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Efficacy of selected botanical oils against the cassava whitefly (*Bemisia tabaci*) and their effects on its feeding behaviour

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Abstract

The control of the whitefly *Bemisia tabaci* relies heavily on the use of synthetic insecticides. There is a need to develop alternative control strategies due to concerns about impact of these insecticides on the environmental and human health, and the threat of insecticide resistance. Botanical oil extracts could potentially be used for the management of whiteflies and other pests. The study reported here therefore aimed to evaluate the efficacy of selected botanical oils against the cassava whitefly, *B. tabaci* and test their effect on its feeding behaviour. Patchouli oil treatment was the most effective at repelling whiteflies in no choice and choice experiments with up to 85% of whiteflies being repelled. Oviposition was also reduced 50–89% in patchouli. Neem was found to be effective at reducing oviposition, nymph and adult emergence by 50%, 70% and 80%, respectively, in a screenhouse no choice experiment. Patchouli significantly reduced the phloem ingestion phase (E2) by 40% and potential drops (pd) by 46% compared to control plants. Neem significantly increased the non-probing duration by 48% and reduced pd by 50% compared to the control. Patchouli and neem were found to be the most effective among the selected botanical oils. These two oils should be further evaluated for efficacy under field conditions to determine suitability for recommendation as biopesticides against the cassava *B. tabaci* whitefly.

KEYWORDS
EPG, neem, patchouli, thyme

1 | INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important staple crops for the developing world, being consumed by 800 million people globally. However, production is severely constrained by arthropod pests, in particular the whitefly, *Bemisia tabaci* (Gennadius) (Parmar et al., 2017). In Africa, the most damaging effect of *B. tabaci* is the transmission of viruses that cause two major destructive diseases: cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Economic losses resulting from CMD and CBSD in sub-Saharan Africa can be up to 97%, valued at more than USD 1 billion annually (Legg et al., 2006, 2011).

Bemisia tabaci is known for its extensive genetic diversity which so far comprises a complex of at least 44 cryptic species (Kanakala & Ghanim, 2019). These cryptic species have mostly been identified based on sequences of the mitochondrial cytochrome oxidase I (COI)

gene (De Barro, 2012; Dinsdale et al., 2010). Cassava-colonizing *B. tabaci* in Africa were categorized, based on COI sequences, into five mitotypes named sub-Saharan Africa 1–(SSA 1–5) with SSA1 further divided into five subgroups (Berry et al., 2004; Legg et al., 2014). Recent studies, using SNP-genotyping through NextRAD sequencing, reassigned them into the six SNP-based haplogroups (phylogenetic classification based on SNP-genotyping) that are sub-Saharan Africa East and Central Africa (SSA-ECA), sub-Saharan Africa East and Southern Africa (SSA-ESA), sub-Saharan Africa Central Africa (SSA-CA), sub-Saharan Africa West Africa (SSA-WA), sub-Saharan Africa 2 (SSA2) and sub-Saharan Africa 4 (SSA4) (Chen et al., 2019; Wosula et al., 2017).

Chemical control through application of synthetic insecticides is the most common method used to manage *Bemisia tabaci* because of their efficacy and convenience (Horowitz et al., 2020). However, their injudicious application has negative impacts on the environment, human health and non-target organisms, especially beneficial insects, including the natural enemies of whiteflies (Horowitz et al., 2020). The greatest challenge to overreliance on synthetic insecticides is the rapid emergence of field-evolved resistance and pest resurgence which threatens the efficacy of existing chemistries (Horowitz et al., 2020). The large-scale use of mostly broad-spectrum insecticides has triggered resistance development in *B. tabaci* to more than 60 active ingredients (Horowitz et al., 2020).

Crop protection products formulated from plant essential oils (EOs) are attracting attention worldwide since they are considered as an alternative to synthetic insecticides that are harmful to the environment and human health, and as a consequence of new stringent insecticide legislation (Isman, 2015; Menossi et al., 2021; Pavela, 2016). However, their natural origin does not necessarily mean essential oils are safe and their off-target effects still need to be considered. Several plant botanical biopesticides are currently in use worldwide to control insect pests in greenhouse, field production and storage conditions (Khater, 2012). A number of these products have been evaluated for control of *B. tabaci* and have proved to some extent to be effective (Menossi et al., 2021; Perring et al., 2018). Neem (*Azadirachta indica* A. Juss.) oil contains >300 biologically active compounds, with the major constituents being triterpenes known as limonoids, the most important of which is azadirachtin (Pascoli et al., 2019). Neem oil-based biopesticides with azadirachtin as the active ingredient have been approved by several countries and are the most common new era botanical oil products for control of agricultural crop pests. They are highly effective against soft-bodied insects and mites, and act as an antifeedant, repellent and repugnant agent, which also induces sterility in insects (Chaudhary et al., 2017; Isman, 2015). Essential oils of *Piper marginatum* Jacq. (Piperaceae) and *Mansoa alliaceae* (Lam.) showed repellency and oviposition deterrence effects and reduced colonization by *B. tabaci* MED on cotton plants to levels that were comparable to an anthranilic diamide insecticide Benevia® (da Silva Santana et al., 2022). Effects of essential oils of garden thyme (*Thymus vulgaris* L.), patchouli (*Pogostemon cablin* [Blanco] Benth.) and lemon-scent gum (*Corymbia citriodora* (Hook.) K. D. Hill & L. A. S. Johnson) were found to cumulatively reduce the survival rate of *B. tabaci* B/MEAM1 by 27–46%, and reduced egg oviposition by 48–74% compared to controls. In addition, *T. vulgaris* caused the greatest contact toxicity, while *P. cablin* had the strongest repellence effect against *B. tabaci* (Yang et al., 2010). Thyme oil is a combination of monoterpenes, the main compounds of this oil are phenol isomer carvacrol and its nature terpenoid thymol which comprise 20–50% (Ahmad et al., 2014). Patchoulol and α-patchoulene are the major constituents that regulate and control patchouli essential oil quality. The major compounds of this essential oil are patchouli alcohol, α-guaiene, α-bulnesene, β-caryophyllene, α-patchoulene, γ-curcumene and some of the minor compounds are pogostone, limonene, cedrene, viridiflorol, γ-himachalene (Pandey et al., 2021). *Thymus pulegioides* L. and *Artemisia absinthium* L. tested on *B. tabaci* repelled 75–80% and caused adult mortality of 53–84% with increasing concentration (Li et al., 2022). Individual and mixed compounds from cumin (*Cuminum cyminum* L.), Cinnamon (*Cinnamomum zeylanicum* L.), Lemongrass (*Cymbopogon citratus* (DC.) Stapf.) and Citronella grass (*Cymbopogon winterianus* Jowitt ex Bor) evaluated for efficacy against *B. tabaci* MED showed these have repellency and toxicity effects (Barkman, 2013). These selected references show that essential oils have been tested on *B. tabaci* for a diverse set of crop systems and geographies, although this has not been done for *B. tabaci* on cassava in Africa. Incorporation of botanical biopesticides into IPM programmes could greatly reduce the quantities of synthetic chemicals applied, and possibly delay resistance development in pests (Khater, 2012).

