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Original Article

Insecticidal activity, Chemical Constituents of *Trachyspermum ammi*, *Withania coagulans* and *Murraya koenigii* ethanloic extracts against *Bemisia tabaci*

Atividade inseticida, constituintes químicos de extratos etanloicos de *Trachyspermum ammi*, *Withania coagulans* e *Murraya koenigii* contra *Bemisia tabaci*

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Abstract

Whitefly is one of the most widespread agricultural pests in the world. Essential oils might be used to control this insect in an environmentally responsible way. The fumigant, repellent, and anti-oviposition activity of ethanol-extracted essential oils of *Trachyspermum ammi*, *Withania coagulans*, and *Murraya koenigii* against *Bemisia tabaci* was investigated in this study. In the experiment, three essential oil concentrations (2.5 mg/mL, 5 mg/mL, and 10 mg/mL) were used. *Trachyspermum ammi* had the highest percentage of whitefly death in laboratory experiments due to its fumigant toxicity; the same tendency was found in contact toxicity and repellent effect. Mortality percent rises as the concentration of essential oil increases with bioassay time. As the concentration of essential oil grows with bioassay time, so does the mortality rate. The most adult whiteflies escaped from the treated plants' greenhouse due to the highest essential oil concentration. The greatest repellency was found with *Trachyspermum ammi* essential oil at 10 mg/mL. The essential oil had the greatest anti-oviposition efficacy against whiteflies. *Trachyspermum ammi* possessed the most potent anti-oviposition deterrent, followed by *Withania coagulans* in second place. *Murraya koenigii* finished third with moderate anti-oviposition, which affects the number of eggs produced in comparison to the control. As a consequence, these three oils might be used as an effective and environmentally acceptable bio-insecticide to control *B. tabaci*.

Keywords: whitefly, essential oils, fumigants, repellence, anti-oviposition, GC-MS.

Resumo

A mosca-branca é uma das pragas agrícolas mais difundidas no mundo. Os óleos essenciais podem ser usados para controlar esse inseto de forma ambientalmente responsável. A atividade fumigante, repelente e antioviposição de óleos essenciais extraídos com etanol de *Trachyspermum ammi*, *Withania coagulans* e *Murraya koenigii* contra *Bemisia tabaci* foi investigada neste estudo. No experimento, foram utilizadas três concentrações de óleo essencial (2,5 mg/mL, 5 mg/mL e 10 mg/mL). *Trachyspermum ammi* teve a maior porcentagem de morte de mosca-branca em experimentos de laboratório devido à sua toxicidade fumigante; a mesma tendência foi encontrada na toxicidade de contato e efeito repelente. A porcentagem de mortalidade aumenta à medida que a concentração de óleo essencial aumenta com o tempo do bioensaio. À medida que a concentração de óleo essencial cresce com o tempo de bioensaio, o mesmo acontece com a taxa de mortalidade. A maioria das moscas-brancas adultas escapou da estufa das plantas tratadas devido à maior concentração de óleo essencial. A maior repelência foi encontrada com óleo essencial de *Trachyspermum ammi* a 10 mg/mL. O óleo essencial apresentou a maior eficácia antioviposição contra moscas-brancas. *Trachyspermum ammi* teve o mais potente impedimento antioviposição, seguido por *Withania coagulans* em segundo lugar. *Murraya koenigii* terminou em terceiro com antioviposição moderada, o que afeta o número de ovos produzidos em relação ao controle. Como consequência, esses três óleos podem ser usados como um bioinseticida eficaz e ambientalmente aceitável para controlar *B. tabaci*.

Palavras-chave: mosca-branca, óleos essenciais, fumigantes, repelência, antioviposição, GC-MS.

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1. Introduction

The whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is one of the most destructive pests on agricultural and horticultural plants in the field and in greenhouses (Lapidot and Polston, 2006; Bográn and Heinz, 2016). Research has determined that there are more than 1,200 species of Aleyrodidae, which are distributed in many countries around the world, and only a very small number of whiteflies cause cost-effective damage to crop (Aslan et al., 2004; Carlos et al., 2016). It is generally known for sucking plant cell sap and generates honeydew, which promotes black mould and spreads a range of plant viruses. Once the number of pests increases, the management of the pests becomes challenging. Synthetic pesticides have been used successfully to regulate several insect species since the 1970 (Aslan et al., 2004). However, excessive use of these chemical poisons has resulted in a slew of environmental issues, including the negative impact on non-target species in the natural agricultural environment, disruption of the ecological balance between pests and predators, pesticide resistance in target species, health risks, and air pollution (Shah et al., 2008). Scientists want to create new methods for successfully controlling pests by using knowledge about the adverse consequences of pesticide use. Because of their repellence, contact and fumigation toxicity, growth regulation, and pathogenicity, numerous aromatic plant essential oils have been introduced for safe and effective management (Buchbauer, 2000). Minimizing the use of synthetic pesticides in favour of developing novel environmentally friendly alternatives, such as utilizing botanical plants and their essential oils to manage insect pest population.

The essential oils EOs also responsible for attracting insects that facilitate pollination, and for protecting plants from harmful microorganisms and pests (Bakkali et al., 2008; Pichersky and Gershenzon, 2002). EOs have traditionally been utilized in stored goods as insect repellents (Koul et al., 2008). Methanol and hexane crude extracts of *Mormodica charantia* from flowers and fruits indicated larvicidal activity against *Aedes aegypti* (Mituiassu et al., 2021). Enzymatic esterification of citronella essential oil (*Cymbopogon winterianus*) with cinnamic acid. Furthermore, the essential oil toxicity against *Artemia salina* before and after esterification, as well as the larvicidal effect against *Aedes aegypti,* were studied (Cansian et al., 2021). Copaiba oil and Neem extract can be used to manage *Sitophilus zeamais* on stored goods since changes in this pest's behaviour can lessen both qualitative and quantitative grain damage (Martins et al., 2024). *Trachyspermum ammi*, also known as Ajacein or Ajwain of the Umbelliferae family and of Egyptian provenance, has been widely utilized as a medication in Ayurveda. It is grown in Afghanistan, Iraq, Iran, Pakistan, and India. The seeds contain 2.0-4.4% brown oil. Thymol (35%-60%) has been reported in many phytochemicals in *T. ammi*. The non-thymol fraction called thyme contains β-pineene (4%-5%), limonene and γ-pineene. P-Propisochlor (50%-55%)) and β-pineene (30%-35%) (Chopra, 1982; Ishikawa et al., 2001; Pruthi 2014). The most important therapeutic effects reported include antioxidants, anti-fever, anti-microbial, anti-spasm, anti-flatulence, sweating, expectorant, anti-fungal, gastric tonic, and diuretic (Salehi-Sourmaghi, 1998). *T. ammi* shoots and leaves of Blue Mint Bush (*Ziziphora clinopodioides*) were shown to be effective against *Anopheles stephensi* larvae. (Torabi-Pour et al., 2017). Adult *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, and *Tribolium confusum* were fumigated by ajwain oil at various concentrations and exposure durations. (Habashi et al., 2011). An important member of Solanaceae, *W. coagulans* weed is a medicinal plant in dry land with strong antibacterial properties. The plant has strong antimicrobial properties. Recently, the antimicrobial effects of leaves, stems and fleshy buds of *W. coagulans* and *W. munifera* have been evaluated, and they have an inhibitory effect on bacterial fusarium wilt, and found that they are effective against bacterial fusarium wilt in tomatoes (Najeeb et al., 2019; Khan et al., 2019). Because of its varied therapeutic potential, withanolides found in *W. coagulans* have piqued the scientific community's attention (Maher et al., 2020). Curry leaf, also known as karipatta (*Murraya koenigii*) in Indian, is a member of the Rosaceae family, which contains over 150 genera and 1600 species (Satyavati et al., 1987). The essential oil in the leaves can produce di-alpha celeryne, Dsabinene, Dpineene, dipentene, D-terpineol and caryophyllene. According to reports, it has antioxidant, antifungal, larvicidal, anticancer, hypoglycemic, anti-lipid peroxidation, hypolipidemic and antihypertensive activities. It is reported to also contain 58-dimethylfuranocoumarin, 6'-dimethyl, 5-hexene, carbazole and sitosterol (Gopalan et al., 1984; Iyer and Uma, 2008; Sumit et al., 2009). The insecticidal properties of several botanicals were examined in this study to identify safe options for whitefly control. Botanical oils were extracted using ethanol, and the chemical composition of *T. ammi*, *W. coagulans*, and *M. koenigii* was determined using GC-MS. The oils were then evaluated for insecticidal activity in both the laboratory and the greenhouse.

