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Experience with Combinations of Fatty Oil and Essential Oil Mixtures against *Trialeurodes vaporariorum* Westw. in the Peter the Great Botanical Garden

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Abstract

Greenhouse whitefly *Trialeurodes vaporariorum* Westw. (Hemiptera, Aleyrodidae) are becoming a very grave risk and have shown resistance to many synthetic insecticides. In recent years, a number of researchers have identified the repellent and insecticidal effects of plant fatty and essential oils. In this sense, the fatty oil of *Azadirachta indica* A. Juss. (Neem or nim from family Meliaceae) was topically applied to plants for protecting them against insects and bacterial diseases. In addition, essential oils produced from many plant species have a wide abiotic effect. In the course of our experimental work, the insecticidal and repellent properties of essential oils combined with *A. indica* fatty oil against greenhouse whitefly were revealed. using a mixture of different oils to protect plants from whitefly, taking into account recommendations for their dosage, showed the effectiveness of this were conducted to determine the potential of a mixture of *A. indica* fatty oil combined with the essential oils of the following species: *Litsea cubeba* (Lour.) Pers., *Cinnamomum verum* J. Presl, *Tagetes minuta* L., *Juniperus communis* L., *Cymbopogon citratus* (DC.) Stapf against whitefly infestation. It was established that the combined action of oils had a repellent and toxic (insecticidal) effect on the phytophage, and also reduced whitefly fertility (two-three times). A mixture of oils (fatty and essential) provided protection for a collection plant with minimal impact on the ecosystem, preventing the settlement and development of the pest population. In the experiments, no manifestations of pest resistance to the treatment of plants with a mixture of oils and their phytotoxicity were observed. Interestingly, after treatment of plants with mixtures of oils, the duration of the protective effect after treatment of plants with mixtures of oils was ranged between 28 and 35 days. Essential oils with a pleasant smell mask the unpleasant smell of *Azadirachta indica* vegetable oil. The prospects of using in enclosed spaces a mixture of different oils that are not toxic to humans to protect plants from whiteflies are shown.

Keywords: Neem Oil, Essential Oils, *Litsea Cubeba*, *Cinnamomum Verum*, *Tagetes Minuta*, *Juniperus Communis*, *Cymbopogon Citratus*, Repellents, Insecticide, *Trialeurodes Vaporariorum*

1. Introduction

One of the ways to protect plants from the greenhouse whitefly *Trialeurodes vaporariorum* Westw. (Hemiptera, Aleyrodidae) may be the use of a mixture of vegetable fatty and essential oils. Unlike synthetic pesticides (Aldrin, Dieldrin, Chlordane, Heptachlor, Hexachlorobenzene and others), at the recommended concentrations (LD50 ranges from 60-150 to 1000-1500

mg/kg depending on the specific pesticide), oils are not toxic to warm-blooded organisms [1-3]. The composition of essential oils includes compounds that exhibit biological activity against a large number of pathogenic microorganisms (bacteria, fungi, viruses). However, in oils obtained from one plant species, the quantitative and qualitative composition of substances can vary significantly depending on the vegetation phase, organ, harvesting season, soil and climatic conditions [4-7]. The instability of the concentration of the main active substances in the composition of essential oils makes it difficult to select the optimal doses to combat the target species. It is also important to take into account the fact that the essential and fatty oils used should not cause a phytotoxic effect in protected plants, especially collection plants kept in greenhouse conditions. In botanical gardens, the fulfillment of this requirement is hampered by the fact that greenhouses are characterized by the representation of a significant floristic diversity, located in protected ground conditions.

For the past 20 years, at the Peter the Great Botanical Garden of the Botanical Institute (BIN) of the Russian Academy of Sciences (RAS), there has been investigation and development of various methods for combating diseases and pests detected on collection plants, both in open and protected ground [8-10].

