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Residual effect of *Citrus sinensis* and *Syzygium aromaticum* essential oils in association with temephos on *Aedes aegypti* Linn. larvae (Diptera: Culicidae) in laboratory

Efeito residual dos óleos essenciais de *Citrus sinensis* e *Syzygium aromaticum* em associação com temefós sobre larvas de *Aedes aegypti* Linn. (Diptera: Culicidae) em laboratório

Efecto residual de los aceites esenciales *Citrus sinensis* y *Syzygium aromaticum* en asociación con temefos sobre larvas de *Aedes aegypti* Linn. (Diptera: Culicidae) en laboratorio

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Abstract

The increase of *Ae. aegypti* resistance to conventional insecticides and growing public concern about the environmental impact has resulted in the development of alternatives to mosquito control. Thus, the use of essential oils (EOs) in combination with larvicides may be a strategy used to reduce the phenomenon of resistance. Controlling larvae with insecticides for a prolonged period is important for interrupting transmission of vector-borne viruses, as long lasting larvicidal formulations reduce health agents visits with a consequent reduction in the global cost for controlling mosquito spread. Previous studies have shown that synergistic interactions between temephos and EOs exhibited increased larvae lethality when compared to temephos alone. Therefore, the goal of this work was to evaluate the residual effects of associations between temephos and EOs of *Citrus sinensis* and *Syzygium aromaticum* homogenized in hydrogel and impregnated in ceramic supports on *Ae. aegypti* larvae. After application of the products in 1,000 mL beakers, 25 larvae were deposited in each beaker and every five days half of the beakers were emptied to

200 mL, the original volume was replaced and new batches of larvae were added. Mortality was observed after 48 h of larval exposure. There was equivalence in the residual effect of association temephos/EOs when compared with temephos alone.
Keywords: Larvicide; Formulation; Ceramics; Mortality ratio; Dengue; Zika.

Resumo
O aumento de resistência do *Ae. aegypti* a inseticidas convencionais e a crescente preocupação pública com o impacto ambiental resultaram no desenvolvimento de alternativas para o controle do mosquito. Assim, o uso de óleos essenciais (OEs) em combinação com larvicidas pode ser uma estratégia utilizada para otimizar o controle do mosquito. O controle de larvas com inseticidas por um período prolongado é importante para interromper a transmissão de vírus transmitidos por vetores, pois as formulações larvicidas de longa duração reduzem as visitas dos agentes de saúde com consequente redução do custo global para o controle da disseminação do mosquito. Estudos anteriores mostraram que as interações sinérgicas entre temefós e OEs apresentaram maior letalidade das larvas quando comparadas ao temefós sozinho. Portanto, o objetivo deste trabalho foi avaliar os efeitos residuais de associações entre temefós e OEs de *Citrus sinensis* e *Syzygium aromaticum* homogeneizados em hidrogel e impregnados em suportes cerâmicos sobre larvas do *Ae. aegypti*. Após aplicação dos produtos em béqueres de 1.000 mL, 25 larvas foram depositadas em cada béquer e a cada cinco dias metade dos béqueres foram esvaziados para 200 mL, o volume original foi reposto e novos lotes de larvas foram adicionados. A mortalidade foi observada após 48 h de exposição larval. Houve equivalência no efeito residual da associação temefós/EOs quando comparado ao temefós isolado.
Palavras-chave: Larvicida; Formulação; Cerâmica; Razão de mortalidade; Dengue; Zika.