2. Material and Methods

2.1. Collection of plant material

The essential oil produced from the seeds of *T. ammi* has insecticidal activity, *W. coagulans* weed is a medicinal plant on dry land with high antiseptic qualities, and *M. koenigii* has antioxidant, antibacterial, antifungal and larvicidal activities. The seeds of *T. ammi* and *W. coagulan* purchased from a traditional medicine shop in Hyderabad Pakistan and leaves of *M. koenigii* were collected from garden (25°25′34″N, 68°32′31″E) of Sindh Agriculture University Tandojam Pakistan. This area is subtropical with an average Temperature is 33.61Cº average. Tandojam typically receives gets approximately 16.51 millimetres (0.65 inches) of rain each year and has 20.65mm wet days. The collected samples were verified at the Department of Plant Breeding and Genetics, Sindh Agriculture University Tandojam, Pakistan.

2.2. Process of ethanol extraction

Three botanicals used were *T. ammi* seed, seed *Withania coagulan* seed and *Murraya koenigii* leaves. Extraction method described by (Su et al., 2009; Wagan et al., 2017) with some modifications was used. The seeds and leaves were cleaned then dried in oven for 48 hours at 45 °C, after that dried material grinded and sieved with 40-mm mesh. The powder of each material was kept separately in containers. The powder was extracted with 95% ethanol (1g powder with 5 mL ethanol), and the mixture was stored in bottles at room temperature (18-25 °C) for 7 days. The bottles were shaken twice a day for mixing and dissolution of the powder in the ethanol. The solution was then filtered through filter paper, and the residuals were dissolved with the same process solution at a ratio 2.5 mL/gram. In a rotary evaporator, the first and second solvents were combined and dried until all of the liquid evaporated. The oil was placed in a brown collecting container and kept at 4 °C. The crude oil (0.01g extract of *T. ammi, W. coagulan,* and *M. koenigii*) was added in 0.3 mL of dimethyl sulfoxide (DMSO); 1% Tween-20 was added to the solution, and the final volume was adjusted to 5 mL at a final concentration of 10 mg/mL (10,000 ppm) by adding double distilled water, the same solution without added oil with 0.3 mL of dimethyl sulfoxide; 1% Tween-20, distilled water to achieve 5 mL volume used as control treatment.

2.3. Laboratory experiment

The laboratory experiment was conducted to confirm mortality and efficacy of different essential oils. Three types of test methods fumigation, contact and repellency were also examined in laboratory. The CRD experiment performed by three essential oils were used against the whitefly, each oil has three concentrations (2.5 mg/mL, 5 mg/mL and 10 mg/mL) with six replications. The studies were carried out in the summer of 2019 at the Lang fang research station in Hebei, Biological control Laboratory of the Institute of Plant Protection Chinese Academy of Agricultural Sciences.

2.3.1. Test 1 Bioassay for fumigant toxicity

To determine fumigant toxicity against the whitefly, the 0.1 mL solution of 2.5 mg/mL, 5 mg/mL, and 10 mg/mL from each essential oil, were applied with the help of micropipette on 6-cm-diameter filter paper discs after soaked separately, dried filter paper were attached on the 50 mL jar caps underside. As a control treatment, the same solution without oil was mixed with 0.3 mL of dimethyl sulfoxide, 1% Tween-20, and distilled water. Twenty adult whiteflies were aspirated into 100 mL glass jars, which were sealed with polythene strips and topped with caps. The jars were incubated in a laboratory setting at 25 2 °C, 50% RH, with a photoperiod of 10 L:14 D. The jars were checked at 2, 6, 12, and 24 hours after the commencement of the bioassay, and the mortality and survival rate was recorded; no contact of insects with the filter paper was seen during the experiment. Each treatment was replicated 6 time.

2.3.2. Test 2 Bioassay for contact toxicity

To determine contact toxicity, the leaves containing almost an equal number of whitefly nymphs were taken from tomato plants along with their petioles and used for this test. The leaves were placed in the cage, while the stem was submerged in water in a jar held inside the cage. Each was treated separately with 0.1 mL of 2.5 mg/mL, 5 mg/mL, and 10 mg/mL solutions. The solution was applied on each leaf through a cotton wick, similarly same volume of control solution (DMSO) mixed with distilled water applied to the control leaf. Nymphal mortality and survival population was observed at 6, 12, 24, and 48 hours after application. Each treatment was replicated 6 time.

2.3.3. Test 3 Bioassay for Repellency effect

Tomato seeds were planted in plastic pots (15 cm diameter) filled with organic materials with an equal ratio of clay loamy soil and organic materials for experimentation. The four potted young tomato plants with 15 to 20 leaves were kept in a cage (thin cloth-made size 3f height, 3ft width, and 3ft length). Tomato plants were sprayed with three essential oils and one control treatment, with dosages of 2.5 mg/mL, 5 mg/mL, and 10 mg/mL of all test solutions with hand sprayer after 30 minutes of application of essential oil, 5-day old adult whiteflies (50 number) were aspirated in all cages. Each treatment was replicated 6 times. The insect's repellent effect and survival was observed at 24 h, 48 h, and 72 h after release.