African farmers are known to use botanical insecticides, but currently they only have access to crude preparations (Isman et al., 2007). The greatest issue with utilizing crude preparations as biopesticides within the field is their chemical instability in the presence of air, light, moisture and high temperature, which causes rapid evaporation and degradation of active components. The incorporation of EOs into controlled release nanoformulations may improve their utility and greatly enhance their effectiveness in comparison with bulk crude formulations. This study explores the activity of essential oils for efficacy against the cassava whitefly. If essential oil formulations are proven to be effective for whitefly control, the scaling of their application could have important beneficial impacts on food security and the livelihoods of smallholder farmers.

2 | MATERIALS AND METHODS

2.1 | Source and formulation of essential oils

Steam-distilled essential oils were sourced from Naissance, United Kingdom except for neem (cold pressed) which was from Hemani, Florida, United States. The oils were formulated in either in chitosan-cholesteryl (1 mg/mL) by sonication (University of Keele, UK) or 1.5% in 4% ethanol with 0.05% Tween 20 (International Institute of Tropical Agriculture – IITA). A suspension of 1 mg/mL chitosan-cholesteryl in distilled was prepared and solubilized using the sonicator set at 15 microns until clear (Soniprep 150plus sonicator—MSE,

Heathfield, UK). The chitosan-cholesteryl suspension was mixed with oil/compound at 10 or 15 mg/mL. The mixture was sonicated at 15 microns until opaque. The oils formulated in chitosan-cholesteryl included citronella (*Cymbopogon nardus* (L.) Rendle), fennel (*Foeniculum vulgare* Mill.), garlic (*Allium sativum* L.) lemon grass, patchouli and peppermint (*Mentha* X *piperita* L.). The oils formulated in ethanol were geranium (*Pelargonium* spp.), neem, patchouli and thyme. Two compounds—linalyl acetate and fenchone—which are found in some essential oils were also tested.

2.2 | Experimental set-up for evaluation of botanical oils efficacy

The oils and compounds were first evaluated in a preliminary screening study whose data are not presented in this study. The screening involved testing oils using the no choice leaf assays under laboratory conditions. The data presented in this study are for oils that were selected for further evaluation. These were patchouli and fennel oils, and linalyl acetate and fenchone compounds formulated in chitosan-cholesteryl in the first experiment. These were tested in a no choice assay leaf assay. In the subsequent experiments, patchouli, which was the most lethal in the first experiment, was selected for formulation in ethanol and tested alongside neem and thyme (neem was selected because of its availability locally and thyme as it had lethal effects comparable to patchouli). These three selected oils were tested in no-choice and choice leaf assays under laboratory conditions, and egg and nymph assays under screenhouse conditions. Choice assays using plants were also carried out under screenhouse conditions. Patchouli is the only oil that was formulated in chitosan-cholesteryl and also in ethanol, but these were tested separately, so there is no direct comparison.

2.2.1 | Whitefly colonies

The *B. tabaci* haplogroup used in this study was sub-Saharan Africa—East and Southern Africa (SSA-ESA—mitotype SSA1-SG3) (Wosula et al., 2020). The whiteflies were collected from cassava plants at Chambezi in Bagamoyo District, Coast Region, Tanzania in May 2019 and introduced to potted cassava plants placed in a 50×50×100 cm netted cages. The cassava plants were grown in 7.5 L pots containing a mixture of soil and farmyard manure at a 4:1 ratio and the whitefly colonies were reared on the cassava plants in the screenhouse at 25–30°C and 65–75% RH. Whiteflies were transferred to fresh 1-month-old cassava plants at intervals of 4–6 weeks to maintain the colonies.

2.2.2 | Whitefly *Bemisia tabaci* adult no-choice leaf assay

Experiment 1: This involved testing two oils (fennel and patchouli) and two compounds (fenchone and linalyl acetate) formulated in chitosan-cholesteryl. The control for this experiment was chitosan-cholesteryl. Cassava leaves were obtained from 4-week-old plants that were established in the screenhouse. The second fully opened leaves from the top were harvested and immediately petioles inserted in a vial containing distilled water. Leaves were allowed to sit on the laboratory bench for 20 min to fully hydrate. The oils were uniformly mist sprayed (four gentle sprayer presses within 15 cm between the leaf and the nozzle) on individual leaves on both adaxial and abaxial surfaces using 3 mL fingertip sprayers and allowed to dry under a fume hood for 10 min. Individual leaflets were cut from whole leaves and immediately inserted in 2 mL Eppendorf tubes with distilled water, and the tubes were sealed with Parafilm. Twenty whiteflies that had emerged within a period of 4 days from 6- to 8-week-old colonies were aspirated into glass vials (7.5 cm × 2.5 cm diameter—Watkins and Doncaster, UK). The tubes containing the sprayed leaflets were placed into the glass vials with whiteflies and sealed with Parafilm perforated with 10–20 pinholes (Figure 1). The vials were replicated five times per treatment and the experiment was conducted three times. Whiteflies that were alive on the leaf surface were recorded at intervals of 3, 24, 48, 72 and 96 h. The total eggs laid on each leaf were recorded once at 96 h.

