Research article

Evaluation of *Artemisia absinthium*. L, essential oil as a potential novel prophylactic against the Asian citrus psyllid *Diaphorina citri* Kuwayama

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Simple Summary: The massive use of synthetic pesticides to manage insect pests has adversely affected humans and the environment. Therefore, botanical pesticides could be helpful as alternative tools for integrated pest management since they have low mammalian toxicity and have minimal risk of developing resistance in target pests. In our current study toxicity of *Artemisia absinthium* essential oil (ABEO) as a novel alternative to synthetic insecticides against *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), a vector of citrus greening disease has been evaluated. The finding of our current investigation suggested that *A. absinthium* essential oil has the potential to be developed as a valuable novel crop protectant against *D. citri*.

Abstract: Interest in developing novel crop protectants has gained attention in the recent decade due to the hazardous effects of synthetic pesticides on humans and the environment. The citrus industry worldwide is threatened by the *D. citri*, the primary vector of phloem-limited bacterium (HLB) or citrus greening. However, there is no cure for citrus greening available. *Diaphorina citri* management largely depends on synthetic insecticides, but their massive application leads to resistance in pest populations. Therefore, alternative pest management strategies are needed. Our results indicated fewer *D. citri* adults settled on plants treated with ABEO than on control 48 h after release. The psyllid fed on citrus leaves treated with ABEO significantly secreted less honeydew than the control. The essential oil showed potent ovicidal activity against the *D. citri* eggs with LC_{50} 5.88 mg/mL. Furthermore, we also explore the fitness of *D. citri* on ABEO-treated and untreated *Citrus × sinensis* by using two-sex life table tools. The results indicated that the intrinsic rate of increase (r) was higher on untreated seedlings (0.10 d^{-1}) than those treated with a sub-lethal dose of ABEO (0.07 d^{-1}). Similarly, the net reproductive rate (R_0) was higher for untreated seedlings (14.21 offspring) than those treated (6.405 offspring). Notably, the ABEO were safer against *Aphis melifera*, with LC_{50} ranging from 31.05 to 55.86 mg/mL, which is relatively higher than the LC_{50} 24.40 mg/mL values against *D. citri*. Our results indicate that ABEO exhibits toxic and behavioral effects on *D. citri* that could be useful for managing this pest.

Keywords: Essential oil, Toxicity, Botanical insecticides, *Artemisia absinthium*, *Diaphorina citri*.

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1. Introduction

The Asian citrus psyllid *D. citri* Kuwayama (Hemiptera: Psyllidae), is a well-known significant citrus pest worldwide. Huanglongbing (HLB) is one of the most devastating diseases of *Citrus* spp worldwide that limits commercial production [1]. The HLB-infected trees show off-season bloom, twig dieback, stunted growth, early fruit dropping and ultimately, death of the whole plant may occur [2,3]. However, no cure for HLB has been reported [4], and controlling *D. citri* is the only effective way to manage HLB [5]. The primary control measures of *D. citri* largely depend on synthetic insecticides [6,7].

Similarly, Florida's citrus industry has been devastated by HLB and, since 2005, lost 74% of production [8]. Insecticidal suppression of *D. citri* has played a disproportionally significant role in HLB management. Eight to 12 insecticide applications are commonly used in one cropping season in China, Florida, Brazil, Mexico and other major citrus-growing countries worldwide [9]. Under such selection pressure, the resistance to organophosphate, pyrethroid, neonicotinoid and carbamate insecticides in *D. citri* has been reported [9,10]. Similarly, the massive use of these synthetic insecticides also negatively affects the environment, mammals, natural enemies, pest resurgence and development of resistance [11]. Therefore, further effort is needed to identify a greater diversity of more sustainable tools to improve HLB management.

Botanical insecticides have long been used as alternatives to synthetic chemical insecticides for pest management because botanicals cause no or minimal threat to the environment or human health [12]. Among the botanical pesticides, essential oils (EOs) are gaining the attention of scientists due to their ability to interfere with the primary metabolic, biochemical, physiological and behavioural functions of insects [13,14]. *Artemisia absinthium* L. (Asteraceae), known as wormwood, is an aromatic and medicinal herb of ethnopharmacological interest [15]. In addition, it has been reported to have toxicity against *Callosobruchus maculatus* and *Bruchus rufimanus* [16], repellent and larvicidal activity against mosquitos (Benelli, 2015; Govindarajan and Benelli, 2016), ingestion toxicity against *Sitona lineatus* [17] and *Drosophila melanogaster* [18], and fumigant toxicity against *Tetranychus urticae* and *Bemisia tabasi* [19].

This study aimed to investigate the toxicity and behavioral effect of *Artemisia absinthium* essential oil against *D. citri*. Furthermore, the effect of Essential oil on the fitness of *D. citri* was also assessed using two-sex life table tools.

2. Materials and Methods

2.1. Insects

The adults of the *Diaphorina citri* were collected from Citrus x aurantium L. grown at South China Agricultural University Guangzhou, China (23◦ 15 N, 113◦ 35 E). The adult psyllids were aspirated very carefully to avoid mechanical injury and were allowed to acclimatize to laboratory conditions 27± 2 ◦C, 65 % ± 5 % R.H., photoperiod 14:10 (Light: Dark) for 72 h before the bioassay. Males and females were separated based on their morphological characteristics. The yellow or orange color of the female abdomen indicates that it contains eggs [20,21].