2.4. Greenhouse experiment

The greenhouse experiment was conducted to evaluate the efficiency of different essential oils against *B. tabaci* on tomato grown in net houses. Tomato seedlings were grown in germination trays before being transplanted in the last week of September 2020. The tomatoes were cultivated on a randomized complete block design with the treatments and one control with three dosages and three replications. Each sub-plot size measured 8 meter, with a row to row and plant to plant spacing of 60 cm × 30 cm. Following established cultural and maintenance techniques, the seedlings were planted in 30 cm raised beds. Thirty days after transplanting, a large number of whitefly adults were released into the net green house. Under 60% relative humidity (RH) and 20 to 30 °C temperature conditions, with no pesticides applied to the plants. A battery-operated knapsack sprayer was employed for foliar spraying of the various plant extracts, with each plot separated by 1 m to prevent chemical interference. The insect repellency and anti-oviposition reactivity was evaluated at 24, 48, and 72 hours post release. Whitefly population was counted on 10 plant randomly selected from each plot. For counting oviposition, 5 leaves were randomly taken from each plant and inspected under a microscope.

2.5. Identification of the chemical components of the essential oil

The essential oils sample of *T. ammi*, *W. coagulans* and *M. koenigi* was subjected to GC-MS analysis. GC-MS analysis was carried out using a 50-GC/320-MS (Varian, Inc., Walnut Creek, California) under the following conditions: compounds were detected using a flame ionization detector and an HP-5MS capillary column (film thickness: 30 m length 0.25 mm inner diameter). The injector oven temperature was initially kept at 60 °C for 2.5 min., then ramped at 10 °C/min to 180 °C and held for 1 minute, then ramped at 20 °C/min to 280 °C and held for 15 minutes for gas chromatography. A split ratio of 1:10 was used to inject one microliter of the diluted in 1% hexane samples. The column pressure was held constant at 100 kPa, and the sample carrier was helium gas, which was passed at a rate of 1.0 mL/min. using the online database, the chemical components of the gas chromatogram were identified.

2.6 Data analysis

Evaluation of the tested materials and techniques was based on the mortality, reduction and oviposition percentage and corrected by Abbot's formula (Abbott, 1925; Equation 1).

$$Corrected\ \%mortality = \frac{\left(1 - n\,in\,T\ after\ treatment\right)}{n\,in\,Co\ after\ treatment} \times 100 \quad \text{(Eq.1)}$$

Where n = insect population, T = treated, and Co = control.

The percentage of insect repellency was determined using the formula according to (Liu et al., 2013). The data on mortality, repellency, and oviposition were submitted to analysis of variance (ANOVA) with Minitab 16.1 software. The means of treated whiteflies were compared using Tukey's LSD test at 5% level of significance to examine the effect of treatments on mortality, survival and oviposition.

3. Results

3.1. Fumigant toxicity of essential oils to Bamisia tabaci

Results showed that application of different concentrations of essential oils extracted from *T. ammi*, *W. coagulan* and *M. koenigii* showed fumigant toxicity (Figure 1) against whitefly adults. Observed significant differences at 2h (F=11.86; $d\,f$=4; P< 0.001), 6h (F= 26.05; df= 4; P = 0.0041), 12h (F=27.33; df= 4; P= 0.0008) and 24h (F=4.08; df=4; P= 0.0013). Significantly caused the mortality in white fly when checked through fumigation method. Generally percent mortality increases with rise in concentration of respective essential oil along with increase in bioassay time. The highest percent mortality of whitefly was observed when *T. ammi* essential oil was applied at the concentration of 10 mg/mL after 24 hours of bioassay. The Different concentrations of *T. ammi* showed (Figure 1) the highest mortality against whitefly. The concentrations 2.5 mg/mL, 5 mg/mL and 10 mg/mL at 2 h brought 30%, 37.6% and 51.7% mortality, which found increasing with time up to 24 h. At 24 h the same concentrations brought 69.87%, 79.65% and 93.94% mortality, respectively. The essential oil of *Withania coagulan* at 2 h brought 29%, 32.4% and 22.5% mortality that increased with time interval and reached to its peak at 24 h with 52.56%, 61.7% and 60.5% mortality, respectively. The minimum mortality was brought by *M. koenigii*, its all three concentrations killed 11.3%, 17.8% and 22.2% adult whiteflies at 2 h. The mortality increased and maximum 32.7%, 51.6% and 54.4% was recorded at 24 h. The survival mean population of *B. tabaci* during the entire fumigation period was lower than the control (Table 1),

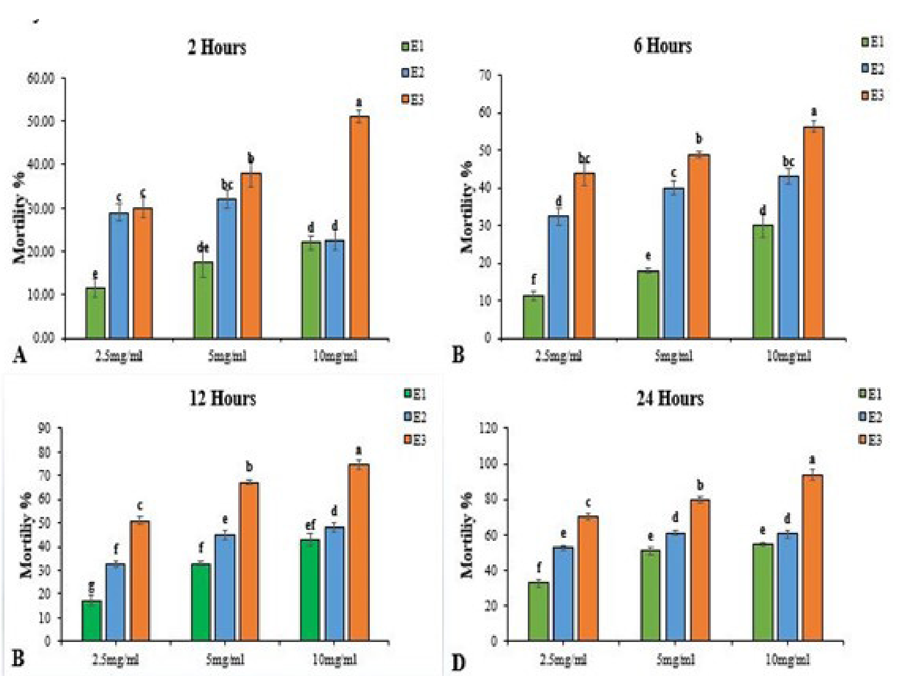

Figure 1. The effect of different ethanol plant extracts oils (E1: *Murraya koenigii* E2: *Withania coagulan* E3: *Trachyspermum ammi*) and their concentrations (2.5 mg/mL, 5 mg/mL and 10 mg/mL) applied through fumigation toxicity method on whitefly at different hours. (A) fumigation toxicity at 2 h; (B) fumigation toxicity at 6 h; (C) fumigation toxicity at 12 h; (D) fumigation toxicity at 24 h of exposure.