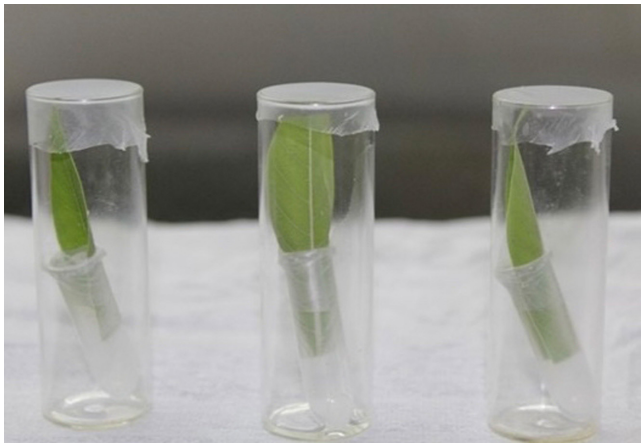

FIGURE 1 Glass vial with cassava leaves inserted in Eppendorf tubes. [Colour figure can be viewed at wileyonlinelibrary.com]

Experiment 2: This involved testing three oils (neem, patchouli and thyme) that were formulated at 1.5% in 4% ethanol with 0.05% Tween 20. The control for this experiment was 4% ethanol. The leaflets were prepared, and whiteflies were collected as stated in the first experiment. The glass vials containing the leaflets and whiteflies (20 per vial) were held in round fit holes in transparent plastic containers (7.5 cm × 10 cm diameter) with lids placed upside down. The whiteflies were confined by covering the plastic containers with perforated bread bags (25 cm × 20 cm) and securing them with rubber bands (Figure 2). There were five vial replicates for each oil treatment with ethanol as the control. The experiment was conducted three times. Whiteflies that were alive on the leaf surface were recorded at intervals of 3, 24, 48 and 72 h. The total eggs laid on each leaf were recorded once at 72 h.

FIGURE 2 Plastic container and bread bag set-up. [Colour figure can be viewed at wileyonlinelibrary.com]

2.2.3 | Whitefly *Bemisia tabaci* adult choice leaf assay

Leaf choice assays were run for three oils (neem, patchouli and thyme) formulated at 1.5% in 4% ethanol with 0.05% Tween 20. The leaflets were prepared, and whiteflies collected as described for the first experiment. Each oil treatment had a set of two leaflets held in individual vials with one leaflet sprayed with oil and the second with ethanol control. Paired control leaves were included as a control. The paired vials containing the leaflets and whiteflies (30 per vial) were held in round fitting holes in transparent plastic containers sealed with bread bags. There were five vial replicates with a set each for the oil and ethanol control and the experiment was conducted three times. Counts were recorded for live whiteflies settling on either oil or the ethanol control for 3, 24, 48 and 72 h, and eggs at 72 h.

2.2.4 | Whitefly *Bemisia tabaci* egg assay

Three selected oils (neem, patchouli and thyme) were tested in this experiment. Three-week-old cassava plants of variety Albert were defoliated leaving only the second leaf from the top 24 h before setting up the experiment. A total of five plants (replicates) were prepared per essential oil treatment and ethanol control. Fifty adult whiteflies emerging within 4 days were collected and confined to the leaf using bread bags for 48 h to lay eggs. The number of eggs laid per plant was counted with the aid of a light microscope. The oils were mist sprayed uniformly on the egg-bearing leaves. The numbers of total instars after 10 days and emerged adults after 24 days post-spraying were recorded in proportion to total eggs. The experiment was conducted three times to give a total of 15 replicates per treatment.

2.2.5 | Whitefly *Bemisia tabaci* nymph assay

The oils and plants were prepared as stated in the 'Whitefly *Bemisia tabaci* egg assay'. Fifty adult whiteflies emerged within 4 days were confined to the leaf using bread bags for 48 h to lay eggs. The whiteflies and bread bags were removed, and plants were confined in cages in the screenhouse for 10–14 days or until the third- and fourth-instar stages had developed. The number of nymphs per plant was counted with the aid of a stereo microscope under laboratory conditions. The oils were mist sprayed uniformly on the instar-bearing leaves. The numbers of emerged fourth-stage nymphs and adults in proportion to total nymphs were recorded at 10 and 14 days.

2.2.6 | Whitefly *Bemisia tabaci* no-choice plant assay

The oils were formulated at 1.5% by emulsifying in 1 mL of 100% ethanol and diluted with distilled water to 4% ethanol with 0.05% Tween 20. The control for this experiment was 4% ethanol. Three-week-old cassava plants of variety Albert were defoliated leaving only the second leaf from the top 24 h before setting up the experiment. The leaves were mist sprayed on both sides using a 100-mL fingertip sprayer. Thirty adult whiteflies that emerged within 4 days were collected and confined to the leaf using bread bags. The total number of whiteflies settling on leaves was recorded at 3 h and early in the morning for five consecutive days. Counts were made of the total number of eggs after 5 days, the number of nymphs on leaves after 10 days and the number of emerged adults at 14 days. There were five replicates per treatment including an ethanol control and the experiment was conducted three times.

2.3 | Probing behaviour of *Bemisia tabaci* on essential oil-treated cassava plants

The probing and feeding behaviours of cassava whitefly on plants treated with essential oils were monitored using the electrical penetration graph (EPG) technique. EPG is a powerful tool used to study the feeding behaviour of piercing–sucking insects including *Bemisia* species. EPG creates an electric circuit through the insect and the plant and measures the fluctuations in voltage in real time while the insect is feeding, producing the waveforms that describe the feeding behaviour in detail (Walker, 2000). The EPG technique has been widely used on *B. tabaci* in studies focusing on virus transmission, host resistance factors and insecticide effects (Civolani et al., 2014; Jiang et al., 2001; Liu et al., 2012; Milenovic et al., 2019; Prado Maluta et al., 2017; Rodríguez-López et al., 2011).

The experiment involved testing three oils (neem, patchouli and thyme) formulated in 4% ethanol with 0.05% Tween20. Each oil treatment had a set of eight plants with four plants sprayed with oil and the other four plants with water control. The oils were uniformly mist sprayed on individual leaves on abaxial surfaces using 3 mL fingertip sprayers and allowed to dry.