2.2. Plant materials extraction procedure

The leaves and flowers of *A. absinthium* were collected from the Skardu Baltistan, Pakistan (N35◦16.775, E075◦38.402, Elevation 2396 meters) in August 2022. The plant species were identified by comparing the voucher specimen PH004 (ART004) Quaid-e-Azam University Islamabad, Pakistan [22]. The GC-MS analysis of *A. absinthium* essential oil was reported in our previous paper [23] (Table 1). It was stored in transparent glass vials (1.5mL) (CNW. Technologies (Shanghai) Inc.) and was kept at 4◦C for further uses.

Table 1. *A. absinthium* essential oil dominant constituents identified through GC/MS (Source Rizvi et al., 2018c).

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Peak #	RT[a]	Compounds Name[b]	Relative %	KI(Exp)[c]
1	12.351	β-myrcene	0.86	1147
2	13.291	Pinocarvone	0.62	1172
3	21.838	α -Gurjunene	1.68	1416
4	22.976	α -Humulene	0.94	1452
5	23.811	α-Copaene	3.51	1478
6	24.054	g-Curcumene	0.45	1486
7	24.754	*epi*-Cubenol	2.67	1508
8	26.599	β -Calacorene	2.10	1570
9	26.737	(-)-Spathulenol	1.94	1575
10	26.861	Germacrene-D-4-ol	3.48	1579
11	27.559	Guaiol	19.34	1602
12	27.837	Thujol	2.69	1620
13	28.356	4-*epi*-Cubedol	1.68	1631
14	28.64	Cubenol	1.89	1641
15	29.011	γ-Eudesmol	1.19	1654
16	29.113	8-*epi*-γ-Eudesmol	1.14	1657
17	29.269	a-Cadinol	2.76	1663
18	29.844	Geranial	8.83	1686
19	31.067	Chamazulene	5.94	1728
20	31.455	1,3-Dicyclopentylcyclopentane	0.93	1746
21	32.355	Fraganol	0.95	1769
22	32.92	Tetrakis(1-methyl)-Pyrazine	2.26	1797
24	36.568	Cubedol	1.16	1941
25	36.748	Geranyl-*p*-Cymene	1.63	1948
26	37.999	Nerolidol-epoxyacetate	1.12	1999
27	38.176	Geranyl-α-terpinene	5.64	2007
28	39.549	Spathulenol	0.83	2066
29	40.341	Heneicosane	1.60	2100
30	40.507	Eugenol	1.21	2102
31	41.557	Carvacrol	5.47	2147
32	41.721	α-Bisabolol	6.17	2166
33	43.784	1-ethyl-4-methoxy-benzene	0.53	2256
34	44.735	Tricosane	1.48	2300
35	44.931	1-Heptatriacotanol	1.03	2309
36	48.786	Pentacosane	2.20	2500
37	52.539	Heptacosane	1.28	2700
38	56.106	Nonacosane	0.80	2899
		Total identified	99.9	
		Oil yield (%)	0.46	
		Monoterpenes	20.42	
		Sesquiterpenes	52.69	
		Others	26.89	

[a] Retention time.
[b] Compounds are listed in order of their retention time.
[c] Retention index relative to C_7-C_{40} *n*-alkanes on a DB-1 (30 m x 0.22 mm i.d., 0.25 µm film thickness).
[e] Identification methods: RI, based on comparison of calculated RI with those reported in Adams or NIST 08 and previous literature.

2.3. Settling behavior of Diaphorina citri

The attractiveness and settling behaviour of *D. citri* adults towards sweet orange seedlings (10-15cm in length) sprayed with different concentrations 0.1, 0.5, 1, 2, 3% w/v of ABEO, diluted in 20% ethanol containing 0.01% Tween80, which corresponds to the dosage of 1, 5, 10, 15, 20 and 30 mg/mL respectively were observed in a choice experiment under controlled laboratory condition. One hundred adults (50 male and 50 female) were released into the centre of cages (60 cm x 30 cm x 60 cm), each cage with six *C. sinensis* seedlings and one from each treatment, including control. Seedlings were sprayed with 1 mL of desired concentrations of ABEO until the product runoff and allowed to dry for one hour under the hood. The flasks were randomly positioned inside the cage but equidistant from each other. There was a total of five replicate cages. The total numbers of *D. citri* adults settling on each seedling were recorded after an interval of 12 and 24 h after release. Within 2 hours after release, the cages were examined to check the mortality due to mechanical injury while aspirating was discarded. 30 mg/mL methanolic extract of Guava was used as a positive control as many reports show that Guava repels *D. citri* [24,25]. The numbers of *D. citri* adults settled on each seedling were compared among various treatments using one-way ANOVA, and means were separated with Tukey's HSD test (SPPSS 17.0).

2.4. Antifeedent activity of A. absinthium essential oil against D. citri

The feeding activity of *D. citri* was evaluated by the amount of honeydew excreted by the adults while they were kept to *C. sinensis* seedlings treated with ABEO. The feeding bioassay arenas consisted of agar-coated mini glass Petri dishes. Briefly, 3 mL of 1.5 % agar solution was added to 60-mm-diameter mini glass Petri dishes to form a solidified bed. Freshly excised leaves from *C. sinensis* grown in the laboratory were used for all bioassays. The leaf disk, average size 5.50 ± 0.3 cm in length, was dipped for 5 sec in the desired concentrations of ABEO and allowed to dry for one hour under the fume hood. Ten CO_2 anesthetized *D. citri* adults were released in each petri dish, and the Petri dishes were capped with a lid lined with 60 mm filter paper. After one hour after release, the petri dish was examined to check the mortality due to mechanical injury while aspirating was discarded. Then the Petri dishes were closed with lab parafilm and turned upside down. The filter papers were removed and immersed in 1% w/v ninhydrin 48 h after release for three minutes and then dried at room temperature [26]. The amino acid present in the honeydew on filter paper reacts with ninhydrin forming dark purple spots. The feeding activities of *D. citri* were assessed by counting the number of purple spots under the stereomicroscope.