The issue of ecologization of the developed protective measures is especially acute in protected ground conditions, where almost every crop has a whole complex of phytophages characterized by a high reproduction rat [11-13]. As a result of many years of entomological surveys of greenhouse collections of protected ground plants of the Botanical Institute of the Komarov Botanical Institute of the RAS it was established the most harmful pests are *Trialeurodes vaporariorum* Westwood, 1856 (family Aleyrodidae), *Frankliniella occidentalis* Perg. (Family Thripidae) and *Tetranychus urticae* Koch. (Family Tetranychidae). *T. vaporariorum*, the common greenhouse whitefly, also contribute to pests that damage a wide range of plants.

The purpose of this work is to assess the combined effect of a mixture of neem fatty oil (*Azadirachta indica*) and essential oils of different plant species on the behavior and breeding potential of the greenhouse whitefly.

Azadirachta indica L. (neem or nim) of the Meliaceae family-is widely used in Chinese, Ayurvedic and Unani medicines, and especially in the Indian subcontinent, for the treatment and prevention of various diseases. Previous evidence has confirmed that A. indica fatty oil and its components play a role in scavenging free radical formation and preventing disease pathogenesis and show a therapeutic role in health management due to its rich source of various constituents [14-28]. The use of dried leaves of *Azadirachta indica* in the control of insects on stored seeds of legumes (cowpea and mung bean) caused the death of *Callosobruchus* sp. Neem leaves have controlling properties as an anti-feed and repellent and cause the death of insects. Neem leaves are an effective insecticide against *Callosobruchus* sp. [29-34]. The most important and biologically active component is azadirachtin, as well as nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, hedunin, salannin and quercetin. The leaves of A. indica contain nimbin, nimbanene, 6-desacetylnimbinen, nimbandiol, nimbolide, ascorbic acid, n-hexocosanol, and the amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol. Quercetin and ß-sitosterol, polyphenolic flavonoids, have been purified from fresh leaves of A. indica, and these compounds are known to have antibacterial and antifungal properties [35], and the seeds contain components such as hedunin and azadirachtin. But it doesn't smell very pleasant. In the practice of protecting plants from insect pests and bacterial diseases, A. indica oil is actively used. Its distinguishing feature is its high sulfur content [36]. The first attempts to add essential oils to the treatment of plants were to mask the unpleasant smell of neem.

The advantage of fatty and essential oils is that they are environmentally friendly. Most oils are not phytotoxic, and the resistance of microorganisms and phytophages to these compounds is not known to occur, as compared to many traditional insecticides [37-43].

Essential oils (those most closely associated with repellency are citronella oil, eucalyptus oil, and catnip oil, but others include clove oil, patchouli, peppermint, and geranium etc) have been found to have repellent properties that prevent the colonization of plants by phytophages treated with the oils [44-46].

It is known that the essential oils of the above species have abiotic, insecticidal and repellant properties [36, 47-50]. According to the literature, fatty oil of *A. indica* and essential oil of *Cinnamomum verum* have a repellant and toxic effect on the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood, 1856), and also reduce its fertility [7, 50-56]. Modern studies show that essential oils of various plant species and their individual components (carvacrol, cinnamaldehyde, citral, thymol, menthol, methyl eugenol, cinnamaldehyde, p-cymene, eucamalol, limonene, linalool, α-pinene and β-pinene, γ-terpinene, geraniol, camphor etc.) have a larvicidal effect and antibacterial activity [55-60].

It is possible to increase the abiotic efficiency of A. indica fatty oil by adding to it essential oils such as *Tagetes minuta* L., *Litsea cubeba* (Lour.) Pers., *Cymbopogon citratus* (DC.) Stapf (synonymous with *Andropogon citratus* DC.), *Juniperus communis* L., and *Cinnamomum verum* J. Presl. The identified components of essential oils of the listed types are presented in Table 1.