EPG experiments were carried out using the Giga-8d DC-EPG device, which has a 1 Giga-ohm input resistance and can record up to eight insects at once (EPG Systems). A 1-cm long, 2.5 µm thick

platinum wire (Sigmund Cohn Corp) was attached to the top of the head of the test insect using electrically conductive silver glue (EPG Systems). The other end of the platinum wire was attached to a 2.5-cm copper wire, which was then soldered to a brass nail inserted into the EPG probe. The platinum wire is thin and flexible which allows the attached insect to move around freely (Milenovic et al., 2019).

One insect was placed on one plant, and plants were used only once. Insects were placed on the abaxial surface of second leaf from the top of the plant. To access the lower surface, leaves were inverted, and their bases were taped to a solid surface with electrical tape. The abaxial surface was used since it is usually the preferred feeding site for whiteflies. Eight plants were recorded at one time for 12 h. Four of the eight were always oil treated, and the other four were water-treated controls. A total of 26 plants were recorded per treatment for neem and patchouli out of which 20 had good waveforms for each, while for thyme, 20 plants were recorded per treatment out of which 15 had good waveforms. The EPG waveforms selected for analysis included the following distinct waveforms (Milenovic et al., 2019):

1. C—the intercellular apoplastic stylet pathway where the insects show a cyclic activity of mechanical stylet penetration and secretion of saliva
2. Pd—potential drops resulting from intracellular stylet puncture occurring during the stylet pathway
3. E1—salivation into phloem sieve elements at the beginning of the phloem phase
4. E2—passive phloem sap uptake from the sieve element
5. G—active intake of xylem sap
6. Np—non-probing, where insect stylets are withdrawn from the leaf

2.4 | Data analysis

Percent whiteflies alive, mortality and number of eggs were subjected to analysis of variance using PROC GLIMMIX (SAS version 9.4; SAS Institute). The essential oil treatment effects were considered significant at $p=0.05$. The LSMEANS statement was used to obtain least squares means and the Tukey–Kramer test at $p=0.05$ was used for pairwise comparison of all treatment means. The Dunnett test was used for comparing treatments with the control in choice experiments. The fixed effect was essential oils, while random effects were set and replication. Treatment means and standard errors were obtained using the PROC MEANS statement in SAS. Raw EPG data were recorded by EPG Systems Stylet+d and manually annotated using EPG Systems Stylet+a software v01.30. The annotated files were imported into the EPG_analysisworksheet_v4.4.3.xls, and data subjected to analysis of variance using PROC GLIMMIX (SAS version 9.4; SAS Institute). The essential oil treatment effects were considered significant at $p=0.05$. The LSMEANS statement was used to obtain least squares means and the Dunnett test was used for comparison of treatments to controls. Treatment means and standard errors were obtained using the PROC MEANS statement in SAS.

3 | RESULTS

3.1 | Efficacy of botanical oils against whiteflies

3.1.1 | Adult no-choice assay

Experiment 1: The percentage of whiteflies settling on treated leaves after 3 h was significantly lower ($p=0.0002$) with the patchouli treatment (14%) compared to the control chitosan treatment (51%), and fenchone, fennel and linalyl acetate (42–51%). At 24 h, the proportion of whiteflies settling on treated leaves was significantly lower ($p=0.0103$) with the patchouli treatment (50%) compared to control (82%), fennel (81%) and linalyl acetate (83%). The percentage totals of live whiteflies at 48 h ($p=0.0028$), 72 h ($p=0.0049$) and 96 h ($p=0.0106$) were all significantly lower on patchouli-treated leaves (54–74%) compared to the control (91–100%). The other three oils were not significantly different from the control. The average number of eggs after 96 h was also significantly lower ($p=0.0107$) on patchouli-treated leaves (100) compared to the control (199) (Table 1).

Experiment 2: The number of whiteflies settling on treated leaves at 3, 24, 48 and 72 h was not significantly different from the control

TABLE 1 Efficacy of nanoformulated essential oils and compounds against cassava *Bemisia tabaci* whiteflies—no choice assay (Means ± SE).*

	% whiteflies settling on leaf					
Treatment	**3 h**	**24 h**	**48 h**	**72 h**	**96 h**	**Eggs 96 h**
Control	51 ± 7.4b	82 ± 7.0b	100 ± 0b	95 ± 3.3b	91 ± 5.4b	199 ± 30.7b
Fenchone	42 ± 6.5b	76 ± 7.8ab	90 ± 4.1ab	89 ± 4.0b	86 ± 3.8b	149 ± 30.2ab
Fennel	51 ± 5.8b	81 ± 5.6b	89 ± 4.6ab	89 ± 4.6b	80 ± 5.0ab	151 ± 30.0ab
Linalyl acetate	49 ± 6.7b	83 ± 8.7b	95 ± 2.6b	94 ± 2.5b	85 ± 5.8ab	180 ± 34.4b
Patchouli	14 ± 3.8a	50 ± 10.9a	74 ± 7.7a	61 ± 11.8a	54 ± 12.5a	100 ± 35.8a
p value	0.0002	0.0103	0.0028	0.0049	0.0106	0.0107

*Means with the same letter within a column are not significantly different ($p=0.05$, Tukey–Kramer test).

($p > 0.05$). The number of eggs after 72 h was significantly lower ($p < 0.0001$) on patchouli (10) and neem (22) treated leaves compared to the control (36). The number of eggs on thyme-treated leaves was not significantly different compared to the control (Table 2).

3.1.2 | Adult choice assay

The two paired control treatments were not significantly different ($p > 0.05$) in the proportion of whiteflies settling on leaves at various hour intervals and eggs at 72 h. The patchouli treatment had significantly fewer whiteflies settling on leaves at 3 h ($p = 0.0001$), 24 h ($p < 0.0001$), 48 h ($p = 0.0018$) and 72 h ($p = 0.0013$), and fewer eggs at 72 h ($p < 0.0001$) compared to its paired control. The thyme treatment had significantly fewer whiteflies settling on leaves at 24 h ($p = 0.0022$), 48 h ($p = 0.0019$) and 72 h ($p = 0.0013$), and fewer eggs at 72 h ($p = 0.0038$) compared to its paired control. The neem treatment had significantly fewer whiteflies settling on leaves only at 72 h ($p = 0.0003$), and fewer eggs at 72 h ($p < 0.0001$) compared to its paired control (Table 3).