2.5. Ovicidal toxicity of ABEO

Ovicidal activities ABEO against *D. citri* were evaluated by confining the eggs containing sprayed *C. sinensis* seedlings with an aerial insect net (25 cm long, 20 cm wide). Sixteen mixed populations (8 male and eight female) of *D. citri* adults were aspirated. The aspirated psyllids were released into gauze nets for five days by covering them with an aerial insect net. After five days, the psyllids were removed from the gauze nets, and the number of eggs was counted under a stereomicroscope. The seedlings were sprayed using Shuaiyu SY-1-8 mini plastic trigger sprayer with the desired essential oil concentrations (approximately 1 mL per seedling). Each foliage shoot on a potted plant was sprayed twice. The plant was sprayed to runoff and allowed to dry for one hour under a fume hood. Then the seedlings were again confined with gauze nets and placed under laboratory condition, and the number of hatched nymphs were counted until all the eggs were hatched. The ovicidal activity was assessed regarding egg mortality rate (E.M.R.) using the formula below.

$$\text{EMR (\%)} = \frac{\text{Number of eggs unhatched}}{\text{Total number of eggs laid}} \times 100$$

2.6. Effect of ABEO on fitness and development of Diaphorina citri

All the conditions were like the ovicidal toxicity bioassay. The *C. sinensis* seedlings were sprayed with LC_{20} concentration of ABEO. Seedlings were allowed to air dry under the hood for one hour and then exposed to five male and female virgin adult psyllids for mating and oviposition. Treated seedlings were covered with an aerial insect net. A hundred eggs were counted in treated and untreated tests on seedlings. The seedlings were observed daily until adult emergence was complete. The data of development time from eggs to adults formation, after adult formation as pre-oviposition, oviposition, and fecundity were calculated using age-stage, two-sex, life table software [27-29], the population growth rate (PGR.) was calculated using the equation [30,31].

$$PGR = {(Nf/NO)}/{\Delta t}$$

Whereas

N_f = Final number of *D. citri*

N_0 = Initial number of *D. citri*

Δt = Total number of days for the experiment

The result with positive values indicated an increasing population, PGR= 0 indicated a stable population, while negative values indicated a decline in population and led towards extinction.

2.7. Toxicity against non-targeted organisms

To evaluate the toxicity of ABEO against *Apis mellifera*, No-choice feeding bioassays were used [32] under laboratory conditions in a plastic container (0.5 L) with a thin net inserted in the cap following the procedure. Briefly, the following concentrations of ABEO (24, 36, 48, 60, and 72 mg/mL) were prepared in 50% sugar solution. Ten healthy foraging workers were introduced into the container. Each concentration was repeated thrice, and the control contained only 50% sugar solution. The mortality data were recorded within 48 h after exposure. The workers' bees were considered dead when they showed no movement upon probing with a camel brush. Each container was considered a single treatment and replicated five times. Imidacloprid (ug/mL) was used as a positive control.

2.8. Statistical analysis

Chi-square goodness of fit tests were used to evaluate the significance of choice between treated and untreated seedlings. The toxicity data was assessed by using Probit analysis to determine the 50% lethal concentration (LC_{50}) and the 90% lethal concentration (LC_{90}) (SPPSS 17.0). According to Levene's test, all data sets were homoscedastic, and the mean difference between treatments was separated by using Tukey's HSD test. The population parameters of *D. citri* were calculated using the TWO-SEX LIFE TABLE program. The values of population and age stage parameters of *D. citri* e.g., R_0, r, k and s_{xj}, f_{xj}, l_x, m_x, e_{xj}, v_{xj}, respectively, were calculated as described in the methodology of Chi (1988), Jaleel et al. (2018), and Saeed et al. (2022). The following equations explain the two-sex life table calculations.

$$lx = \sum_{j=1}^{k} S_{xj} \quad (1)$$

$$m_x = {\sum_{j=1}^{k} S_{xj} f_{xj}} \Big/ {\sum_{j=1}^{k} S_{xj}} \quad (2)$$

$$R_{\circ} = \sum_{x=0}^{\infty} l_x m_x \tag{3}$$

$$\sum_{x=0}^{\infty} e^{r(x+1)} l_x m_x = 1 \tag{4}$$

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{k} s'_{iy} \tag{5}$$

$$v_{xj} = \frac{e^{r(x+1)}}{S_{xj}} \sum_{i=x}^{\infty} e^{-r(x+1)} \sum_{y=j}^{k} s'_{iy} f_{iy} \tag{6}$$

3. Results

3.1. Effect of ABEO on settling behavior of Diaphorina citri

Concentration and time-dependent effects in settling *D. citri* adults were observed. The settling behavior of *D. citri* adults was not significantly different among the various ABEO concentrations tested compared with the control at 24 h (F= 18.98; *df* =4, 24; *P*= 0.243) after release. However, significant differences were observed after 48 h (F= 66; *df*= 4, 24; *P*= 0.005) and 72 h (F= 86; *df*= 4, 24; *P*= 0.001 after release **(Figure. 1)**. After 72 h, more *D. citri* adults were observed on control plants than on any of the ABEO treatments. However, no considerable repellant effect of 30 mg/mL methanolic extract of Psidium guajava was observed as a positive control during the settling behavior cage trails.

