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EFFECTS OF *Litsea cubeba* (Lour.) Pers. ESSENTIAL OIL AND ITS MAIN COMPONENT TO THE DEVELOPMENT OF THE GREENHOUSE WHITEFLY *Trialeurodes vaporariorum* Westw.

E.A. STEPANYCHEVA, M.O. PETROVA, T.D. CHERMENSKAYA✉

All-Russian Research Institute of Plant Protection, 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail stepanycheva@yandex.ru, mar34915696@yandex.ru, tchermenskaya@yandex.ru (✉ corresponding author)

ORCID:

Stepanycheva E.A. orcid.org/0000-0002-0224-758X

Chermenskaya T.D. orcid.org/0000-0001-5791-491X

Petrova M.O. orcid.org/0000-0003-3710-3292

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Abstract

Trialeurodes vaporariorum Westw. (Hemiptera, Aleyrodidae) causes significant economic damage to vegetable and ornamental crops due to phloem sap feeding and the transmission of viral diseases. High reproductive potential of the phytophage and multiple treatments with chemicals lead to the emergence of resistance to various insecticides. Therefore, it becomes necessary to search for new effective environmentally safe plant protection products. Plant essential oils are of considerable interest in this regard. In this work, for the first time, we obtained information about the effectiveness of *Litsea cubeba* essential oil and its main component, citral, as fumigants and repellents for controlling the number of greenhouse whiteflies. As the problem of reducing the insecticidal load is especially acute in greenhouses, the aim of our study was to examine mechanisms of action of *L. cubeba* essential oil and citral on *T. vaporariorum* — one of the most harmful phytophages for greenhouse crops. Obtaining information on the effectiveness of the tested samples will serve as the basis for the development of a new protective tactic against the greenhouse whitefly. The whiteflies were lab-reared on bean (*Phaseolus vulgaris* L.) plants at 24 ± 1 °C and a 16 h light period. The essential oil of *L. cubeba* and citral were obtained from the Crop Research Institute (Prague, Czech Republic). For testing, 1 % solutions of *L. cubeba* essential oil or citral were prepared by dissolving 100 µl of the substance in 900 µl of ethanol, followed by the addition of 9 ml of water with stirring. Concentrations of 0.5, 0.25, and 0.125 % were obtained by sequential dilution with water. The phytotoxicity of *L. cubeba* essential oil and citral was pre-assessed. The pest preimaginal stages were treated to assess the effect of the essential oil on egg hatching and the larvae development. The influence of *L. cubeba* essential oil and citral on the choice of plants by *T. vaporariorum* for feeding and oviposition under free choice was also investigated. When studying the fumigation effect, the number of live, dead individuals and laid eggs was assessed. The experimental data were analyzed with one-way analysis of variance (one-way ANOVA), the mean values were compared using the Tukey's HSD test. Differences between the means were considered significant at $p \leq 0.05$. When the *T. vaporariorum* eggs were treated with solutions of *L. cubeba* essential oil, none of the tested concentrations affected the vital parameters of the phytophage during the entire preimaginal period of development. A similar pattern occurred after the treatment of larvae. The obtained results show the absence of both direct toxic effects and aftereffects during treatment at the embryonic and larval stages of whiteflies. When whitefly adults were kept on plants treated with 0.25 % concentration of *L. cubeba* oil, the number of laid eggs significantly decreases (by 25 % compared to the control). Oil volatiles at 0.25 % concentration had a repellent effect and reduced the offspring numbers. The preference index was -18.7, and the number of laid eggs decreased by almost 40 %. The fumigation effect of the *L. cubeba* oil on the greenhouse whitefly was most noticeable. *L. cubeba* oil (9.0 and 6.0 µl/l) caused the 90 % death of adults and a decrease in the number of eggs by 98.2 and 93.8 %, respectively, compared to control. Citral had no repellent effect but its fumigation activity was not inferior to that of essential oil. The maximum used citral concentration of 6.0 µl/l led to 85.9 % mortality of adults and a decrease in the number of eggs by more than 90 %. Our findings suggest prospects of the *L. cubeba* oil application as a fumigant and repellent against *T. vaporariorum* in greenhouses.