Table 1: The main components of the essential oil of the studied plant species

Plant species (family)	Essential oil components	References
Tagetes minuta L. (Asteraceae)	Myrcene, ocimene, terpinolene, terpinene, limonene, β-pinene, thujene, sabinene, caryophyllene, aromadendren, linalool, geraniol, α-terpineol, eudeemole, nonanal, decanal, citral, phenylacetaldehyde, dimethyloctanone	[61-63]
Juniperus communis L. (Cupressaceae)	α-Pinene 42%, myrcene 5%, limonene 9%, sabinene 16%, β-pinene 3%, γ-terpinene, camphene, limonene, p-cymene, cis-ocymene, carene, caryophyllene, humulene, germacrene D, germacrene B, element, cubeben, terpinen-4-ol).	[47, 48, 64]
Litsea cubeba (Lour.) Pers. (Lauraceae)	Citral (60-85%)	[65, 66]
Cinnamomum verum J.Presl (Lauraceae)	Essential oil from the bark contains cinnamaldehyde (up to 80%), (-)-limonene, β-phellandrene, α- and β-pinenes, camphene, caryophyllene, n-cymene, linalool, eugenol, nonanal, benzaldehyde, hydrocinnamon, cumic, salicylic and methyl salicylic aldehydes, camphor, methylcoumarin. The essential oil from the leaves contains more than 80% eugenol, α- and β-phellandrenes, dipentene, α- and β-pinenes, caryophyllene, (-)-linalool, α-terpineol, geraniol, cinnamic alcohol, sesquiterpene alcohols, piperitone, safrole, benzaldehyde, cinnamaldehyde, benzyl benzoate.	[76-71]
Cymbopogon citratus (DC.) Stapf (*Andropogon citratus* DC.) (Poaceae)	Geranial - 45%, neral - 33%, limonene (D) - 3%, geraniol - 3%, geranyl acetate - 2%, beta-caryophyllene - 2%)	[50, 52- 55]

2. Materials and Methods

Experimental work was carried out on collection plants in the subtropical greenhouses of the Peter the Great Botanical Garden of the BIN RAS in the period from 2019 to 2022, in the winter-spring (December – April) period. Before processing, samples of model plants were taken (3 specimens of the following species: *Gerbera jamesonii* Bolus ex Hook.f. (Asteraceae = Compositae), *Lantana camara* L. and *Duranta stenostachya* Tod. (Verbenaceae = Lamiaceae = Labiatae), *Acanthus mollis* L. (Acanthaceae), *Brugmansia versicolor* Lagerh (Solanaceae). On 10 leaves of each species, the number of adult insects was counted before treatment. The following variants of experiments were used in the work: 1) treatment of model plants only with a solution of neem oil; 2) treatment of model plants with a mixture of neem oil and essential oil; 3) control, model plants were sprayed with pure water. Accounting for the presence/absence of the greenhouse whitefly (*T. vaparariorum*) was carried out after treatment on days 3, 7, 10, 14, 21 and 30. The effectiveness of pest control was determined by the presence of adults, since the mixture of oils acts mainly on adults, while the effect on eggs and puparia (pest dormancy; or false cocoon) will be noticeable within 7 or 10 days after treatment. Treatment with a mixture of fatty and essential oils was determined by the formula:

$$C = 100 (Ba-Ab) / Aa,$$

Where:

C is the percentage of pest mortality adjusted for control;

A and a are the total number of individuals in the experimental variant and control, respectively;

B and b are the number of dead individuals in the experimental variant and control, respectively.

A working solution for treating plants with a mixture of oils (*A. indica* fatty oil and essential oils) was prepared based on 50 ml of *A. indica* fatty oil per 10 liters of water. TWEEN-80 was used as an emulsifier (at the rate of 10-15 ml per 10 l of water) or polysorbate. Essential oils were used at a concentration of 0.06%. Experiments on the processing of collection plants were carried out with a manual sprayer at an air temperature of 12-16 ° C. Working fluid consumption 800 l/ha. The results are presented in Table 2.