M. koenigii (7.5 ± 1.1), *W. coagulan* (6.5 ± 0.6) and *T. ammi* (01 ± 0.6) at different dosage.

3.2. Contact Toxicity of essential oils to Bamisia tabaci

The essential oils of *T. ammi*, *W. coagulan* and *M. koenigii* at different concentrations also demonstrated their contact toxicity (Figure 2) against nymphal stage of the whitefly. The toxicity increased within passage of time highest mortality at 48 h of the bioassay test. *T. ammi* brought 84.57% mortality at (10 mg/mL), as compared to *W. coagulan* (66.05% at 10 mg/mL) and 53.70% (10 mg/mL) in *M. koenigii* was observed (Figure 2). The significant differences in contact

Table 1. In-vitro survival rate of *Bamisia tabaci* application different concentration of ethanol based essential oils at different hours through fumigation toxicity test in laboratory conditions.

Essential oils	Concentrations (mg/mL)	2h	6h	12h	24h
Murraya koenigii	2.5	17.7 ± 0.6 b	16.4 ± 0.2 b	14.3 ± 0.6 b	10.5 ± 0.6 c
	5	16.5 ± 0.4 c	15.6 ± 0.4 b	12.5 ± 0.5 bc	8.4 ± 1.1 d
	10	15.6 ± 0.6 c	13.3 ± 0.4 c	10.2 ± 0.4 de	7.5 ± 1.1 de
Withania coagulans	2.5	14.2 ± 0.4 d	12.5 ± 0.6cd	11.7 ± 0.4 cd	7.4 ± 0.3 de
	5	13.6 ± 0.2 de	11.4 ±0.2de	10.2 ± 0.3 de	6.7 ± 0.0 e
	10	15.5 ± 0.9 c	10.8 ± 1.2de	9.2 ± 0.1 e	6.5 ± 0.6 e
Trachyspermum ammi	2.5	14 ±1.2 de	10.4 ± 0.6 ef	8.5 ± 1.2 e	4.7 ± 0.4 f
	5	12.4± 0.2 e	9.7 ± 0.4 ef	6.1 ± 0.6 f	3.5 ± 0.4 f
	10	9.8 ± 0.4 f	8.3 ± 1.2 f	4.5 ± 0.9 f	01 ± 0.6 g
Control	2.5	20 ±0.0 a	18.5 ± 0.0 a	17.3 ± 0.3 a	15.6 ±0.7 b
	5	20 ± 0.3 a	19 ± 0.6 a	18.6 ± 0.0 a	17.2 ±0.5 a
	10	20 ± 0.3 a	19 ± 0.9 a	17.8 ± 0.0 a	16.5 ±0.9 ab
Significance Level		*df*=2, *F*= 5.46, *P*=0.0119	*df*=2, *F*= 5.77, *P*=0.0096	*df*=2, *F*= 15.39, *P*=0.0001	*df*=2, *F*= 8.97, *P*=0.0014

Means followed by same letters within colum are non-significantly different from each other, (LSD; P=0.05) test.

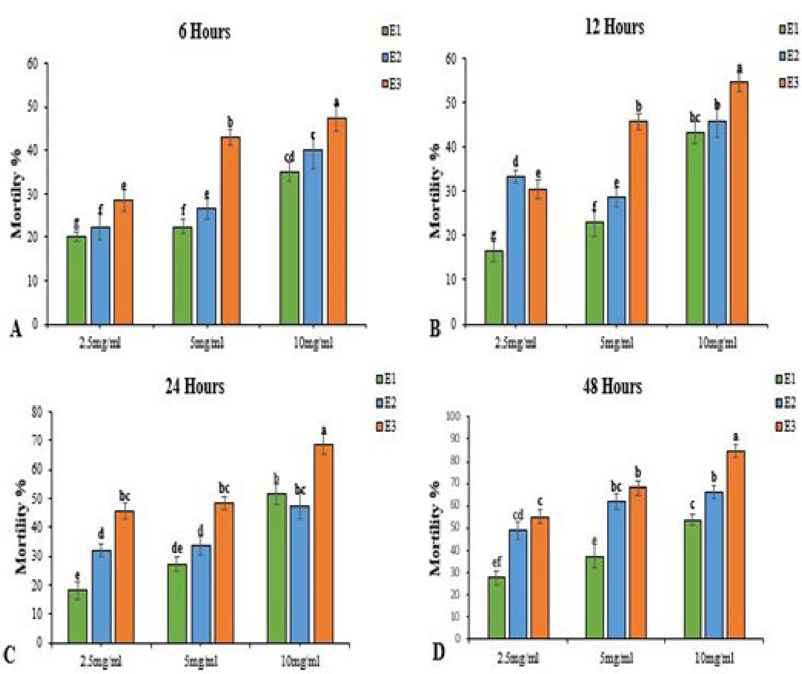

Figure 2. The effect of different ethanol plant extracts oils (E1: *Murraya koenigii* E2: *Withania coagulan* E3: *Trachyspermum ammi*) and their concentrations (2.5 mg/mL, 5 mg/mL and 10 mg/mL) applied through contact toxicity method on whitefly at different hours. (A) contact toxicity at 6 h; (B) contact toxicity at12 h; (C) contact toxicity at 24 h; (D) contact toxicity at 48 h, of exposure.

toxicity were obtained between the concentrations and three essential oils (*F*=9.90; *df*= 4; *P*= 0.0011), 12h (*F*=26.96; *df*= 4; *P*=0.0022), 24h (*F*=4.83; *df*=4; *P*= 0.0003) and 48 h (*F*=6.92; *df*=4; *P*< 0.0001). Each essential oil had adverse effects on survival rate (Table 2) of whitefly. During the contact toxicity highest survival was reported in control treatment. An inverse relation between concentration and survival rate was observed in all tasted essential oils.

3.3. Repellency of essential oils to Bamisia tabaci

The survival rate of *B. tabaci* was significantly declined at a 72-h exposure interval when essential oils were sprayed on potted plants as compared with control (Table 3). Maximum numbers of whiteflies survival from 2.5 mg/mL dosage of *M.koenigii* at 24-h (14.68 ± 0.6) and *W. coagulan* (13.8 ± 0.4). The ANOVA findings revealed significant differences in repellency (Figure 3) for all essential oils at

Table 2. In-vitro survival rate of *Bamisia tabaci* application different concentration of ethanol based essential at different hours through contact toxicity method.