Resumen
El aumento de *Ae. aegypti* resistencia a los insecticidas convencionales y la creciente preocupación pública por el impacto ambiental ha resultado en el desarrollo de alternativas para el control de mosquitos. Así, el uso de aceites esenciales (AE) en combinación con larvicidas puede ser una estrategia utilizada para reducir el fenómeno de la resistencia. El control de larvas con insecticidas durante un período prolongado es importante para interrumpir la transmisión de virus transmitidos por vectores, ya que las formulaciones larvicidas de larga duración reducen las visitas de los agentes de salud con la consiguiente reducción del costo global para el control de la propagación de mosquitos. Estudios previos han demostrado que las interacciones sinérgicas entre temephos y AE exhibieron una mayor letalidad de larvas en comparación con temephos solo. Por tanto, el objetivo de este trabajo fue evaluar los efectos residuales de asociaciones entre temefos y AE de *Citrus sinensis* y *Syzygium aromaticum* homogeneizados en hidrogel e impregnados en soportes cerámicos sobre larvas de *Ae. aegypti*. Luego de la aplicación de los productos en vasos de 1.000 mL, se depositaron 25 larvas en cada vaso y cada cinco días se vació la mitad de los cubiletes a 200 mL, se repuso el volumen original y se agregaron nuevos lotes de larvas. La mortalidad se observó después de 48 h de exposición larvaria. Hubo equivalencia en el efecto residual de la asociación temefos/AE cuando se comparó con temefos solo.
Palabras clave: Larvicida; Formulación; Cerámica; Tasa de mortalidad; Dengue; Zika.

1. Introduction

With the emergence of resistance to commercial larvicides, research actions into new effective and ecologically safe products are alternatives to mosquito control. Plant EOs represent strong natural candidates in the discovery of new larvicidal agents (Şengül Demirak & Canpolat, 2022). Within this scope, the use of EOs as larvicidal agents have been studied because they are potential sources of biologically active substances that have the potential to reduce environmental impacts and health risks caused by application of chemical insecticides (Şengül Demirak & Canpolat, 2022).

EOs are believed to act in different ways on insects causing repellency, inhibition of oviposition and feeding, developmental disorders, deformations, infertility, and mortality with the advantage of being obtained from renewable resources and are biodegradable. Development of insect resistance to these substances, composed of the association of several active principles is a process that occurs very slowly (Şengül Demirak & Canpolat, 2022). Studies on the insecticidal properties of plants lead us to a possible vector control with low environmental impact and low cost.

Chemical and biological larvicides containing a variety of active principles such as the organophosphate temephos have been developed and recommended to control *Ae. aegypti* spreading (Norris et al., 2019). This class of insecticide irreversibly inhibits the enzyme acetylcholinesterase, important in the cholinergic transmission of nerve impulses in the mosquito CNS and in the regulation of levels of acetylcholine (G. P. C. dos Santos et al., 2022). Inhibition occurs due to a stable bond established between a hydroxyl group of the active site of the enzyme with the phosphorus atom of these compounds. Phosphorylation of serine present in the active site leads to an accumulation of acetylcholine, causing cholinergic hyperstimulation in insects with consequent hyperexcitability, tremors, paralysis, which can culminate in the death of the insect. However, the frequent and increasing use of organophosphates, in addition to causing an environmental impact, has also selected resistant populations in several locations around the world (Lesmana et al., 2022).

Eradicating larvae from breeding sites with insecticides for a prolonged period is important in controlling virus transmission. The residual effect of these products on mosquito breeding sites should last long enough to avoid additional costs with frequent re-inspections or inefficacy of vector-borne disease programmes (Hendrichs et al., 2021). Residual effect is understood as the ability of a larvicide to maintain lethal doses for a target organism for a certain period of time (Elliott et al., 1978).

To promote reduction in the use of synthetic larvicides, formulations containing more than one pesticide, aiming at a synergistic effect, have been sought as an alternative to vector control (Junkum et al., 2021; L. Santos et al., 2022). To date, there are no studies in the literature assessing the residual effect of associations between temephos and EOs with larvicidal activity. In view of this fact, the residual effect of associations between temephos and EOs of *C. sinensis* and *S. aromaticum*, using a controlled release device is presented.

2. Methodology

2.1 Essential oil extraction

C. sinensis fruit peels, in a single batch, were oven-dried (40°C for 24 h) and pulverized in a knife mill. Clove buds were obtained from the local market and pulverized in a knife mill. Both EO were obtained by hydrodistillation using a Clevenger-type extractor device for 3 h (Clevenger, 1928), placed in a glass vial and stored in a freezer at -20ºC until further use.