Table 2: Evaluation of the biological effectiveness of the combined action of *Azadirachta indica* fatty oil mixed with essential oils

Blend with essential oil	Species	Average number of whiteflies per cm^2	Whitefly mortality rate (day)					
			3	7	10	14	21	30
Litsea cubeba	*Gerbera* sp.	10.8	33.3	52.5	66.6	76.8	90.5	82.1
	Acanthus mollis L.	25	28.2	48.6	58.3	71.1	95.6	84.1
	Lantana camara L.	28	27.6	54	65	75	80,1	75,2
	Brugmansia versicolor Lagerh.	22.5	33.3	68.5	72.1	88.8	93.4	80.3
	Duranta stenostachya Tod.	20.1	38.3	52.4	70.3	80.6	92.2	82.2
Cinnamomum verum	*Gerbera* sp.	8.5	30.3	48.0	59.7	69.8	82.5	73.7
	Acanthus mollis L.	15.3	30.1	41.5	54.6	65.5	80.4	71.1
	Lantana camara L.	20.1	40.1	60.5	64.8	71.5	84.3	75.1
	Brugmansia versicolor Lagerh.	22.5	30.,1	50.1	65.3	72.5	81.1	70.2
	Duranta stenostachya Tod.	12.8	27.1	42.5	59.5	68.7	83.1	73.4
Tagetes. minuta	*Gerbera* sp.	8.6	24.5	37.5	53.5	70.1	80.1	72.1
	Acanthus mollis L.	10.2	32.5	45.6	65.3	76.8	84.2	70.1
	Lantana camara L.	20.1	30.3	48.1	60.2	73.5	81.3	70.8
	Brugmansia versicolor Lagerh.	22.8	30.1	49.1	66.2	72.5	85.6	72.4
	Duranta stenostachya Tod.	12.5	26.1	42.5	65.2	70.2	79.9	69.5
Juniperus communis	*Gerbera* sp.	9.5	28.6	39.5	59.8	72.8	82.4	77.8
	Acanthus mollis L.	10.8	35.5	47.6	66.7	75.3	86.6	78.9
	Lantana camara L.	15.6	29.1	45.3	62.7	76.8	85.2	76.6
	Brugmansia versicolor Lagerh.	20.1	31.4	49.4	67.9	78.8	89.9	79.4

	Duranta stenostachya Tod.	10.2	29.6	46.6	64.7	77.8	91.3	81.2
Cymbopogon citratus	*Gerbera* sp.	10.4	29.5	40.5	66.3	84.1	91.4	86.8
	Acanthus mollis L.	18.5	30.1	48.2	60.3	78.3	89.7	80.1
	Lantana camara L.	16.8	34.1	41.5	69.4	82.5	95.1	86.3
	Brugmansia versicolor Lagerh.	32.5	33.3	49.5	68.3	78.4	91.1	83.3
	Duranta stenostachya Tod.	19.6	38.2	48.5	66.9	79.3	92.1	85.1

3. Results and Discussion

As can be seen from Table 2, in the conditions of protected ground of subtropical greenhouses of the Peter the Great Botanical Garden of the BIN RAS, the mixture of *A. indica* fatty oil and *L. cubeba* essential oil has the best insecticidal and repellent effect. Mortality of adult whitefly reaches 90%. A somewhat smaller effect (approximately 80%) was observed for a mixture of *A. indica* fatty oil and essential oils of *Juniperus communis* and *Cymbopogon citratus*. A lesser effect (mortality of adults about 70%) was found in mixtures with *Cinnamomum verum* and *Tagetes minuta* essential oils. All these essential oils performed a repellent function and masked the smell of *A. indica* oil, while at the same time enhancing the repellant effect. The effectiveness of the mixture of oils begins to decline from 25-30 days. By this time, new imagoes are reflected. Therefore, on the 25th day, the next treatment of plants should be carried out (Fig. 1-5).

As can be seen from the presented results obtained for the first time, it can be seen that a mixture of vegetable and essential oil is much more effective than using each oil separately. The use of a mixture of oils prolongs the protective effect for plants, thus having a prolonged effect. Treatment with a mixture of different oils has both an insecticidal and repellent effect. We have observed that these treatments reduce bacterial and fungal diseases in greenhouse plants.