3.1.3 | Egg assay

After treating eggs, nymphs still emerged but differences in survival were observed. The percentage of nymphs that emerged 10 days after spraying the eggs was significantly lower ($p < 0.0001$) with neem and patchouli treatments compared to the control, while thyme was not different from the control. Nymphs were reduced by 20% compared to the control with the most effective oil being the neem treatment (Figure 3a). The percentage of adults that emerged after 24 days post spraying was significantly lower ($p < 0.0001$) with all three oils with patchouli having the least compared to the control. The most effective oil, patchouli, reduced emerged adults by 37% followed by neem (30%) compared to the control (Figure 3a).

3.1.4 | Nymph assay

The percentage of sprayed nymphs that reached the fourth stage was significantly reduced ($p = 0.0002$) by 38% for patchouli and 50% for neem compared to the control. The percentage of adults that emerged from the fourth-stage instars was significantly lower ($p < 0.0001$), being reduced by 34% for patchouli and 39% for neem compared to the control (Figure 3b).

3.1.5 | No-choice plant assay

The number of whiteflies that settled on plants treated with neem and patchouli was significantly fewer (33–68%) ($p < 0.0001$) compared to the control at 3, 24, 48, 72, 96 and 120 h. Whiteflies settling on thyme-treated plants were significantly fewer (41–54%) ($p < 0.0001$) compared to the control at 72, 96 and 120 h (Figure 4a). The number of immature stages that emerged was significantly fewer ($p < 0.05$) on neem-treated plants compared to the control. Fifty-six per cent less eggs were laid, and there were 70% fewer nymphs and 80% less adults in neem. Patchouli had significantly fewer adults emerging (61% less) compared to the control (Figure 4b).

3.2 | Probing behaviour of *Bemisia tabaci* on essential oil-treated cassava plants

Waveform events associated with host suitability and virus transmission were selected for analysis. Whiteflies on plants treated with neem had a significantly longer ($p < 0.05$) non-probing (np) total duration (1.9 times), and np mean duration per event (2.6 times) compared to the control plants. The total potential drop (pd) duration for neem oil was significantly longer ($p < 0.05$) in the control (two times) compared to the neem-treated plants. The total duration of phloem ingestion (E2), though not significant, was 1.4 times longer in the control compared to neem-treated plants. The other events including xylem ingestion (G), stylet pathway (C) and phloem salivation (E1) did not differ significantly in neem-treated plants compared to controls (Table 4). Whiteflies on plants that were treated with patchouli had significantly shorter ($p = 0.05$) total phloem ingestion (E2) duration, mean E2 events, potential drop (pd) duration and mean pd events compared to the control plants. The other parameter associated with host suitability is duration to first probe. Although there were no statistically significant differences in duration to first probe when comparing the three oils to their controls, on patchouli

TABLE 2 Efficacy of three essential oils against cassava *Bemisia tabaci* whiteflies—no choice assay (Means ± SE).*

	% whiteflies settling on leaf				
Treatment	**3 h**	**24 h**	**48 h**	**72 h**	**Eggs 72 h**
Control	13 ± 3.4a	36 ± 4.1ab	32 ± 4.1a	24 ± 3.5a	36 ± 8.1c
Neem	10 ± 2.5a	37 ± 2.7ab	31 ± 3.9a	20 ± 3.9a	22 ± 6.0b
Patchouli	9 ± 2.8a	28 ± 4.6a	22 ± 4.8a	19 ± 4.4a	10 ± 2.8a
Thyme	13 ± 5.4a	47 ± 6.2b	36 ± 4.7a	29 ± 5.7a	42 ± 16.6c
P value	0.3233	0.0451	0.1396	0.0636	<0.0001

*Means with the same letter within a column are not significantly different ($p = 0.05$, Tukey–Kramer test).

TABLE 3 Efficacy of three essential oils against cassava *Bemisia tabaci* whiteflies—choice assay (Means ± SE).*

		% whiteflies settling on paired leaves				
Oil	**Treatment**	**3 h**	**24 h**	**48 h**	**72 h**	**Eggs 72 h**
Control	Control-1	35.2 ± 2.8a	8.7 ± 1.8a	32.2 ± 3.4a	24.0 ± 2.9a	87.2 ± 14.2a
	Control-2	40.2 ± 2.8a	12.4 ± 1.9a	32.9 ± 2.5a	24.0 ± 2.5a	102.6 ± 15.1a
	p value	0.2200	0.1923	0.8662	0.9987	0.4667
Neem	Neem	6 ± 1.2a	30 ± 3.9a	24 ± 2.9a	11 ± 2.1b	27 ± 6.2a
	Control	9 ± 1.9a	36 ± 4.4a	35 ± 4.8a	33 ± 5.0a	104 ± 13.9b
	p value	0.2230	0.3384	0.0675	0.0003	<0.0001
Patchouli	Patchouli	2 ± 0.6a	8 ± 2.2a	8 ± 2.3a	8 ± 2.0a	12 ± 4.6a
	Control	13 ± 3.5b	49 ± 5.7b	40 ± 6.3b	28 ± 4.4b	111 ± 21.6b
	p value	0.0001	<0.0001	0.0018	0.0013	<0.0001
Thyme	Thyme	6 ± 1.3a	17 ± 3.4a	16 ± 3.1a	10 ± 2.2a	25 ± 9.0a
	Control	12 ± 3.0b	40 ± 4.6b	38 ± 4.2b	30 ± 4.4b	120 ± 19.2b
	p value	0.0990	0.0022	0.0019	0.0013	0.0038

*Means with the same letter within a column for each treatment comparison are not significantly different ($p = 0.05$, Dunnett test).

(31.6 ± 10.4a), the duration was 2.7 times longer compared to the control (11.3 ± 3.1a), while on neem (47.0 ± 17.6a), the duration was 2.4 times longer compared to the control (19.5 ± 5.5a).

4 | DISCUSSION

The findings from this study show that three oils (neem, patchouli, thyme) were effective in controlling the cassava *B. tabaci* whitefly through reduced adult settling on leaves, eggs laid and fewer emerged nymphs and adults compared to controls. Neem oil stood out as an oviposition deterrent since in some experiments, there was no difference in whitefly settling on treated versus control leaves, but eggs laid were significantly fewer on treated leaves compared to the control. The essential oil fennel as well as the two compounds linalyl acetate and fenchone were found to have no significant effect on cassava *B. tabaci*.