Essential oils	Concentrations (mg/mL)	6h	12h	24h	48h
Murraya koenigii	2.5	16 ± 1.0 b	15 ± 1.8 b	13.5 ± 0.6 b	10.5 ± 1.2 b
	5	15.5 ± 1.4 b	13.5 ± 1.2 bc	11.5 ± 0.7 bc	9.4 ± 0.6 bc
	10	13 ± 0.8 cd	10.5 ± 1.3 de	8.5 ± 0.6 ef	7.5 ± 0.3 cd
Withania coagulans	2.5	15.5 ± 0.9 b	12 ± 0.7 cd	11.2 ± 0.7 cd	7.4 ± 0.4 cd
	5	14.7 ± 0.9 bc	12.5 ± 0.9 c	10.5 ± 0.6 de	5.7± 0.9 e
	10	12 ± 0.6 de	10 ± 0.4 ef	9.2 ± 0.7 def	5.5 ± 0.9 de
Trachyspermum ammi	2.5	14.3 ± 0.3 bc	12.5 ± 0.2 c	9 ± 1.2 ef	6.5 ± 0.6 de
	5	11.4 ± 0.7 de	9.5 ± 0.4 ef	8.2 ± 0.4 f	4.8 ± 0.4 e
	10	10.5 ± 0.6 e	8.4 ± 0.3 f	5.5 ± 1.0 g	2.5 ± 0.6 f
Control	2.5	20 ± 0.0 a	18 ± 0.7 a	16.5 ± 0.3 a	14.5 ± 1.1 a
	5	20 ± 0.0 a	17.5 ± 0.3 a	15.8 ± 0.9 a	15 ± 0.6 a
	10	20 ± 0.3 a	18.5 ± 0.3 a	17.5 ± 0.3 a	16.2 ± 0.2 a
Significance level		*df*=2, *F*= 17.78, *P*=0.0052	*df*=2, *F*= 15.72, *P*=0.0001	*df*=2, *F*= 10.36, *P*=0.0007	*df*=2, *F*= 9.04, *P*=0.0014

Means followed by same letters within colum are non-significantly different from each other, (LSD; P=0.05) test.

Table 3. In-vitro survival rate of *Bamisia tabaci* application different concentration of ethanol based essential at different hours through repellence method.

Essential oils	Concentrations (mg/mL)	24h	48h	72h
Murraya koenigii	2.5	14.6 ± 0.6 c	9.7 ± 0.5 de	13.7 ± 1.7 cd
	5	10.5 ± 0.3 d	12.7 ± 0.8 cd	9.5 ± 0.8 e
	10	10.2 ± 0.4 d	11.7 ± 1.5 d	12.2 ± 0.3 d
Withania coagulans	2.5	13.8 ± 0.4 cd	14.1 ± 0.6 c	13.3 ± 0.4 cd
	5	9.3 ± 1.2 e	10.4 ± 04 d	8.5 ± 0.2 ef
	10	5.2 ± 0.6 f	7 ± 1.1 e	7.4 ± 0.4 f
Trachyspermum ammi	2.5	13.2 ± 1.2 cd	9. ± 1.6 de	11.6 ± 0.7 d
	5	6.8 ± 0.3 ef	13.2 ± 0.9 c	9.6 ± 1.5 e
	10	3.4 ± 0.7 g	5.8± 0.4 f	7.1 ± 0.4 f
Control	2.5	18 ± 0.2 b	16.7± 0.5 b	14.2 ± 1.4 c
	5	16.5 ± 1.6 b	15.4± 0.8 bc	25.6 ± 2.6 a
	10	23.4 ± 2.4 a	20.5± 0.4 a	18.5 ± 1.9 b
Significance level		*df*=2, *F*= 60.93, *P*=0.0007	*df*=2, *F*= 27.25, *P*=0.0004	*df*=2, *F*= 8.72, *P*=0.0016

Means followed by same letters within colum are non-significantly different from each other, (LSD; P=0.05) test.

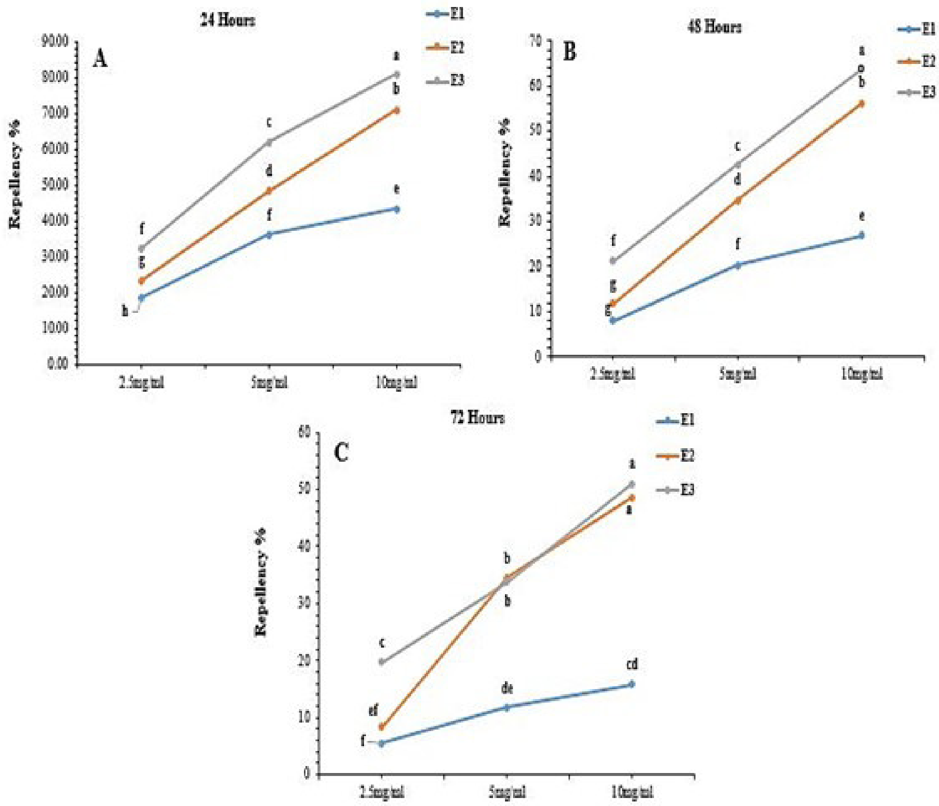

Figure 3. The effect of different ethanol plant extracts oils (E1: *Murraya koenigii* E2: *Withania coagulan* E3: *Trachyspermum ammi*) and their concentrations (2.5 mg/mL, 5 mg/mL and 10 mg/mL) applied through repellency method in cage on whitefly at different hours. (A) Repellency at 24 h; (B) repellency at 48 h; (C) repellency at 72 h, of exposure.

24 h (F =30.59; df = 4; P = 0.0031), 48 h (F = 16.92; df = 4; P = 0.0043), and 72 h (F =19.06; df = 4; P = 0.0086) of the bioassay. *T. ammi* had the highest repellency with 10 mg/mL at 24, 48, and 72 h, with mean values of 80.83%, 63.75% and 56.67%, respectively. The moderate repellency shown against whitefly with used 10 mg/mL concentration of *W. coagulan* essential oil at 24, 48, and 72 h with mean values of 61.94%, 42.50%, and 33.79%, in the same way used 5 mg/mL at 24, 48, and 72 h with mean values of 61.94%, 42.50%, and 33.79%. *M. koenigii* demonstrated the least repellency with 10 mg/mL at 24, 48, and 72 hours, with mean values of 43.33%, 26.88%, and 15.85%, percent, respectively, followed by 5 mg/mL. All the essential oil showed low repellency by 2.5 mg/mL concentration and with the passage of time repellency action was reduced.