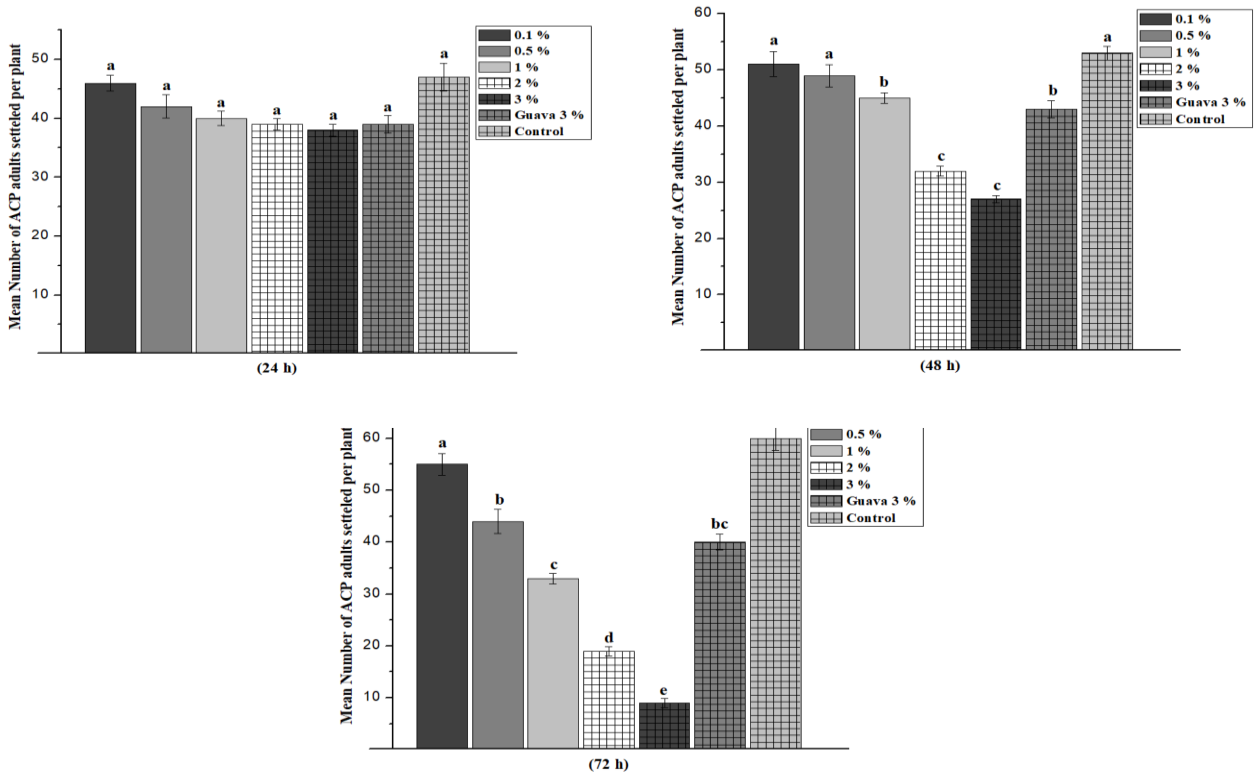

Figure 1. Settling preference of ***D. citri*** adults on sweet orange seedlings treated with various concentrations of ABEO 24, 48, and 72 h after release of adults. Bars within a panel not labeled by the same letter are significantly different according to Tukey's test ($P < 0.05$). A 3 % methanolic extract of Guava was used as a positive control.

3.2. Effect of ABEO on Diaphorina citri feeding

The feeding activity of *D. citri* measured by the amount of honeydew extraction was presented in **(Figure. 2)** The data indicated a concentration-dependent antifeedant effect of ABEO on the feeding activity of *D. citri*. Except for 1 mg/mL of ABEO assessed, all the treatments 5, 10, 20, and 30 mg/mL significantly reduced the amount of honeydew excretion compared to the control (F=84.47; *df*= 4, 24; P<0.0001). However, there was a reduction of 92 and 86% honeydew excretion by ABEO at 20 and 30 mg/mL. However, the effect of ABEO on *D. citri* feeding in term number of honeydew droplets recorded per filter paper disc is lower than cyantraniliprole, a synthetic anthranilic diamide insecticide, which caused an 80 % reduction in honeydew droplets secretion by *D. citri* at 0.1 µg/mL [33].