Keywords: essential oil plants, citral, *Trialeurodes vaporariorum*, whitefly, toxicity, fumigation, repellent effect

Greenhouse whitefly *Trialeurodes vaporariorum* Westw. (Hemiptera, Aleyrodidae) can affect 859 plant species from 469 genera and 121 families [1]. Suppression of plant development and a decrease in yield resulted from phloem sap feeding and the sooty fungus infection developed on the honeydew that are secreted by the whitefly. *T. vaporariorum* actively transmits plant viral diseases [2], therefore, even with a small number of pests, frequent use of insecticides is necessary, which provokes the development of whitefly resistance [3].

In the search for new effective means of protection, environmentally low-hazardous substances are preferable. Vegetable essential oil that have various effects on phytophages, from direct toxicity to the regulation of behavior and development of arthropods, are of considerable interest [4, 5]. There are approx. 3,000 ethereal plants in the world that produce and accumulate essential oils, but only 200 species, containing a sufficient amount of the product of the required quality are of commercial importance. Among the ether-bearing plants cultivated in Russia, crops grown for grain and flower-herbaceous raw materials predominate (fruits of coriander, anise, fennel, cumin, dill, flowers and green mass of lavender, wormwood, hyssop, sage, rose, mint, oregano). However, only 6-8% of the world's essential oils derive from the Russian Federation.

Currently, many facts confirm the biological activity of plant essential oils against whiteflies [6, 7]. Vegetable oil from *Litsea cubeba* (Lour.) Pers. (family Lauraceae) with insecticidal properties are promising [8-10]. The natural ranges of this deciduous shrub or a small tree plant are southern China, Japan, Southeast Asia, the mountainous regions of Taiwan, Thailand, northeast India, Korea, Vietnam and Indonesia. Despite the dependence of the chemical composition of the oil on the location of *L. cubeba* and the plant parts used [11], 59 of its components were identified. Citral is the main component of this oil, regardless of the growing zone [12].

This work, for the first time, submits information on the effectiveness of the *L. cubeba* essential oil and citral as fumigants and repellents to control the greenhouse whitefly population.

The aim of our work was to reveal mechanisms of action of *Litsea cubeba* essential oil and its main component, citral, on the whitefly *Trialeurodes vaporariorum*.

Materials and methods. The whitefly was grown under laboratory conditions on bean plants (*Phasöolus vulgaris* L.) at 24 ± 1 °C and a 16 h light period. The essential oil of *L. cubeba* and citral were obtained from the Crop Research Institute (Prague, Czech Republic).

For tests, 1% solutions of *L. cubeba* essential oil or citral were prepared by dissolving 100 µl of the substance in 900 µl of ethanol, followed by the addition of 9 ml of water with stirring. Concentrations 0.5, 0.25 and 0.125% were obtained by sequential dilution with water.

Before starting the experiments, the phytotoxicity of *L. cubeba* essential oils and citral was evaluated to determine the maximum possible concentration. Bean plants were sprayed with solutions of substances and observed for 7 days.

The exact number of whiteflies in each replication (live and dead) was counted. The abundance of the daughter generation was calculated per one original individual.

When studying the effect of *L. cubeba* essential oil on the embryonic stage of *T. vaporariorum*, bean plants grown individually in 200 ml cups were placed in cages with whitefly adults for colonization. After 1 day, adults were removed. After counting the eggs laid, the plants were treated with a 0.25% oil solution until the drops closed, the control plants were treated with water. The effect was assessed by the number of hatched larvae, formed puparia and hatched adults.

To evaluate the effect of the essential oil on the larvae, the plants were colonized as in the previous experiment, but after the adults were removed, the plants were placed in a clean box for 9 days. Appeared larvae were counted before treatment with 0.25% oil solution (in control with water). Further counts were carried out as described above.

Under conditions of forced maintenance of *T. vaporariorum* imago, bean plants in the experiment were treated with 0.25% oil solution, in the control with water. Plants were individually placed in 10 l cylinders, 30 whitefly adults were released into the cylinders without separation by sex. The cylinders were covered with mill gas to prevent the escape of the phytophage and normal ventilation. After 1 day, the number of adults and eggs laid on the plants was counted. Further counts were carried out as in the test with egg treatment.

To study the effect of the *L. cubeba* essential oil and citral on the choice of plants for feeding and oviposition by *T. vaporariorum*, 2 experimental and 2 control plants were placed in Plexiglas cages (60×60×60 cm) with ventilation holes, and 60 adult whiteflies were released to each cage. After 1 day, the number of phytophages on plants and the number of eggs laid were counted. The influence of the test samples on the attraction of adults was assessed by the preference index (PI): $PI = (X_c - X_t)/X_{tot}$, where X_c is the number of individuals on the control plant, X_t is the number of individuals on the test plant, and X_{tot} is the total number of attracted individuals.