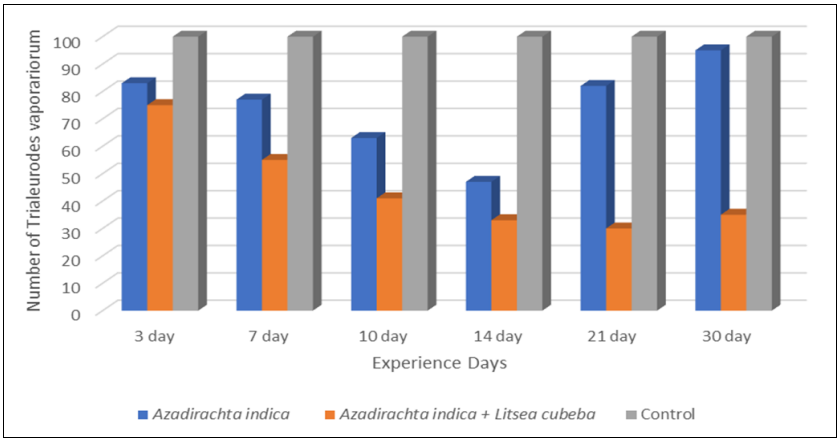

Fig 1: Effectiveness to reduce the abundance of Trialeurodes vaporariorum Westw. of the combined action of *Azadirachta indica* fatty oil mixed with essential oils of *Litsea cubeba*

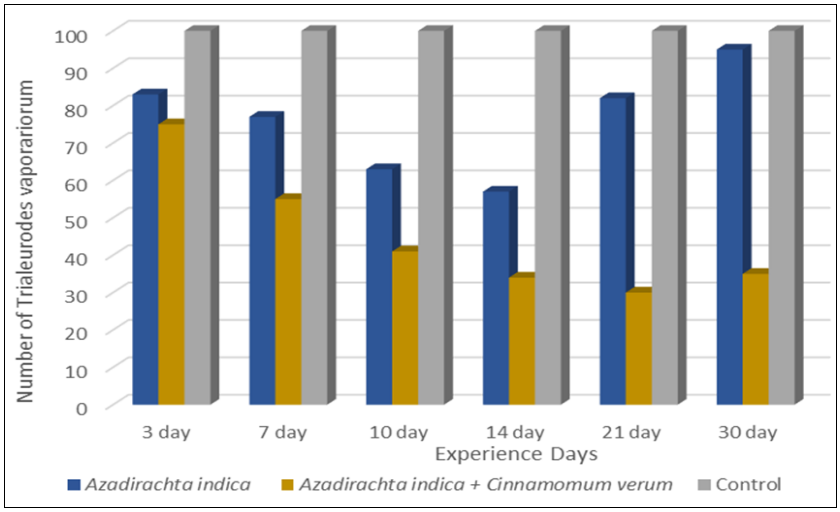

Fig 2: Effectiveness to reduce the abundance of Trialeurodes vaporariorum Westw. of the combined action of *Azadirachta indica* fatty oil mixed with essential oils of *Cinnamomum verum*

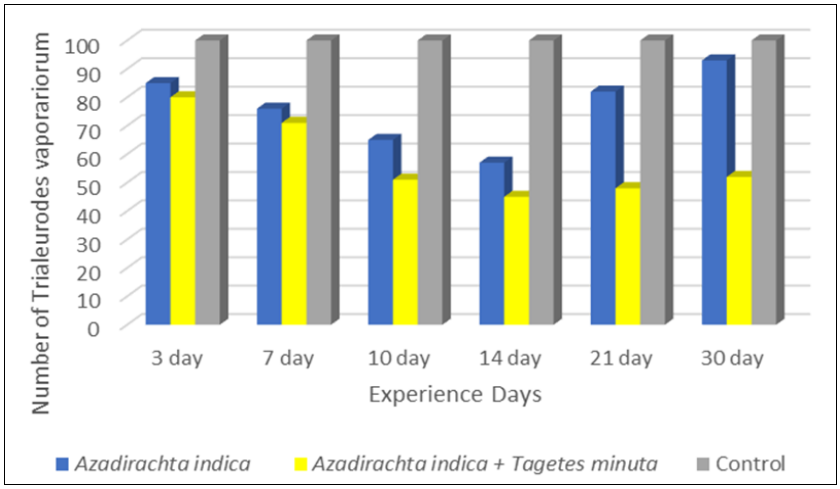

Fig 3: Effectiveness to reduce the abundance of Trialeurodes vaporariorum Westw. of the combined action of *Azadirachta indica* fatty oil mixed with essential oils of *Tagetes minuta*