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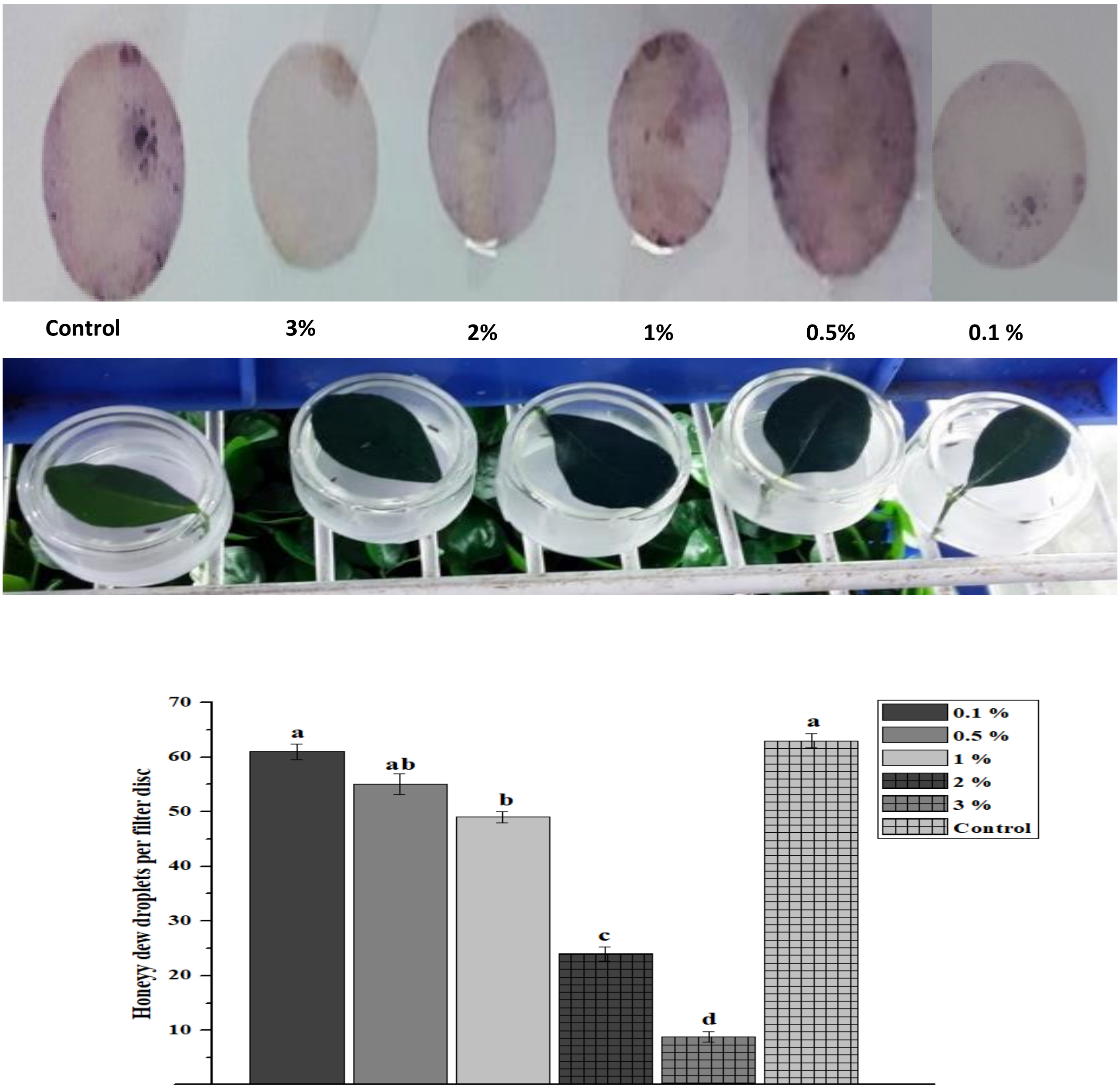

Figure 2. Effect of ABEO on ***D. citri*** adult feeding as measured by the number of honeydew droplets produced. Citrus leaf discs treated with various concentrations of ABEO in 20% ethanol+ 0.05 % or 20% ethanol+ 0.05 % Tween 80 (as control) were exposed to 10 ***D. citri*** adults. Bars not labeled by the same letter are significantly different from one another according to Tukey's test ($P < 0.05$).

3.3. Effect of ABEO on eggs hatchability of D. citri

A concentration-dependent response of ABEO on eggs hatchability of ***D. citri*** was observed. The ABEO has shown potent ovicidal activity with LC_{50} 5.88 mg/mL **(Table. 2)**. The number of eggs hatchability per plant was significantly lower than the control except for 1 mg/mL ABEO (F=63.82; *df*= 5, 29; P <0.0023). Whereas sweet orange potted plants treated with 30 mg/mL ABEO only 11.75% of eggs could hatch into adults, followed by 5, 10, and 20 mg/mL, only 30.44, 72.46 and 83.65% of eggs were able to hatch into adults. However, ABEO ovicidal activity is lower than cyantraniliprole, a synthetic anthranilic diamide insecticide, which caused complete inhibition of ***D. citri*** egg's hatchability at 0.025 µg/mL [33].

Table 2. Ovicidal activity of ABEO on ***D. citri*** eggs hatchability measured by number ***D. citri*** adult emergence.

Treatment	% of egg mortality	LC50 (95% CL) mg/mL	Slope ± SE	X^2 (*d.f.*)	*P*-value
Control	7.76 ± 1.37	5.88 (2.27-12.09)	1.40 ± 0.134	16.83 (3)	0.326
1 mg/mL	14.78 ± 1.87				
5 mg/mL	29.00 ± 1.67				
10 mg/mL	55.67 ± 0.89				
15 mg/mL	80.42 ± 0.65				
30 mg/mL	92.44 ± 0.28				

[a] Eggs containing sweet orange potted plant were sprayed with 1 mL of different concentrations of ABEO.
[b] 50% lethal dose.
[c] 90% lethal dose.
[d] 0.025 µg/mL of cyantraniliprole caused complete inhibition of eggs' hatchability.

3.4.1. Population parameters

The intrinsic rate of increase (*r*) was higher on untreated sweet orange (0.10 d^{-1}) than treated with LC_{20} concentration of ABEO (0.07 d^{-1}). Similarly, the net reproductive rate (R_0) was higher for untreated *C. sinensis* seedlings (14.21 offspring) than treated (6.40 offspring) with LC_{20} concentration of ABEO **(Table 3)**.