2.2 Analytical conditions

The essential oils obtained by water distillation were analyzed by GC/MS using a Shimadzu QP5050A equipped with a DB-5MS fused silica column (30 m • 0.25 mm; film thickness 0.25 lm), under the following conditions: helium as carrier gas at 1.0 mL/min; injector split at 250°C (split ratio 1/20); detector at 280°C, column temperature program 80°C during 1.5 min, with 4°C increase per min. to 180°C, then 10°C/min to 300°C, ending with a 10 min isothermal at 300°C. The mass spectra were obtained at 70 eV, scanning speed 0.85 scan/s from 40 to 550 Da. Peak normalization was used to calculate percentage composition. Peak identification was assigned on basis of comparison of their retention indices relative to an *n*-alkane homologous series obtained by co-injecting the oil sample with a linear hydrocarbon mixture. Components of the essential oil were identified by computerized matching of the mass spectra with the ones stored in NIST21 and NIST107 GC/MS mass spectral library data system, as well as by search of published spectral data (Adams, 2007).

2.3 Formulation and characterization of larvicidal devices

Pluronic was slowly added to jacketed beakers containing distilled water at 5ºC under mechanical agitation in Ultraturrax until adequate homogeneity was obtained (approximately 1 h) to prepare pluronic-based hydrogel at 20% in water (Peng et al., 2016). Then, the oil/temephos mixture was slowly added with continuous agitation at 5ºC to obtain a final concentration of 27.2 mL/L of EO and 16 mg/L of temephos. The polymeric solution continued to exhibit the characteristics of a hydrogel, such as homogeneity, viscosity, and transparency.

Three different models of porous ceramics (Santana et al., 2007) were characterized according to their adsorption capacity of distilled water and the formulation. The basic composition of all ceramics is alumina (Al_2O_3) and quartz (SiO_2) differing only in porosity, working as a supporting device for the formulation. Cubic ceramic fragments measuring ±1x1x1 cm (96 units total) were cut from larger blocks. Despite having lower adsorption in all samples (average 33.34% of water; 51.4% of formulation), ceramic 3 was chosen due to its ability to maintain integrity in the system. Therefore, ceramic 3 was selected for density, porosity and adsorption tests of the pure hydrogel for subsequent impregnation of the EO/T mixture. The ceramic supports were impregnated at 5°C with the pluronic-based hydrogel solutions for 24 h prior to use.

2.4 Rearing of *Ae. aegypti* larvae

Ae. aegypti (Rockefeller) eggs were kept dry, adhered to strips of paper, in insectary. Approximately 2000 eggs were placed in a rectangular polyethylene container containing one liter of filtered water and approximately 260 mg of crushed cat food (13 mg/100 larvae/day). The container was kept at a temperature of 26±2°C in order to allow the eggs hatching and mosquito development into adults, which were used to obtain eggs.

Approximately 2000 eggs were placed in a rectangular polyethylene container containing filtered water and 150 mg of crushed cat food per 100 larvae per day. The container was kept at a temperature of 26 ± 2°C and relative humidity (60 ± 10%), to allow hatching and development of larvae, when 3rd-instar larvae were collected according to size and morphological characteristics.

2.5 Residual effect

Initially, all beakers were filled to 1,000 mL and kept at rest for 48 h for acclimatization, only the evaporation water was replaced. After the storage period, six T/EO impregnated ceramics containing 1.7 mL/L of EO and 1 mg/L of T were added to 16 beakers containing one liter of filtered water. Eight were submitted to water renewal and eight were not subjected to water renewal. All beakers were properly identified with the name of the product to be applied, replicate number, and whether or not it would be subjected to water renewal. After this period, the ceramics impregnated with T/EO and those impregnated only with Pluronic-based hydrogel (negative controls) were carefully deposited with the aid of tweezers, in their respective beakers, after removing the excess product with a paper towel. Commercial temephos (100 mg formulated in sand, equivalent to 1 mg of pure temephos) was added to the positive control beakers. Then, the first batch of 25 3rd-instar larvae of *Ae. aegypti* Rockefeller strain was added to each beaker to start the test, in such a way that 800 larvae were exposed to the larvicides and 200 larvae were exposed to controls. Dead larvae were removed with the aid of a plastic Pasteur pipette.