The attractiveness of plants for the development of offspring was determined by the decrease in the number of eggs (%) = $[(X_c - X_t)/X_c] \times 100$, where X_c is the number in the control, X_t is the number in the test [13].

To assess the fumigation effect of the *L. cubeba* essential oil and citral on *T. vaporariorum*, the essential oil or citral was diluted in ethanol to a certain concentration and applied to filter paper (dispenser), 10 µl per repetition. The dosages were 9.0. 6.0. 4.5. 3.0 and 2.25 µl/l air. In the control, 10 µl of ethanol was applied to the dispenser. After the solvent had evaporated (in 2 min), the dispenser was attached to the inside of the lid of a 265 ml plastic container; a bean leaf was placed on the bottom, the petiole of which was in an Eppendorf tube with water. After the release of phytophage adults (30 individuals) into the container, it was tightly closed with a lid. After 1 day, live and dead individuals and laid eggs were counted. There were 10 repetitions in each treatment. Citral was evaluated at concentrations used for *L. cubeba* essential oil.

Mortality was calculated by the O. Schneider-Orelli formula [14]. The effect of test samples on the number of eggs was calculated by the W.S. Abbot formula [13].

Statistical processing was carried out using the MicroCal Origin program, version 3.01 (<https://microcal-origin.software.informer.com/>). Mean values (M) and standard errors of means ($\pm SEM$) were calculated. Experimental data were analyzed using one-way analysis of variance (one-way ANOVA), mean values were compared using Tukey's HSD test. Differences between the means were considered significant at $p \leq 0.05$.

Results. The evaluation of the phytotoxicity of the tested samples showed that the maximum concentration that did not adversely affect the bean plants was 0.25%.

When whitefly eggs were treated with *L. cubeba* essential oil during the entire preimaginal period, none of the tested concentrations affected the vital parameters of the phytophage. Death at the studied stages did not statistically differ from the control. A similar pattern occurred after the treatment of larvae. The average total death for the entire period of observation (before emergence of

adults) did not exceed 13% and did not differ significantly from that in the control (Table 1).

When adults of the whitefly were kept on plants treated with *L. cubeba* oil, a statistically significant decrease (by 25%) in the number of eggs laid occurred compared to the control at a concentration of 0.25% ($F = 4.55915$, $p = 0.04674$). The total death did not differ significantly over the treatments ($F = 3.66306$, $p = 0.07167$) (see Table 1).

1. Stages of ontogeny of *Trialeurodes vaporariorum* Westw. under the influence of the *Litsea cubeba* (Lour.) Pers. essential oil (0.25% solution, $M \pm SEM$; a lab test)

Treatmen	Original num- ber of indi- viduals	Number of laid eges per 1 imago	Laid egg decrease, %	Numer of dead individuals.			Average dead indi- viduals
				embryonic stage	larvae stage	puparia	
Embryonic stage:							
test	254			3.30±1.62	2.30±1.21	1.30±0.78	6.90±2.17
control	270			2.30±1.01	1.90±0.89	3.00±0.85	7.20±1.72
Larvae stage:							
test	225				8.06±0.85	4.35±1.13	12.4±1.04
control	218				5.91±1.05	5.34±1.40	11.2±1.75
Imafo stage:							
test	301	0.68±0.07*	25.3	7.30±1.56	6.50±0.92	4.20±1.47	17.9±1.39
control	282	0.91±0.08		4.90±1.22	5.00±0.68	4.80±1.22	14.7±0.91

* Differences from control are statistically sifnificant at $p \leq 0.05$ (see the “Materials and methods” section).

At 0.25% concentration of essential oil, the phytophage gave preference to control plants for feeding and laying eggs, the PI was 18.7, and the number of eggs laid decreased by almost 40%. After treatment of plants with 0.125% essential oil, the repellent effect was almost completely absent and all parameters did not significantly differ from the control ($F = 0.88411$, $p = 0.35953$ for the distribution of imagoes, $F = 0.37043$, $p = 0.55037$ for the number of eggs). Citral had no effect on the whitefly behavior (Table 2).