Several studies have reported the efficacy of essential oils from various plant parts against *B. tabaci* and other herbivores, and in most cases, they have had a significant effect with varying activities such as toxicity, irritability or repellence and as a deterrent to egg laying (Aslan et al., 2004; Baldin et al., 2013; Chae et al., 2014; da Silva Santana et al., 2022; Drabo et al., 2017; Kim et al., 2011; Pereira et al., 2018; Yang et al., 2010; Yarahmadi et al., 2013). Findings from this study demonstrate that some of the tested essential oils are effective against *B. tabaci* whiteflies either through repellence, oviposition deterrence or biocidal action with patchouli and neem oils being the most promising. Testing the performance of essential oils on different cryptic species of *B. tabaci* is critical. In this study, we tested against the cassava *B. tabaci* SSA-ESA (mitotype SSA1-SG3).

Patchouli and thyme have been reported to be effective against all stages of *B. tabaci* mitotype MEAM1 with whitefly survival

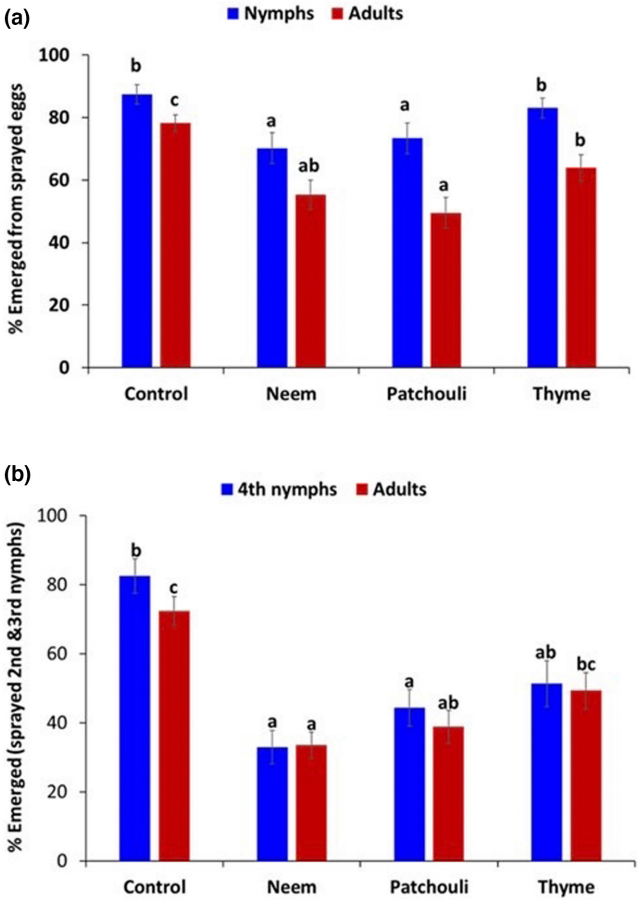

FIGURE 3 Effect of three essential oils on cassava *Bemisia tabaci* nymph and adult emergence from sprayed eggs (a) and nymph and adult emergence from sprayed second- and third-stage nymphs (b). Means with the same letter for treatments within each whitefly stage are not significantly different ($p = 0.05$, Tukey–Kramer test). [Colour figure can be viewed at wileyonlinelibrary.com]

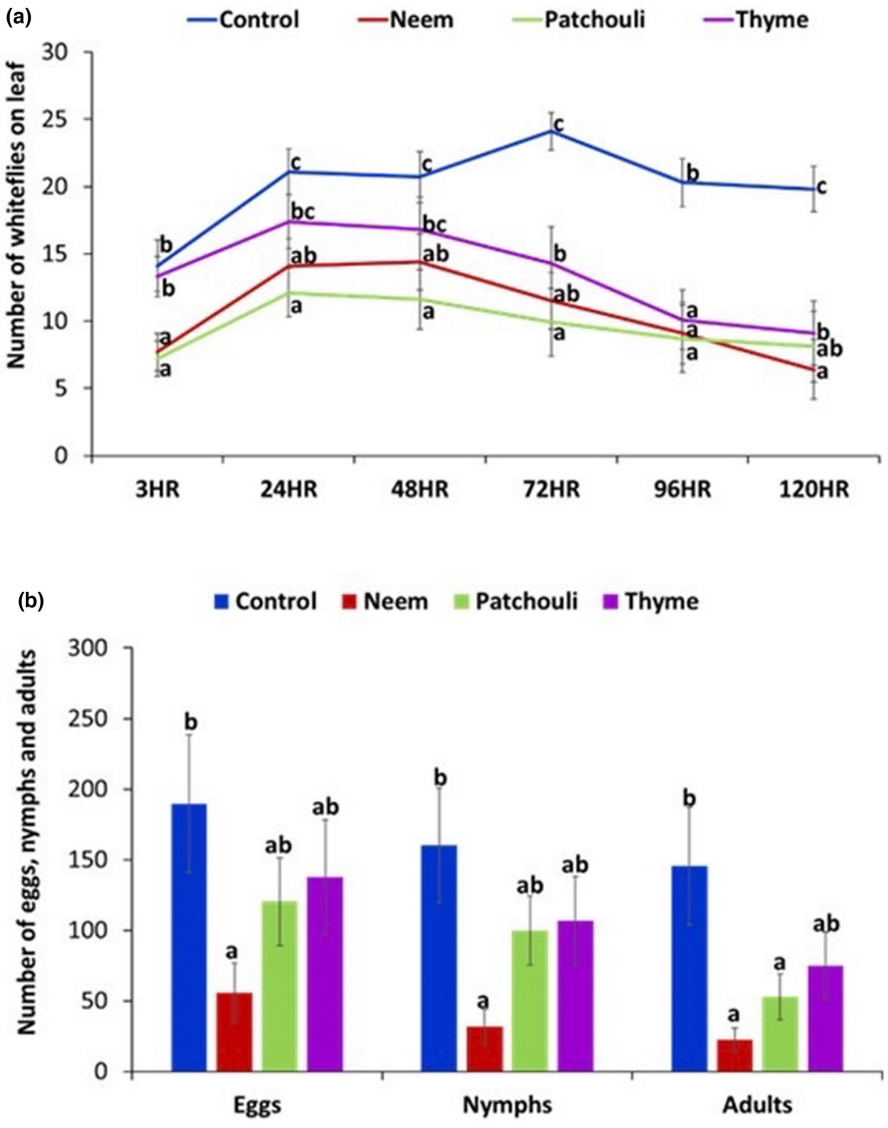

FIGURE 4 Effect of three essential oils on cassava *Bemisia tabaci* adults settling on plants (a) and eggs laid, nymph and adult emergence (b). Means with the same letter for treatments (a, b) and within each whitefly stage (b) are not significantly different ($p = 0.05$, Tukey–Kramer test). [Colour figure can be viewed at wileyonlinelibrary.com]