3.4. Greenhouse experiment

3.4.1. Repellant effect of essential oils to Bamisia tabaci

In greenhouse experiment, the maximum repellency was observed at 10 mg/mL concentration of essential oil of *T. ammi* at 24h (73.93%), which lower down at 48 h (58.48%) and then 72 h (41.54%) of exposure. *W. coagulan* showed moderate repellency effect at 24 h (57.39%), 48 h (44.52%), and 72 h (26.15%), respectively. The lowest repellency effect observed (Figure 4) with the use of *M. koenigii* at 24 h (46.50%) 48 h (29.33%) and 72 h (18.46%). Following that, the 5 mg/mL and 2.5 mg/mL, the proportion of repellency dropped. There were significant differences in repellency across treatments at 24 h (F = 2.20; df = 4; P=0.0002), 48 h (F= 1.63; df = 4; P=0.0001), and 72 h (F= 1.96; df = 4; P=0.0029).

3.4.2. Oviposition deterrence effect of essential oils to Bamisia tabaci

The *T. ammi* at 10 mg/mL exhibited the highest deterrence in oviposition capacity among all treatments studied at 24 h (57.83), 48 h (40.79) and 72 h (29.20) eggs per female with the deterrence of (4.1%), (4.2%) and (2.3%), respectively as compared to control (Figure 5). *W. coagulan* ranked 2nd the eggs per female were recorded at 24 h (40.84), (22.76) and (17.40) with deterrence of (3.4%), 48 h (1.8%), and 72 h (1.8%) in the fecundity, respectively. *M. koenigii* ranked 3rd with moderate anti-oviposition effect 24 h (33.01), 48 h (14.47), and 72 h (12.98) as compared to control (Figure 5), the deterrence in the fecundity was observed as (2.5%), (1.2%) and (1.2%), respectively., There were substantial differences between the control and the treatment groups at 24 h (F =6.75; df = 4; P = 0.0003), 48 h (F = 7.82; df = 4; P < 0.0001) and

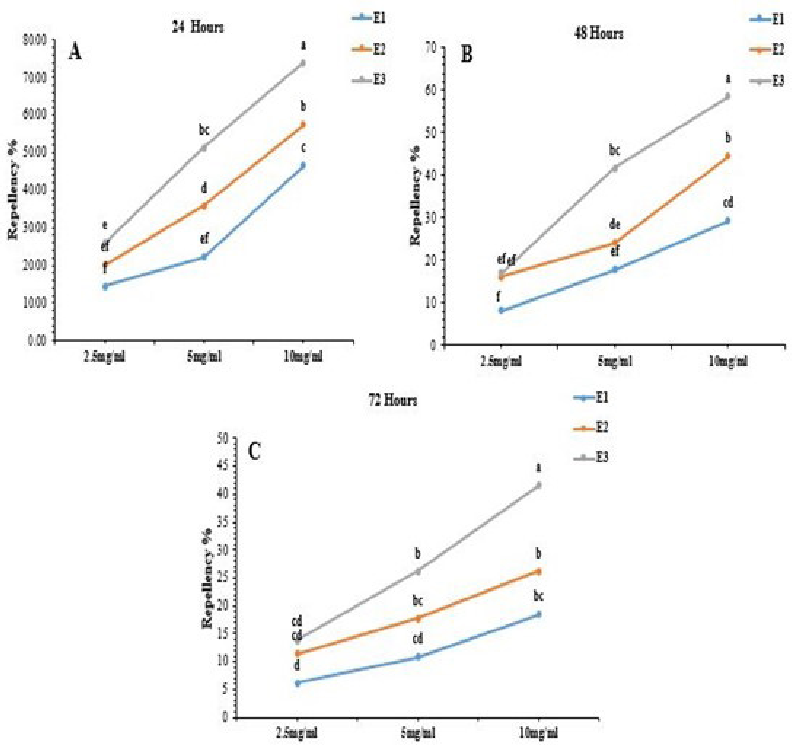

Figure 4. The effect of different ethanol plant extracts oils (E1: *Murraya koenigii* E2: *Withania coagulan* E3: *Trachyspermum ammi*) and their concentrations (2.5 mg/mL, 5 mg/mL and 10 mg/mL) applied through greenhouse repellency method on whitefly at different hours. (A) repellency at 24 h; (B) repellency at 48 h; (C) repellency at 72 h, of exposure.

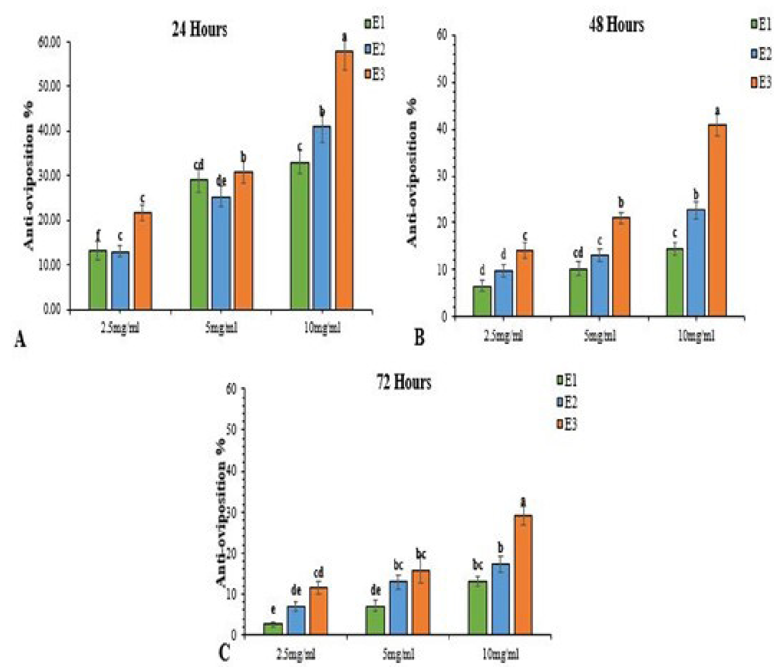

Figure 5. The effect of different ethanol plant extracts oils (E1: *Murraya koenigii* E2: *Withania coagulan* E3: *Trachyspermum ammi*) and their concentrations (2.5 mg/mL, 5 mg/mL and 10 mg/mL) applied through Anti-oviposition effect in greenhouse method on whitefly at different hours. (A) Anti-oviposition (%) at 24 h; (B) Anti-oviposition (%) at 48 h; (C) Anti-oviposition (%) at 72 h, of exposure.

72 h (F = 1.80; df = 4; P = 0.0032). The moderate effect was observed with 5 mg/mL concentration and the lowest oviposition deterrence observed by the use of 5 mg/mL concentrations of all oils. The exposure time for all tested oil was significant in terms of reducing the oviposition capacity of whitely female.