Diaphorina citri, comprehensive age-stage survival rate (sxj) on treated and untreated *C. sinensis* seedlings, was determined. Our findings showed the possibility of a freshly hatched larva making it to age *x* and stage *j* **(Figure. 3)**. Because development rates varied across individuals on treated and untreated seedlings. The projected curves exhibited completely different layouts at each developmental stage. Individual survival rates rapidly dropped with age increased and showed an inverse relationship between treated and untreated seedlings **(Figure. 3)**. The developmental time of *D. citri* female was longer, and the survival rate was shorter on untreated than treated. While in the case of males, the development was shorter, and the survival rate was longer on untreated than on treated *C. sinensis* seedlings **(Figure. 3).**

The highest value of age-stage specific fecundity (f_{xj}) was higher on untreated sweet oranges as compared to treated **(Figure. 3)**. There is a direct relation in age-specific maternity (l_x*m_x) of *D. citri*. As the survival rate increases, fecundity increases in treated and untreated cases. However, the constant peak point of age-specific maternity (lx*mx) of D. citri was higher on untreated sweet oranges than on treated *C. sinensis* seedlings with ABEO **(Figure 4)**.

The effects of treated and untreated sweet oranges on the population's expected average life expectancy (exj) at egg, nymph, and adults stages of *D. citri* were determined in **(Figure. 5)**. The longevity of the newly hatched *D. citri* eggs was longer on untreated sweet oranges as compared to treated. The maximum point of the graph on untreated sweet oranges eggs at zero age day their life expectancy (e_{xj}) was higher on untreated sweet oranges as zero age day **(Figure. 5)**. With heterogeneity in various developmental phases, an increasing tendency in female adult expectancy was identified on untreated sweet oranges in comparison to treated with ABEO. Overall, all stages of the highest life expectancy (exj) of *D. citri* were observed on untreated sweet oranges **(Figure. 5).** Age-stage reproductive value (v_{xj}) of *D. citri* in **(Figure. 6)** explains an individual's contribution to the future population (i.e., the population forecasting scale) at age *x* and stage *j*.

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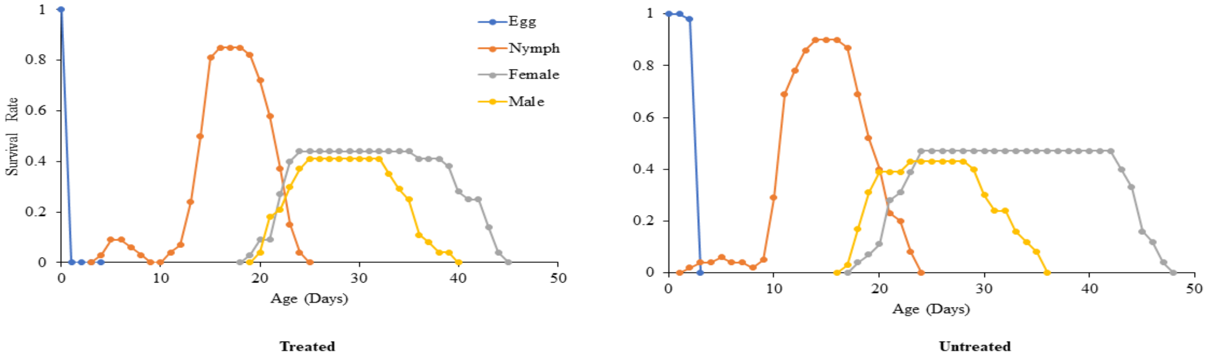

Figure 3. Effect of ABEO on the age-stage-specific survival rate (s_{xj}) of the *D. citri* on sweet orange seedlings in comparison to untreated sweet orange seedlings.

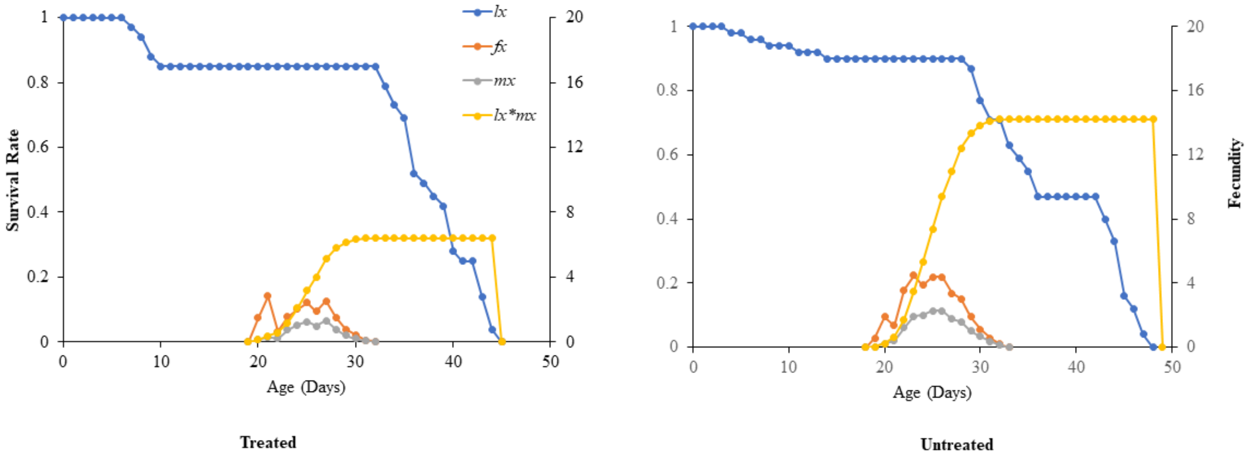

Figure 4. Effect of ABEO on the age-specific survival rate (l_x), female age-specific fecundity (f_x), age-specific fecundity (m_x), and age-specific maternity (l_x*m_x) of the *D. citri* on sweet orange seedlings in comparison to untreated sweet orange seedlings.