reduced by 38–46%, and egg reduction of 59–75% within 24 h. Patchouli was reported to have a pronounced repellent effect, while thyme was more effective with contact toxicity (Yang et al., 2010). The repellent effect of patchouli was also observed in our study with fewer settling whiteflies and eggs laid. Patchouli was the most effective treatment in the choice assay and reduced whitefly settlement by an average of 51%, and eggs were 89% fewer compared to controls.

Neem oil had a weaker settling deterrent effect compared to patchouli and thyme, but treatment with it resulted in 54–60% fewer eggs compared to the other oils which is an indication of its effectiveness in preventing oviposition. Neem oil has recently been reported to have no repellent effect on the whitefly haplogroup SSA-ESA used in this study (Mrisho et al., 2021). The significant reduction in eggs despite no difference in numbers of whiteflies settling on neem treated and control leaves could be attributed to the active ingredient azadirachtin having an antibiotic effect deterring oviposition by inhibiting oogenesis and synthesis of ovarian ecdysteroid (Chaudhary et al., 2017) or to the lipidic nature of the essential oil that may interfere with the action of the glue-like substance that attaches egg pedicels on the leaf surface (Byrne & Bellows Jr, 1991; Pereira et al., 2018). Azadirachtin is not volatile and therefore would not be expected to be repellent. The significant reduction in hatched eggs and nymphs that emerged as adults on neem-treated plants compared to the control could be attributed to disruption of moulting and reduced growth and development. Azadirachtin is known to inhibit feeding, disrupt moulting, reduce growth and development and cause high mortality in immature stages (Campos et al., 2016; Carvalho et al., 2012; Kumar & Poehling, 2007). Fewer adult female adults of the jasmine whitefly (*Aleuroclava jasmini* Takahashi) were reported to alight on neem oil-treated paper mulberry (*Brousson etiapapyrifera* L.) in comparison to the control (Khederi et al., 2019). In addition, female adult jasmine whiteflies exposed to neem oil had their oviposition levels significantly reduced by up to 80% compared to the control (Khederi et al., 2019). Neem extracts and neem-based biopesticides have been reported to cause significant mortality in *B. tabaci* and have been widely utilized to manage this pest (Kumar et al., 2019; Lynn et al., 2010; Pinheiro et al., 2009; Younas et al., 2021). Neem is a broad-spectrum contact biopesticide that has

TABLE 4 Mean total duration, mean individual and mean number of selected waveform events (Means ± SE).*

Treatment	G	E1	E2	C	np	pd
Mean total duration of waveform events in minutes						
Neem-treated	84.7 ± 22.8a	3.2 ± 0.8a	162.9 ± 24.1a	231.0 ± 24.0a	259.1 ± 35.1b	3.5 ± 0.8a
Neem-control	65.3 ± 18.2a	2.6 ± 1.0a	224.6 ± 29.2a	285.8 ± 20.5a	135.7 ± 19.5a	7.0 ± 1.0b
p value	0.6714	0.4454	0.1583	0.0563	0.0045	0.0007
Patchouli-treated	179.8 ± 28.1a	3.5 ± 1.5a	119.8 ± 22.2a	235.7 ± 22.5a	169.9 ± 29.5a	2.4 ± 0.6a
Patchouli-control	106.5 ± 18.3a	2.8 ± 1.7a	201.5 ± 26.0b	270.3 ± 17.4a	134.5 ± 11.6a	4.5 ± 0.6b
p value	0.5251	0.1446	0.0375	0.1811	0.322	0.0384
Thyme-treated	103.8 ± 24.3a	8.3 ± 2.0a	260.1 ± 46.3a	258.2 ± 36.1a	120.1 ± 24.7a	3.6 ± 0.8a
Thyme-control	49.6 ± 11.2a	7.9 ± 4.3a	311.8 ± 53.8a	278.5 ± 38.2a	77.7 ± 12.6a	6.4 ± 1.1a
p value	0.3535	0.2357	0.7181	0.9403	0.3509	0.0739
Mean duration of individual waveform events in minutes (pd in seconds)						
Neem-treated	40.5 ± 9.1a	1.5 ± 0.4a	87.1 ± 18.8a	14.5 ± 3.4a	16.5 ± 3.2b	7.5 ± 0.7a
Neem-control	26.1 ± 4.5a	0.8 ± 0.3a	86.6 ± 13.5a	10.8 ± 0.8a	6.2 ± 0.8a	6.8 ± 0.5b
p value	0.3006	0.0707	0.7214	0.8923	0.0037	0.4189
Patchouli-treated	72.2 ± 16.2a	9.6 ± 8.9a	76.8 ± 12.2a	22.5 ± 4.8a	16.3 ± 3.6a	8.1 ± 1.3a
Patchouli-control	36.5 1 ± 3.8a	0.7 ± 0.1a	101.1 ± 18.8a	14.5 ± 2.4a	8.2 ± 1.1a	6.0 ± 0.2a
p value	0.0760	0.7884	0.3233	0.2207	0.4168	0.4454
Thyme-treated	49.1 ± 11.4a	1.1 ± 0.2a	161.0 ± 48.6a	10.4 ± 2.2a	5.0 ± 0.8a	5.4 ± 0.5a
Thyme-control	33.6 ± 12.3a	1.5 ± 0.53a	184.1 ± 61.6a	8.7 ± 1.1a	2.6 ± 0.2a	5.6 ± 0.3a
p value	0.2919	0.7749	0.8212	0.8111	0.1151	0.4474

Abbreviations: G, xylem ingestion; E1, Phloem salivation; E2, phloem ingestion; pd, potential drops; np, non-probing.