Every five days, new batches of 25 larvae were introduced into all beakers, and 80% of the water in beakers with water renewal was replaced to simulate average dilution in household use. The water in negative control beakers was also renewed. Only the evaporated water was replaced in beakers without water renewal. Mortality (indicator of the residual effect of larvicides) was obtained after 48 h exposure (WHO, 2005).

The criterion used to define larvae as dead was the reduction of winding movements and inability to surface, when compared to control larvae. The number of live and dead larvae were counted to exclude the occurrence of cannibalism. The whole experiment lasted 95 days.

2.6 Statistics

Minitab softwareTM (version 15) was used to calculate larval mortality ratios (equivalent to the relative risks) and respective confidence intervals (95% CI). Mean, standard deviation, minimum and maximum exposure times of each replicate were calculated, in addition to calculating the mortality ratio (MR) and 95% confidence interval. Mortality ratio values above 1 mean the 1st group is more effective, values below 1 mean the 2nd group is more effective. Real differences in MR appear when the CI (95%) does not cross the value 1.

3. Results and Discussion

3.1 Essential oil extraction

The essential oils of *C. sinensis* and *S. aromaticum* were obtained in 9.65% and 20% yield, respectively. Their composition, retention indices, and percentage, listed in order of elution in the DB-5MS column, are given in Tables 1 and 2. Ferhat et al. (2006) obtained an average of 0.39% yield for extracting oil from fresh peels of *C. sinensis* fruit by hydrodistillation. In the present work, the EO was obtained from the dried fruit peels, which may have contributed to obtaining higher yield. Higher EO yields than the ones obtained from the literature were also obtained for dry flowering buds of *S. aromaticum* (Selles et al., 2020).

Seven compounds, representing 100% of *C. sinensis* EO were identified by GC/MS, while five compounds, representing 100% of *S. aromaticum* EO were identified.

GC/MS data showed D-limonene as the major constituent (88.53%), in addition to other monoterpenes such as myrcene (6.93%) and α-pinene (1.58%). Sharma and Tripathi (2008) identified 10 constituents and found 84.2% of D-limonene in orange peel EO (Table 1).

Table 1. Essential oil composition from fruit peels of *Citrus sinenis* (L.) Osbeck characterized by GC/MS.

Peak	RT (min.)	Compound	CG/MS (%)	RI exp.	RI lit.
1	6.783	α-pinene	1.58	931	932
2	8.042	Sabinene	0.63	970	969
3	8.600	Mircene	6.93	988	988
4	9.250	δ-3-carene	0.45	1008	1008
5	10.008	D-limonene	88.53	1029	1024
6	12.575	Linalool	1.43	1099	1107
7	16.542	*n*-decanal	0.45	1206	1201
Total			100.0		

RT: Retention titme; RI exp: Experimental Retention Index calculated against *n*-alkanes applying the Van den Dool equation; RI lit: Literature Retention Index. %: Compound percentage. Source: Authors (2022).

Eugenol (74.62%) was identified as the major compound in the EO of *S. aromaticum* in addition to eugenyl acetate (19.74%), *E*-cinnamaldehyde (3.30%), Ar-Turmerone (1.32%), and β-caryophyllene (1.02%) appeared as minor components. A number of studies report eugenol in *S. aromaticum* EO in concentrations varying from 34.1 to 88.5% (Table 2).

Table 2. Essential oil composition from dry flowering buds of *Syzygium aromaticum* (Mistarcea).