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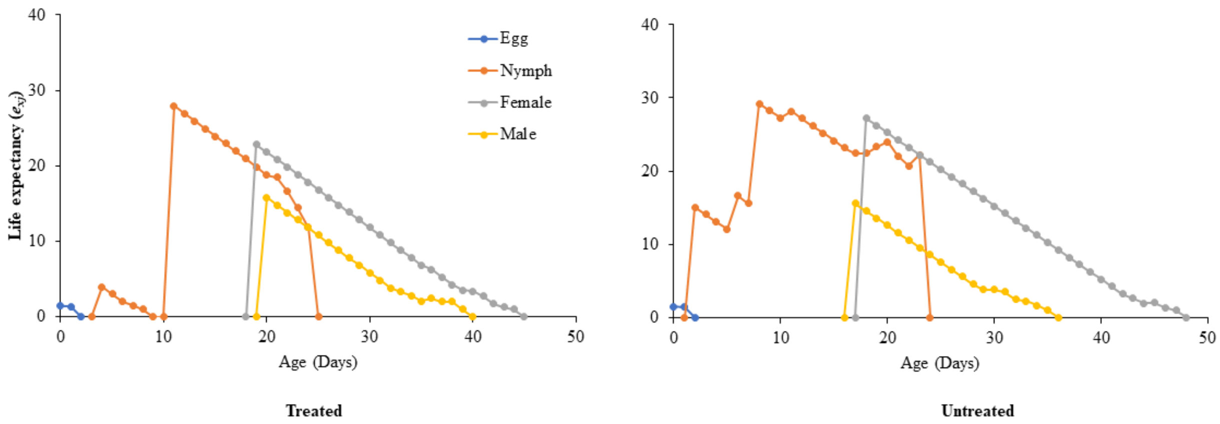

Figure 5. Effect of ABEO on the life expectancy (e_{xj}) of the *D. citri* on sweet orange seedlings compared to untreated sweet orange seedlings.

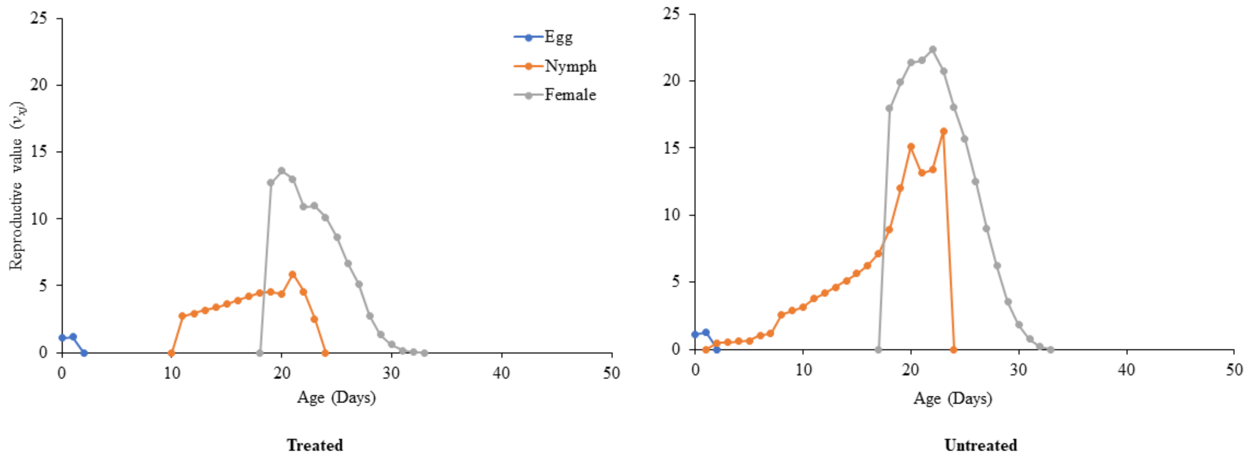

Figure 6. Effect of ABEO on the reproductive value (v_{xj}) of the *D. citri* on sweet orange seedlings in comparison to untreated sweet orange seedlings.

Table 3. Effect of ABEO on reproductive and population parameters of the *D. citri* on treated sweet orange seedlings compared to untreated sweet orange seedlings.

Traits	Treated	Untreated
r (per day)	0.07	0.10
λ (per day)	1.07	1.11
GRR (offspring)	7.53	16.02
R_0 (offspring/individual)	6.40	14.21

r; The intrinsic rate of increase (per days)

λ; The finite rate of increase (per days)

GRR; Gross reproductive rate (offspring)

R_0; The net reproductive rate (offspring/individual)

3.5. Toxicity of ABEO against Aphis mellifera

The ABEO caused toxicity against *A. mellifera* at significantly higher concentrations, with LC_{50} and LC_{90} 31.05 and 55.86 mg/mL, which is too high compared to the lethal concentration of ABEO against *D. citri* 5.20 µg/insect [15] **(Table 4)**. Therefore, ABEO can be considered safe against *A. mellifera.*

Table 4. Toxicity of *A. absinthium* essential oil against *Aphis mellifera.*

Concentration mg/mL	Exposed[a]	% Mortality±SD	LD50(LCL-UCL)[b]	LD90(LCL-UCL)[c]	X^2 (d/f)[d]	P-value
24	64	21.65±0.87	31.05(25.58-34.69)	55.86(46.01-67.81)	0.94(2)	0.23
36	60	35,32±0.59				
48	67	52.01±0.87				
60	67	65.34±0.60				
72	63	84.32±0.51				
0	65	4.11±0.24				
Imidacloprid (ug/mL)	60	98±0.11	(17.21)11.45-24.34	71.32 (86.32-92.32)	4.11 (3)	0.51

[a] Total number of bees treated.

[b] 50 % lethal dose.

[d] 90% lethal dose.

[e] $\chi 2$ chi square, *d.f.* degrees of freedom.