*Means with the same letter within columns for treatment versus control for each essential oil are not significantly different ($p = 0.05$, Dunnett's test). C-Stylet pathway and sheath salivation.

been used to control pests in various crops. Neem is one of the least toxic biopesticides to humans and is less harmful to non-target organisms compared to other botanical biopesticides. Neem is also compatible with other biological control agents such as entomopathogens (Campos et al., 2016). Pests are also less likely to evolve resistance to neem-based biopesticides which can contain more than 200 allelochemicals (Chaudhary et al., 2017; Forim et al., 2013).

A study has shown that *B. tabaci* MEAM1 is more susceptible than *B. tabaci* MED to thyme, cinnamon bark and clove bud oils (Kim et al., 2011). This trend is comparable to what has been observed for insecticides where *B. tabaci* cryptic species are reported to vary in susceptibility, with some such as MED having a very high potential to develop resistance to numerous insecticides (Horowitz et al., 2020). However, other essential oils such as vetiver, catnip, summer savoury, lemon balm, lemongrass, basil and black sesame were reported to be effective across *B. tabaci* cryptic species tested (Chae et al., 2014; Drabo et al., 2017; Kim et al., 2011).

Botanical biopesticides should preferably be developed from plant extracts containing a mixture of active compounds and not based on individual dominant components to minimize the risk of pest resistance development. A study carried out on aphids to test the efficacy of azadirachtin or refined neem seed extract showed that after 40 generations, the population exposed to the pure component developed resistance, while that on whole neem extract did not (Feng & Isman, 1995). The different components can have a synergistic action (Tak & Isman, 2017).

Botanical biopesticides suffer from low persistence and effectiveness under field conditions due to rapid degradation and volatility when exposed to sunlight. Nanoformulation technology offers an opportunity to enhance slow release characteristics, improve the stability of active ingredients, use reduced doses and limit degradation loss of these products under field conditions (Campos et al., 2016; Chaudhary et al., 2017; de Oliveira et al., 2014). It can also reduce plant toxicity and harm to non-target organisms (Campolo et al., 2017; Campos et al., 2019). In this study, the results for nanoformulated and ethanol dissolved patchouli were comparable despite the fact that these were tested in separate experiments. This could be attributed to the short duration of data collection and lack of exposure to weather elements or to differences in the percentage of oil in the two formulations. Several studies have tested the efficacy of nanoformulated essential oils compared to natural oils against *B. tabaci* and other pests. There are instances where natural oils performed better compared to nanoformulated oils (Carvalho et al., 2012; Pascoli et al., 2020) and where there were no significant differences in performance (Christofoli et al., 2015; Pereira et al., 2018; Peres et al., 2020) or inconsistences with the natural oil outperforming the nanoformulated and vice versa (Campos et al., 2016). The lack of consistent

differences between nanoformulated and natural products could be attributed to testing under laboratory conditions that do not cause rapid oil degradation due to high temperatures, wind and UV radiation. The benefits of nanoformulation may therefore be better assessed by testing under natural field conditions where high temperatures and UV radiation degrade and lower the efficacy of natural oils.

Results from the experiments to examine the probing behaviour of cassava whitefly on plants sprayed with patchouli and neem suggest that these oils deter/repel this pest. The longer time taken to first probe, shorter phloem ingestion (E2), shorter potential drop (pd) duration in patchouli and neem, longer non-probing duration in neem and fewer E2 events in patchouli indicate that these essential oils interfered with whitefly probing through deterrence. Other studies testing host plants and the effect of insecticides show that reduced E2 durations, increased np duration and delay in initiating the first probe signal unsuitability of the plants for whitefly feeding (Civolani et al., 2014; Garzo et al., 2020; Maluta et al., 2020; Milenovic et al., 2019). Neem and patchouli treatments also gave rise to reduced pd durations in comparison to control plants. Potential drops are associated with the ability to transmit viruses so the shorter they are the less likely whiteflies are able to acquire and inoculate viruses (Garzo et al., 2020; Maluta et al., 2020). These findings show that these essential oils are likely to reduce virus transmission.

5 | CONCLUSION

This study demonstrates that three botanical oils (patchouli, thyme and neem) could be effectively developed and incorporated into IPM packages for the management of cassava whiteflies. Neem could be particularly suitable since neem plants are commonly found growing in sub-Saharan Africa, derived products can easily be produced on a large scale and the major active compound azadirachtin is abundant hence allowing for low production costs compared to patchouli and thyme (Forim et al., 2013). Synthetic pesticides can harm human health, non-target organisms and the environment, and induce resistance in target pests, meaning that it is unsustainable to manage pests solely through the application of synthetic insecticides (Carvalho, 2017; Struelens & Silvie, 2020). Botanical essential oils are among the control strategies considered for insect pest management as an alternative to synthetic pesticides (Chaudhary et al., 2017), and they are already being effectively delivered as commercial components of IPM programmes in some parts of the world (Anonymous, 2023). The potential of nanoformulation technology in improving efficacy of botanical oils against cassava whitefly should be tested under field conditions. The increasing commercialisation of cassava production systems in the developing world means that there are important new opportunities for the development of botanical essential oils as key components of IPM systems for the management of cassava whiteflies and whitefly-transmitted viruses.

AUTHOR CONTRIBUTIONS

Massoud Amour was involved in conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; software; writing—original draft; writing—review and editing. Everlyne N. Wosula was involved in conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; software; writing—original draft; writing—review and editing; supervision. Latifa Mrisho was involved in conceptualization; validation; visualization; writing—review and editing; funding acquisition. Clare Hoskins and David Buss were involved in conceptualization; methodology; validation; visualization; writing—review and editing. Toby Bruce was involved in conceptualization; validation; visualization; writing—review and editing; funding acquisition. Flora Stephano was involved in conceptualization; validation; visualization; writing—review and editing; supervision. James P. Legg was involved in conceptualization; investigation; validation; visualization; writing—review and editing, supervision, funding acquisition, resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at the IITADataBank https://doi.org/10.25502/bxr6-c172/d.

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