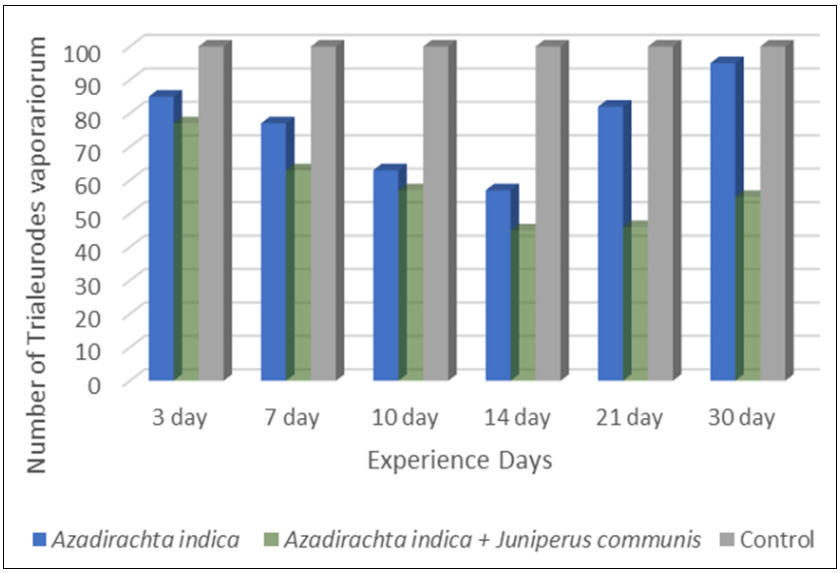

Fig 4: Effectiveness to reduce the abundance of Trialeurodes vaporariorum Westw. of the combined action of *Azadirachta indica* fatty oil mixed with essential oils of *Juniperus communis*

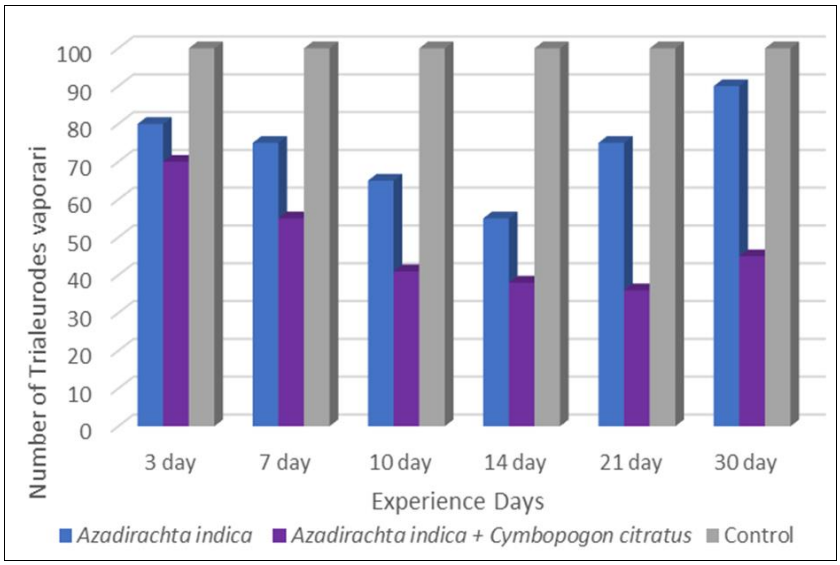

Fig 5: Effectiveness to reduce the abundance of Trialeurodes vaporariorum Westw. of the combined action of *Azadirachta indica* fatty oil mixed with essential oils of *Cymbopogon citratus*