3.5. GC-MS analysis of three essential oils

The components of *M. koenigii*, *W. coagulan*, *T. ammi* oils were analyzed by GC-MS analysis to study their insecticidal toxicity against adults and nymphs of *B. tabaci*. The components and their percentages in the essential oils are illustrated in (Table 4). The constituents analyzed were: Caryophyllene (27.45%), α-Pinene (14.22%), α-Caryophyllene (7.25%) and α-Selinene (5.36%) as the major constituents of *Murraya koenigii* oil. Similarly, Cetyldimethylamine (18.56%), methyl ester (7.66%), Butyloctyl phthalate (3.45%), Glycerine 1-3 diesterate (3.23%), and Methyl hexadecadienoate (2.54%) in *Withania coagulan*, and Thymol (32.43%), γ-Terpinene (11.45%), Isobornylisobutyrate (9.87%), p-Cymene (5.65%), and Silphine (4.39%) were the major Components in *T. ammi* oil.

Table 4. Chemical composition of *Trachyspermum ammi*, *Withania coagulans* and *Murraya koenigii* by GC-MS.

Trachyspermum ammi	**%**	**RETENTION TIME**
Sabinene	0.79	10.43
b-Pinene	2.32	10.76
Myrcene	1.28	11.32
Verbenene	2.35	11.54
Menthatriene	0.57	11.78
Foeniculin	0.89	12.17
o-Cymene	3.11	12.65
p-Cymene	5.65	12.82
a-Pinene	3.86	13.34
γ-Terpinene	11.45	13.71
Isobornylisobutyrate	9.87	14.45
Umbellulone	0.52	16.83
Tertradecanal	0.74	20.14
Thymol	32.43	21.86
Davanone	1.69	23.34
Myristiain	2.25	27.46
Thymolhydroquinone	0.74	34.54
Manool	0.56	37.19
Thymyl acetate	1.93	41.56
Ionone	3.34	42.34
Silphine	4.39	45.34
Cedrane	1.35	49.56
Withania coagulans		
2,4- Ditert butyl phenol	2.35	5.343
1-Pentadecene	1.44	6.276
1-Nitro- Naphthalene	0.76	6.843
Methyl tetradecanoate	1.327	7.565
Tetradecanoic acid	2.453	8.461
Benzyl Benzoate	0.95	9.632
Phenanthrene	0.67	10.782
Neo Phytadiene	0.53	11.263
1,7 pentatriacontene	1.38	11.768
Cetyldimethylamine	18.56	12.164
2-(4-tert butyl benzyl) butanol	1.89	12.483
Palmitic acid, methyl ester	7.66	12.856
Butyl octyl phthalate	3.45	13.475
9(10H)-Anthracenone	0.57	14.131
9-Octadecenoic acid, methyl ester	2.65	14.785
Mentha camphor	2.12	15.364
Octadecanoic acid	3.45	15.898
9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	0.86	16.567

Table 4. Continued...

Trachyspermum ammi	**%**	**RETENTION TIME**
Glycerol 1,3 disterate	1.18	16.876
Methyl hexadecadienoate	2.54	17.453
Glycerine 1,3 diesterate	3.23	17.882
Cholesterol	2.27	18.345
Stigmasterol	1.42	19.637
Fucosterol	0.98	21.323
1,2-Benzenedicarboxylic acid	0.56	22.567
Murraya koenigii		
α-Phellandrene	0.70	9.954
α-Pinene	14.22	10.327
β-Pinene	4.65	11.856
o-Cymene	1.16	12.292
D-Limonene	5.04	12.684
γ-Terpinene	0.66	13.497
β-Linalool	1.32	13.959
Bornyl acetate	2.32	14.153
β-Bourbonene	1.99	14.735
Caryophyllene	27.45	16.334
Germacrene	0.87	16.946
α-Caryophyllene	7.25	17.856
β-Selinene	2.79	18.254
α-Selinene	5.36	19.246
γ-Cadinene	0.69	19.734
δ-Amorphene	1.52	20.356
Spathulenol	1.73	20.659
Caryophyllene oxide	7.74	21.392
Viridiflorol	1.23	22.738
Phytol	1.38	23.271

4. Discussion

The essential oils or botanicals extracts used for years. In the present study essential oil of three plants were used against whitefly on tomato crop. Botanicals or essential oil have long been marketed as attractive alternative to synthetic chemical insecticide for pest management. The plant bio-substance act as repellent or antifeedants and oviposition deterrent for many insect species at commercial level have ensured the variation in behavior of some insects (Isman, 2006). The botanicals are a less effective and time taking management but a well appropriate alternative to chemicals for defeating pest population (Khater, 2012). The whole-plant n-hexane extract *Chenopodium botrys* n-hexane extract showed significant larvicidal, pupicidal, adulticidal, oviposition deterrent, and adult emergence inhibiting actions against *Culex quinquefasciatus*. It demonstrated 100% adulticidal action. The maximum concentration 10 mg/mL demonstrated 71.3 ± 4.4% repellence and inhibition activity occurs throughout adult emergence (Ilahi et al., 2021). In over study the three tested essential oils extracted from *T. ammi*, *W. coagulan* and *M. koenigii* with different concentrations were tested by different methods. During present studies, the results of the laboratory experiment revealed the maximum fumigant toxicity of essential oil of *Trachyspermum ammi* that brought (95.00 ±3.0), mortality in whitefly followed by *W. coagulan* with (82.50 ± 5.2) mortality and *Murraya koenigii* revealed moderate (47.85 ± 3.2) toxicity. The similar fumigant toxicity was reported by (Wagan et al., 2017) for the ethanol-extracted essential oil of *Acorus tatarinowii*, which had 98.8% fumigant toxicity to whitefly after 8 hours in a bioassay. Another study found that essential oil of *G. jasminoides*, its four chemical components, showed the highest fumigant toxicity against whitefly adults. Their toxicity increased with increasing time up to 9 hours after the start of the bioassay, with essential oil having the highest toxicity (81.48%), (Wagan et al., 2018). The work of other researcher discovered that efficacy of five botanicals against *Myzus persicae*, including *Citrullus colocynthis*, *Nicotiana tobaccum*, and seeds of *Trachyspermum ammi*, *Azadirachta indica*, and *Withania coagulans*. Botanical oils were extracted from powder using ethanol oil extraction and extracted via boiling and their insecticidal activity was evaluated in a laboratory and a greenhouse. *T. ammi* caused the most deaths (81.7%), followed by *C. colocynthis* (76.5%), *N. tobaccum* (63.0%), *A. indica* (56.5%), and *W. cagulans* (50.0%) (Hyder et al., 2022). The toxic effect *Trachyspermum ammi* essential oils was tested against larval and adult stage of *Tribolium castaneum* by using similar method: A filter paper strip fumigated with solutions of various concentrations of essential oils produced in acetone (4, 8, 12, 16, and 20 mL in 100 mL) (Chaubey, 2007). At various doses and exposure durations, Ajwain oil was found to have fumigant action against adults of *Oryzaephilus surinamensis, Rhyzopertha dominica,* and *Tribolium confusum* (Habashi et al., 2011).