4. Discussion

Botanicals insecticides are plant-derived materials and include pyrethrin, azadiractin and neem oil, garlic, capsaicin, and vegetable oil. Botanicals are generally short-lived in the environment, as they are broken down rapidly in the presence of light and air [34]. These include plant extracts and essential oils, which are eco-friendly, biodegradable, and nontoxic to mammals [15]. Essential oils from various plants are reported having antifeedant, repellent, and toxic against many insect pests [35]. These comprise various compounds but are dominated mainly by monoterpenes and *sesquiterpenes [36]. These m*onoterpenes and sesquiterpenes can be lethal against insects in different ways. For example, limonene decreased oviposition in mite *Oligonychus ununguis* [37], carvacrol and thymol showed contact toxicity against *Pochazia shantungensis* [38], α-pinene, (-)-limonene and (-)-carvone showed fumigant and antifeedant activities against *Solenopsis invicta* and Meloidogyne incognita [39], thymol showed substantial contact toxicity against *Blattella germanica* (Yeom et al., 2012), and 1,8-cineole showed both contact and fumigant toxicities against *Sitophilus oryzae* and *Ectomyelois ceratoniae* [40]. Carvacrol showed contact toxicity against *D. citri* [15].

Plant volatile plays a crucial role in herbivores' host location and recognition [41]. Odors and plant colors mediate how the herbivores' insects find and recognize their potential host [42]. The citrus psyllid mainly relies on its olfactory and visual cues to locate and evaluates its host plants [43]. The volatile compounds emitted from non-host plants mask the host plant odor perceived by the phytophagous insects, which results in avoidance and non-preference of host plants[44]. Regarding the repellent activity of ABEO against *D. citri*, results indicated a concentration-dependent effect. The adults strongly preferred settling on the control *C. sinensis* seedlings to the treated seedlings. The psyllids settling was not significantly reduced 24 h after release compared to the control. However, after 48 and 72 h of release, only a few adults were observed on the treated plant compared to the control as *D. citri* were able to identify host plants volatiles from the mixture of non-host volatiles in the open atmosphere in a short period, approximately nine h [45]. A literature report indicated that the host finding and recognition ability of *D. citri* were reduced when non-host plant semiochemicals were used [44,46]. Many non-host plants have shown repellent activities against *D. citri,* including Guava [3,24,47], *Allium* spp [48,49]. HLB bacteria can only multiply in the body of the eukaryotic host [50]. The transmission of HLB bacterium from infected to uninfected tree primarily takes by nymphs and adults of *D. citri* [51]. Here we found that ABEO reduces the feeding activity of *D. citri*, measured as the number of purple spots on the treated leaf disc. ABEO at 20 and 20 and 30 mg/mL reduced 72.86 and 85.5% honeydew secretion compared to control. To get more accurate data on the antifeedant activity of ABEO against *D. citri*, further investigation should be conducted using EPG (Electrical penetration graph) technology.

Essential oils are effective against several insect species. They act as growth inhibitors, toxins, deterrents, repellents, and toxicants [52]. Essential oil of azadirachtin and *Piper aduncum* against nymph and adults of *D. citri* caused 90-100% mortality in nymph and below 80% in adults having the edge of nontoxic to ectoparasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) [53]. Similarly, the EOs from *Syzygium aromaticum, Eucalyptus obliqua , Tithonia diversifolia and Citrus limonial* showed considerable toxicity and repellent effects against *D. citri* [54,55]. Primarily *D. citri* management was prodigiously focused on controlling adult psyllids. For this, many classes of insecticides have been utilized [56]. Limited literature is available regarding the developmental and ovicidal products against *D. citri*. The current investigation showed that potted *C. sinensis* seedlings treated with ABEO give a concentration-dependent ovicidal activity. Only 11.75% and 30.44 eggs were able to hatch into adults when confined with the dry residue of ABEO at 20 and 30 mg/mL, while in control and 10 mg/mL concentration, the hatching percentages were 93.45 and 93.78%, respectively. The result showed that ABEO has ovicidal activity against *D. citri*. Neurotoxicity is the possible mode of action of EOs against insects [57,58]. The EOs are generally composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes, which have a wide range of hydrophilic-hydrophobic properties which are able to easily penetrate insect cuticles and interfere with their physiological functions [59,60].

Despite the most promising properties of EOs as a natural insecticide, many technical issues are raised for their broader application due to their rapid volatility, and poor water solubility [13]. There are many challenges and constraints related to the commercialization of essential oils, including strict legislation [61], low persistence of effects, and lack of quality and insufficient quantities of raw materials for affordable prices [62]. Their rapid degradability and low persistence may significantly reduce the toxicity of EOs [62]. However, the efficacy and persistence of EOs can be enhanced by encapsulation, nanoparticles and nano gel formulation, and cyclodextrins (CDs) [63]. Overall, EOs have the potential to develop eco-friendly candidates for novel pest management, which should be a top priority to preserve ecosystems from contamination.

Essential oils and plant extracts are safer for the environment, humans, and non-targeted organisms than synthetic insecticides [64]. In our current investigation, the toxicity ABEO was evaluated against *A. mellifera*. The result indicated that EOs have lower toxicity, as the LC_{50} value was higher than 31.5 mg/mL compared to the LC_{50} value of 5.20 µg/insect against *D. citri*.

5. Conclusions

The current study concluded that *A. absinthium* essential oil showed repellent ovicidal activities against adult citrus psyllids, with minimal toxicity against honeybees tasted as non-targeted organisms under laboratory conditions. Further research should persuade regarding its broader application and impact on natural enemies. It is concluded that the *A. absinthium* essential oil might be developed as a novel prophylactic against *D. citri* with the edge of being environment friendly.

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