Peak	RT (min.)	Compound	CG/MS (%)	RI exp.	RI lit.
1	18.908	*E*-Cinnamaldehyde	3.30	1272	1270
2	21.742	Eugenol	74.62	1352	1359
3	24.025	β-Caryophyllene	1.02	1418	1419
4	27.142	Eugenyl acetate	19.74	1514	1522
5	31.700	Ar-Turmerone	1.32	1663	1669
Total			100		

RT: Retention titme; RI exp: Experimental Retention Index calculated against *n*-alkanes applying the Van den Dool equation; RI lit: Literature Retention Index. %: Compound percentage. Source: Authors (2022).

3.2 Residual effect

Chemical control of *Ae. aegypti* immature forms is an important element in controlling vector-borne diseases, along with lethal effect, the residual effect is one of the most important characteristic of a larvicide with regard to cost-effectiveness. The duration of the residual effect may have serious implications for *Ae. aegypti* control and therefore the incidence of dengue epidemics. To contribute to more effective larvicides, combinations between temephos and EO were incorporated into Pluronic F-127, a surfactant that aids controlled release because in aqueous solution at room temperature or higher, the solution will change its liquid state to a non-fluid hydrogel (Schmolka & Lundsted, 1986). Such hydrogel may help slowly releasing larvicides for longer periods, which will result in less frequent applications compared to conventional formulations.

Using larval mortality percentage and larval mortality ratio as a quantitative indicator, the formulations were analyzed for their residual effects. Water temperature ranged between 27 and 30ºC, pH (6.5) was recorded daily showing no changes after application of the products. There was no larvae mortality in the control group.

3.2.1 Residual effect of the mixture temephos/*C. sinensis* essential oil (TCSEO)

Table 3 shows larval mortality ratios (MR) as quantitative indicators of larvicidal efficacy over 60 days for the experiment with water renewal and 95 days for the experiment without water renewal, with their corresponding confidence intervals of the mortality ratio and MR (95% I.C.).

Table 3: Mortality percentage of temephos and TCSEO.

Water renewal?	**Days**	**Mortality (%)**		**Mortality Ratio (95% CI²)a/b**
		TCSEO[a]	**Temephos[1b]**	
Yes	1	100.0	100.0	-
	5	100.0	100.0	-
	10	100.0	100.0	-
	15	100.0	100.0	-
	20	100.0	100.0	-
	25	100.0	100.0	-
	30	100.0	100.0	-
	35	91.5	81.0	1.13 (0.89–1.36)
	40	53.5	28.5	1.87 (1.37–2.38)
	45	24.5	5.5	4.45 (2.69–6.21)
	50	13.0	0	0.00 (0.00–0.00)
	55	2.0	0	0.00 (0.00–0.00)
	60	0	0	0.00 (0.00–0.00)
No	1	100.0	100.0	-
	5	100.0	100.0	-
	10	100.0	100.0	-
	15	100.0	100.0	-
	20	100.0	100.0	-
	25	100.0	100.0	-
	30	100.0	100.0	-
	35	100.0	100.0	-
	40	100.0	100.0	-
	45	100.0	100.0	-
	50	100.0	100.0	-
	55	100.0	100.0	-
	60	100.0	100.0	-
	65	100.0	100.0	-
	70	100.0	100.0	-
	75	95.0	98.5	0.96 (0.76–1.15)
	80	85.0	62.5	1.36 (1.07–1.64)
	85	67.0	3.0	22.33 (16.98–27.67)
	90	9.0	0.0	0.00 (0.00–0.00)
	95	0.0	–	–

[1]: Formulated 1% temephos in sand; adding 100 mg/L (1 ppm pure temephos equivalent). [2]: 95% CI = Mortality Ratio 95% Confidence Interval. Source: Authors (2022).

During the first 30 days of exposition there was no difference in larval mortality in the experiments with water renewal (Table 3), therefore both positive control and test exhibited 100% larval mortality. Experiments with water renewal maintained mortality percentage above 80% on day 35. However, on the 40th day TCSEO showed better performance, killing 53.5% of larvae, while temephos mortality decreased to 28.5%.