The findings of contact toxicity tests revealed that oil produced from *T. ammi* seeds applied at a concentration of 10 mg/mL had the maximum contact toxicity with (80.30±4.3) against whitefly nymphs, followed by *W. coagulan* seeds with (73.20 ± 4.6) and *M. koenigii* oil showed lowest efficacy with (42.00±3.7) at 48 hour. Similar reductions in *B. tabaci* nymphs were seen after treatment with 0.3 percent aqueous extract of neem kernels (Price and Schuster, 1991). The essential oil of *Myristica fragrans* was extracted using ethanol, maximum concentration (10 mg/mL) resulted in significant mortality (72.50 ± 4.23), whereas minimal dosage led in the lowest nymph mortality (Wagan et al., 2017). The essential oil of *Gardenia jasminoides* and its constituents were evaluated against the nymphal stage of the whitefly using a contact toxicity method. Toxicity was the highest during the first three hours of the test, then gradually decreased. (Wagan et al., 2018). *Thymus vulgaris* oil showed the strongest contact toxicity on nymph and adults of *Bemisia tabaci* (Yang et al., 2010; Barkman, 2013).

The use of essential oils offered a strategy for pest management in greenhouses. Ethanol-essential oils derived from *W. coagulan*, *T. ammi* and *M. koenigii*, protected the plants by repealing the adult whiteflies and also disrupted oviposition at 24 h and slightly low at 72 h on treated plants. (Subramaniam and Murugan, 2013) mentioned that the ethanol-extracted oil from *Myristica fragrans* seed was discovered to repel malarial vector mosquito, *Anopheles stephensi*, from the test subject for up to 210 minutes. *T. ammi* showed strong repellent activity tested in laboratory conditions found (80.83±5.6) at high concentration 10 mg/mL. sequentially *W. coagulan* essential oils indicated (70.94±5.1) at the same concentration. The lowest repellent activity observed in the oil of *Murraya koenigii.* In greenhouse experiment determined *T. ammi* ethanol-essential oil with 10 mg/mL had the strongest repellency effect at 24-h, 48-h, and 72-h with mean values of 80.83%, 63.75% and 56.67%, moderate repellency showed by *W. coagulan* and *M. koenigii* showed minimum repellency with 10 mg/ mL. The percentage of repellency slightly decreased over time. Similar results were found in essential oils of *Thymus vulgaris* with three concentrations, 0.125%, 0.25% and 0.5% was observed greater repellency against *B. tabaci biotype B* with increasing dose of essential oils. No phytotoxicity was observed on plants treated with oils (Yang et al., 2010). *Cuminum cyminum and Tymus vulgaris* essential oils repelled greenhouse whiteflies the best with 66.11% and 62.46% repellency, respectively, for the first three days of a bioassay and *Tymus vulgaris* at six days (Dehghani & Ahmadi, 2013). Thymol was found in *T. ammi* seed oil and has been documented to be extremely poisonous to *Spodoptera litura* larvae. Thymol, when tested against fourth-instar *A. stephensi* larvae at a dosage of 100 g/mL, resulted in 100.0% death, whereas *T. ammi* seed oil resulted in 63.0% larval mortality at the same dose (Pandey et al., 2009). Anti-microbial effect of different parts including leaves, stem and succulent shoots of *W. coagulans* and *Withania somnifera* were evaluated against bacterial wilt pathogen and found effective in the management of bacterial wilt pathogen in tomato (Najeeb et al., 2019; Khan et al., 2019). In over study observed repellency test in laboratory conditions showed maximum repellency up to 72 hour after the beginning of the bioassay, whereas in greenhouse conditions no longer than 48-h with concentration of 10 mg/mL and 5 mg/mL of *T. ammi* and *W. coagulan.* However, the lower concentration 2.5 mg/mL of three used oils had no effective repelling activity.

The oviposition is an important factor of insect population that can be influenced by plant extracts, resulting in reduced reproduction. The findings of this study demonstrated that essential oils produced from plants *T. ammi, W. coagulan* and *M. koenigii* had efficiently reduced oviposition rate of *B. tabaci. T. ammi* with 10 mg/mL concentration had the strongest oviposition deterrence among all treatments tested, *Withania coagulan* ranked 2nd and *Murraya koenigii* ranked 3rd with moderate anti-oviposition effect. The anti-oviposition determined the number of eggs as compared to control. Similar findings were obtained in terms of anti-oviposition activity, with the 10 mg/ml concentration of *Myristca fragrans* ethanol-extracted oil having the highest anti-oviposition activity among those tested at 24 and 48 hours of the bioassay, on *Bemisia tabaci* (Wagan et al., 2017). The females of the Whitefly deposited significantly lesser number of eggs on oil-treated tomato plants, the female avoided to oviposit on the treated host plant (Yang et al., 2010). After 24 hours of exposure, ethanol-extracted *M. fragrans* essential oil displayed an anti-oviposition action 83.40% against the brown plant hopper (Su et al., 2009). Furthermore, (Dehghani and Ahmadi, 2013) found that the essential oil from *Achillea millefolium* decreased oviposition by 67.77% in a three-day bioassay. *Gardenia jasminoides* essential oil demonstrated the highest anti-oviposition efficacy in the bioassay, at 59.5~65.84% at 48 hours (Wagan et al., 2018).

Our chemical analysis of three essential oils showed major constituents. All essential oils had already been recognized as combinations of various chemical substances, major Components discovered in *T. ammi* included thymol, γ-terpinene, isobornylisobutyrate, p-Cymene, and silphine. The impact of thymol derivatives on a variety of insects, including *Culexpipiens* (Traboulsi et al., 2002), Aedesaegypti (Waliwitiya et al., 2009), and *Sitophilus granarius* (Linnaeus) (Coleoptera), has been investigated previously (Ziaee, 2014). Thymol has also been shown to have a negative impact on *Sitophilus zeamais* walking behavior (Oliveira et al., 2018). The major components were Cetyldimethylamine, methyl ester, Butyloctylphthalate, Glycerine 1-3 diesterate and Methyl hexadecadienoate identified in *W. coagulan*. Hexadecanoic acid methyl ester is a type of fatty acid ester, it can inhibit the growth of pathogenic bacteria (Anneken et al., 2006; Suresh et al., 2014). *M. koenigii* main components were caryophyllene, α-pinene, and α-selinene. Previous research on other Premna species revealed that their chemical compositions were high in caryophyllene (Renjana & Ethoppil 2013; Sadashiva et al., 2013). In reality, the essential oil of *P. angolensis* leaves contains α -pinene, a chemical component with insecticidal effects against *Tribolium confusum, Tribolium castaneum, Sitophilus zeamais,* *Callosobruchus maculatus,* and *Rhyzopertha dominica* (Noudogbessi et al., 2009; Ketoh et al., 2006).

5. Conclusion

In conclusion, ethanol-extracted essential oils of *Trachyspermum ammi, Withania coagulans*, and *Murraya koenigii* effectively regulated the adult and nymphal phases of whiteflies and have an influence on oviposition by whiteflies. In both laboratory and greenhouse studies, the highest dose produced the most action (toxic, repellent, and oviposition-deterrent) as well as the longest duration of activity. Further study should be conducted to determine the efficacy of these essential oils against other agricultural pests in greenhouse and open field IPM programs.

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