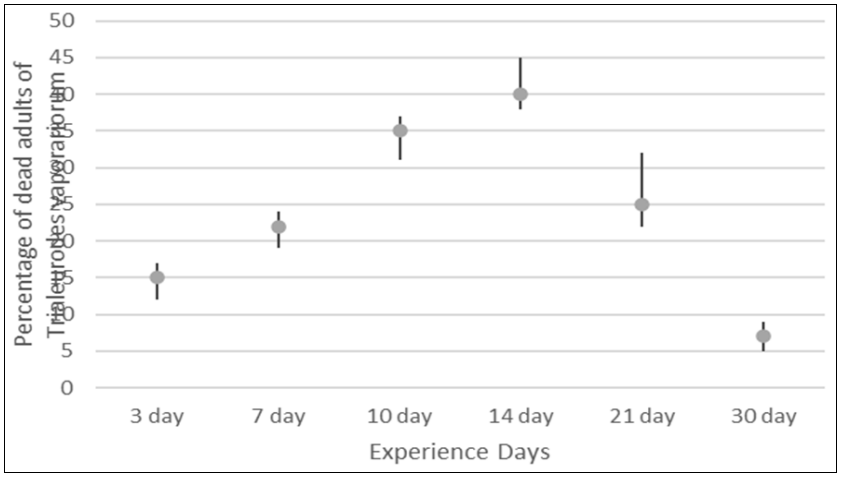

Fig 6: Reducing the number of adult Trialeurodes vaporariorum after treatment with fatty oil *Azadirachta indica*.

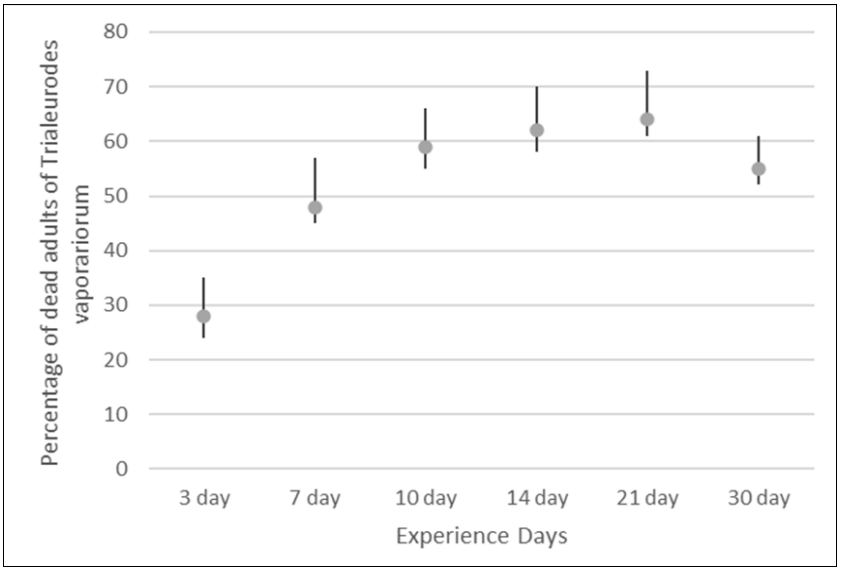

Fig 7: Reducing the number of adult Trialeurodes vaporariorum after treatment with essential oil of *Litsea cubeba*.

In Fig. 6 and 7 show the dynamics of the effect of *A. indica* fatty oil on the number of adult *T. vaparariorum*. As can be seen from these graphs, the combined use of fatty and essential oils (for example – *Litsea cubeba* essential oil) has a greater effect with a prolonged action.

4. Conclusion

Experience has shown that the use of a mixture of *A. indica* fatty oil and essential oils, especially *L. cubeba*, *J. communis* and *C. citratus*, reduces the number of greenhouse whiteflies. Therefore, to protect collection plants in greenhouse conditions, it is possible to recommend and use a mixture of fatty oil *A. indica* together with essential oils of different plant species.

For the protection of plants (collectible in botanical gardens, cultivated food plants), especially in greenhouse conditions (greenhouses), the use of a mixture of oils gives a good result. Mixtures of fatty and essential oils are not toxic to humans, do not have a cumulative effect, and are environmentally friendly.

The results obtained provide a basis for expanding the search for essential oils of different species from different families to expand the range of effects on insect pests and protect plants, including against bacterial and viral diseases.

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6. Conflict of interest

The authors have no conflict of interest.

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