**2. Free choice of bean (*Phasiolus vulgōris* L.) plants by greenhouse whitefly *Trialeu-
rodes vaporariorum* Westw. for feeding and oviposition depending on the concen-
tration of the *Litsea cubeba* (Lour.) Pers. essential oil and citral ($M \pm SEM$; a lab
test)**

Concentration, %	Imago distribution on plants, number of individuals		Preference index	Number of eggs		Decrease in egg number, %
	test	control		test	control	
Essential oil						
0,25	13.9±1.48*	20.3±1.26	-18.7	10.5±1.92*	17.1±2.18	38.6
0,125	15.8±2.01	18.5±2.06	-7.9	10.3±1.78	11.7±1.46	12.0
Citral						
0,25	15.3±1.75	19.7±2.12	-12.6	8.5±1.28	10.9±1.54	22.0
0,125	16.6±2.57	17.7±3.60	-3.2	8.8±1.22	9.2±1.27	4.3

* Differences from control are statistically significant at $p \leq 0.05$ (see the “Materials and methods” section).

**3. Fumigation action of the *Litsea cubeba* (Lour.) Pers. essential oil and citral on the
viability of the greenhouse whitefly *Trialeurodes vaporariorum* Westw. adults and
the abundance of daughter generation ($M \pm SEM$; a lab test)**

Dosage, μ l/l	Total number of individuals	Imago death rate, %	Death rate including control, %	Egg number per I imago	A decreas in egg number, %
Essential oil					
9.0	290	97.1 \pm 1.22*	97.1	0.02 \pm 0.006*	98.2
6.0	275	91.3 \pm 2.93*	90.3	0.03 \pm 0.011*	93.8
4.5	288	54.2 \pm 7.53*	51.5	0.06 \pm 0.019*	90.6
3.0	284	3.0 \pm 1.57	0.6	0.53 \pm 0.039	7.0
Citral					
6.0	287	86.0 \pm 5.33*	85.8	0.03 \pm 0.014*	94.3
4.5	272	73.9 \pm 3.77*	72.7	0.07 \pm 0.029*	87.5
3.0	267	10.1 \pm 2.87	6.9	0.31 \pm 0.072*	57.8
2.25	290	7.0 \pm 2.35	6.3	0.35 \pm 0.056*	45.9

* Differences from control are statistically sifnificant at $p \leq 0.05$ (see the “Materials and methods” section).

When studying the fumigation effect of test samples on *T. vaporariorum*, *L. cubeba* oil (9.0 and 6.0 $\mu\text{l/l}$) caused the death of 90% of adults and a 98.2 and 93.8% decrease, respectively, in the number of eggs compared to the control. At a concentration of 4.5 $\mu\text{l/l}$, about half of the tested insects died, while the number of eggs in the experiment decreased by 90.6%. After another 1.5-fold reducing the dosage, the effects were completely leveled (Table 3). The use of citral at the maximum concentration (6.0 $\mu\text{l/l}$) with this mode of exposure led to 85.9% death of adults and a decrease in the number of eggs by more than 90%. A sharp decrease in toxicity, similar to *L. cubeba* oil, was found at 3.0 $\mu\text{l/l}$, and the negative effect on fertility remained (see Table 3).

The scientific literature provides sufficient information on the mechanisms of action of the essential oil of *L. cubeba* and citral on arthropods. Thus, *L. cubeba* essential oil is characterized by pronounced contact toxicity for some species of *Coleoptera*, e.g., adults of *Lasioderma serricorne* (LD_{50} 27.33 $\mu\text{g}/\text{cm}^2$) and *Liposcelis bostrychophila* (LD_{50} 71.56 $\mu\text{g}/\text{cm}^2$), *Tenebrio molitor* larvae and beetles (LD_{50} 21.2 $\mu\text{g}/\text{cm}^2$), *Sitophilus zeamais* [8, 9]. For *Trichoplusia ni* caterpillars, *L. cubeba* oil showed moderate toxicity (LD_{50} 112.5 μg per larva) [10].

In our experiments, the essential oil of *L. cubeba* (0.25%), when 1-day-old eggs and 1-2-day-old whitefly larvae were treated, did not have a negative effect. Observation of the treated individuals before the emergence of adults did not reveal any differences in mortality between the experiment and control. However, when assessing the effect of oil (0.25%) on the phytophagous imago and their offspring, a decrease in the fertility of the whitefly on the treated plants was shown, and further development during the preimaginal period did not differ in the experiment and control.

Repellent property of the *L. cubeba* essential oil was clearly demonstrated on the beetles *Sitophilus zeamais* and *Tribolium castaneum* [15], mosquitoes *Aedes albopictus* [16], termites [17], and ants *Monomorium pharaonis* [18]. Citral acts as repellent against the mosquito *Aedes albopictus* [19] and the beetle *Lasioderma serricorne* [20].

