adult whitefly compared to the individual treatments. This existing literature suggests that the combinations of extracts result in promising efficacy against insect pests.

Table 5
Mortality of the whitefly adults 24 h post-treatment with binary mixtures of aqueous plant extracts at their LC25 and LC50 values

Extract A	Extract B	Lethal Concentration	Final concentration of extract (ppm)	Adult mortality (%)				χ^2	Effect
				Individual extract		Binary Mixtures			
				Observed A	Observed B	Expected	Observed		
Azadirachta indica	*Phyllanthus acidus*	LC25:LC25	14785.06: 26135.52	22.5	17.5	36.1	30	1.02	Additive
Phyllanthus acidus	*Cymbopogon citratus*	LC25:LC25	26135.52: 19722.84	17.5	25	38.1	25	4.52	Antagonistic
Azadirachta indica	*Cymbopogon citratus*	LC25:LC25	14785.06: 19722.84	22.5	25	41.9	52.5	2.70	Additive
Azadirachta indica	*Phyllanthus acidus*	LC50:LC50	31838.95: 40224.70	30	25	47.5	32.5	4.74	Antagonistic
Phyllanthus acidus	*Cymbopogon citratus*	LC50:LC50	40224.70: 32692.80	25	30	47.5	17.5	18.95	Antagonistic
Azadirachta indica	*Cymbopogon citratus*	LC50:LC50	31838.95: 32692.80	30	30	51.0	60	1.59	Additive
Azadirachta indica	*Phyllanthus acidus*	LC50:LC25	31838.95: 26135.52	30	17.5	42.3	45	0.18	Additive
Phyllanthus acidus	*Cymbopogon citratus*	LC50:LC25	40224.70: 19722.84	25	25	43.8	50	0.89	Additive
Azadirachta indica	*Cymbopogon citratus*	LC50:LC25	31838.95: 19722.84	30	25	47.5	47.5	0.00	Additive
Azadirachta indica	*Phyllanthus acidus*	LC25:LC50	14785.06: 40224.70	22.5	25	41.9	55	4.11	Synergistic
Phyllanthus acidus	*Cymbopogon citratus*	LC25:LC50	26135.52: 32692.80	17.5	30	42.3	72.5	21.66	Synergistic
Azadirachta indica	*Cymbopogon citratus*	LC25:LC50	14785.06: 32692.80	22.5	30	45.8	37.5	1.49	Additive

Synergistic interaction often relies on the content, interaction between the different bioactive compounds, susceptibility of the target pest, as well as concentrations and ratios of individual components. A previous study on the synergistic interaction of thymol with *Piper ribesioides* extracts and isolated active compounds against *Spodoptera exigua* concluded that the synergism between plant extract and secondary metabolite depends on the composition of the extract and specific doses used (Kumrungsee et al., 2022). The higher potency of combined extracts at lower concentrations offers economic advantages in developing a cost-effective pest control approach as well as reducing the risk of resistance development. Smallholder farmers with limited technical resources can benefit from pest management strategies based on locally available resources.

CONCLUSION AND IMPLICATION OF THE STUDY

This study shows the potential of plant-derived insecticides, with all evaluated extracts achieving over 50% mortality against adult whiteflies. The aqueous extracts of *A. indica*, *C. citratus* and *P. acidus* exhibited promising pesticidal activity against adult whitefly with lower LC50 values. Additionally, the bioassay performed also revealed synergistic interaction between the mixtures of *P. acidus* and *C. citratus*, as well as *A. indica* and *P. acidus* under laboratory conditions.

The synergistic combinations of these plant extracts highlight their potential for optimising biopesticide formulations against *B. tabaci*. Utilising natural insecticides promotes sustainable agricultural practices and is generally safer for application on edible plants, as they are less likely to persist in the environment. Ultimately, incorporating biopesticides into small- scale farming can potentially reduce reliance on synthetic pesticides and minimise their associated risks.

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