Our experiments on the behavioral responses of the whitefly also revealed a decrease in the attractiveness of plants treated with the 0.25% essential oil of *L. cubeba* for both feeding and oviposition. At the 0.125% dosage, the revealed effects leveled out. Citral, even at the maximum concentration of 0.25%, did not cause significant changes in the behavior of the phytophage compared to the control. It is possible that higher concentrations of oil and citral would also have had an effect on the greenhouse whitefly *T. vaporariorum*, but the phytotoxicity did not allow an increase in the dosage for treatment. The phytotoxicity in oils was reported earlier [21, 22].

The essential oil of *L. cubeba* and citral showed the greatest efficiency in fumigation, both having a direct toxic effect on adults and reducing the abundance of the daughter generation. *L. cubeba* essential oil has fumigation properties against several harmful arthropods. The examples are the beetles *Lasioderma serricorne* and *Liposcelis bostrychophila* (LD_{50} 22.97 and 0.73 mg/l , respectively) [8], the ants *Solenopsis invicta* (more than 90% death at a dosage of 5.33 $\mu\text{l}/\text{cm}^3$) [23], the larvae of the gall midge *Camptomyia corticalis*, a pest of shiitake mushrooms (LC_{50} 3.46 mg/cm^3) [24], and tobacco whitefly *B. tabaci* (100% mortality at 2.4 $\mu\text{l}/\text{cm}^3$) [25].

Fumigation properties were also described for citral against the cabbage moth *Plutella xylostella* (LC_{50} for adults 1.65 mg/l , for larvae of the 1st age 0.35 mg/l , for eggs 4.28 mg/l) [26], beetles *Tenebrio molitor* [9], ants *Solenopsis invicta* (more than 90% death at a dosage of 5.33 $\mu\text{l}/\text{cm}^3$) [23]. We have previously

identified similar properties of *L. cubeba* against the dangerous quarantine pest *Frankliniella occidentalis* which is often present together with the whitefly on the same crops in greenhouses [27, 28]. The concentrations we studied were significantly lower than those reported in the literature, and the death rates of the whitefly were similar to those reported. The toxicity of the tested samples was comparable at the same concentrations, but, unlike *L. cubeba* essential oil, citral reduced the number of eggs even at lower dosages.

A comparison of the whitefly behavioral response and viability under the influence of two volatile products, the essential oil and citral, did not reveal a common pattern in the effects of a single substance (citral) or the multicomponent oil of *L. cubeba* on fecundity of the phytophage. Some authors suggested that the biological activity of vegetable essential oils is due to the synergistic effect of the compounds that make up the composition [23, 29]. Therefore, it is not always possible to expect that a single substance, even if it is significantly dominant in an essential oil, will be more active than the original product itself. The delayed emergence of pest resistance to essential oils and various mechanism of their action may be due to the multicomponent nature of these bioactive substances.

Thus, at the initial stages of embryonic and larval development of greenhouse whitefly *Trialeurodes vaporariorum*, the *Litsea cubeba* essential oil at a concentration of 0.25% did not have either a direct toxic effect or an aftereffect. Contact of adults with plants treated with essential oil at the same concentration caused a 25.3% decrease in the number of eggs laid. Oil volatiles of the oil at the 0.25% concentration had repellent effects (the preference index accounted for 18.7) and reduced the offspring abundance (38.6% reduction in egg count). In 0.25% citral, these properties were less pronounced. The oil was the most effective against the greenhouse whitefly during fumigation. At a dosage of 4.5 $\mu\text{l/l}$, more than 50% of whitefly adults died and the number of eggs decreased by 90.6%. The same properties were characteristic of citral (4.5 $\mu\text{l/l}$) with the estimated values of 72.7 and 87.5%, respectively. The fumigation and repellent effect of *L. cubeba* essential oil and citral on the whitefly that we have revealed indicates their ability to reduce the abundance of the phytophage. Our findings prove that *L. cubeba* oil is promising as a fumigant and repellent against *T. vaporariorum* in greenhouses where the phytophage develops year-round in 10-16 generations, regardless of weather conditions, and there are strict phytosanitary requirements for the applied protective preparations. Further studies in greenhouses will substantiate the effectiveness of *L. cubeba* essential oil in more details. The mode of applications will also depend on the test sample properties. Fumigation activity can prevent the spread of whiteflies during the transportation of plant materials and crops, while repellent action can reduce plant colonization by phytophages.

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