A second experiment without water renewal was carried out (Table 3), which shows 100% larvae mortality in positive control and test experiments until day 70. On day 75, the mortality percentage remains above 90%. The TCSEO mixture showed better efficacy, (85% and 67% mortality on days 80 and 85, respectively), while temephos killed 62.5% and 3% of the larvae in the same period.

Table 4 exhibits the best performance of TCSEO used alone by comparing the average mortality between temephos and TCSEO with water renewal. The water renewal method was used to simulate a situation of dilution similar to that of the most important deposits in households, such as water tanks, drums, buckets and cisterns for personal use, a method that directly interferes with the persistence of larvicides in addition to degradation and, therefore, reducing the mortality period due to dilution of the larvicide.

Table 4: Average mortalities and mortality ratios for temephos and TCSEO.

Water renewal?	**Average mortality (%)**		**Mortality Ratio**
	TCSEO[a]	**Temephos**[1b]	**(95% CI[2]) a/b**
Yes	68.03	62.69	1.08 (0.82–1.34)
No	87.80	87.57	1.00 (0.98–1.02)

[1]: Formulated 1% temephos in sand; adding 100 mg/L (1 ppm pure temephos equivalent). [2]: 95% CI = Mortality Ratio 95% Confidence Interval. Source: Authors (2022).

Table 4 shows that mean mortality ratio between temephos and TCSEO groups with or without water renewal were not statistically different.

3.2.2 Residual effect of the mixture temephos/*S. aromaticum* essential oil (TSAEO)

During the first 30 days of exposure, the mortality ratio was nearly the same between temephos and TSAEO (MR approximately =1), in the experiment with water renewal (Table 5). The mortality of the formulation containing the mixture TSAEO was 100% up to 45 days, and 88 and 37% on days 50 and 55, respectively. In the experiment using only temephos, there were 41% dead larvae on day 45, and then thereafter no effect was observed.

Table 5: Mortality percentage of temephos and TSAEO.

Water renewal?	Days	Mortality (%)		Mortality Ratio (95% CI[2]) a/b
		TSAEO[a]	Temephos[1b]	
Yes	1	100.0	100.0	-
	5	100.0	100.0	-
	10	100.0	100.0	-
	15	100.0	100.0	-
	20	100.0	100.0	-
	25	100.0	98.0	1.02 (1.00–1.02)
	30	100.0	100.0	-
	35	100.0	97.5	1.02 (1.00–1.02)
	40	100.0	99.5	1.00 (0.80–1.19)
	45	100.0	41.0	2.43 (1.95–2.90)
	50	88.0	0.00	0.00 (0.00–0.00)
	55	37.0	–	0.00 (0.00–0.00)
	60	0.00	–	0.00 (0.00–0.00)
No	1	100.0	100.0	-
	5	100.0	100.0	-
	10	100.0	100.0	-
	15	100.0	100.0	-
	20	100.0	100.0	-
	25	100.0	100.0	-
	30	100.0	100.0	-
	35	100.0	100.0	-
	40	100.0	100.0	-
	45	100.0	100.0	-
	50	100.0	100.0	-
	55	100.0	100.0	-
	60	100.0	100.0	-
	65	100.0	100.0	-
	70	100.0	100.0	-
	75	100.0	99.5	1.00 (0.80–1.19)
	80	68.0	77.0	0.88 (0.67–1.08)
	85	58.0	32.0	1.81 (1.34–2.27)
	90	33.0	0.0	0.00 (0.00–0.00)
	95	0.0	–	-

[1]: Formulated 1% temephos in sand; adding 100 mg/L (1 ppm pure temephos equivalent). [2]: 95% CI = Mortality Ratio 95% Confidence Interval. Source: Authors (2022).

Results from temephos and TSAEO without water renewal show 100% larval mortality until day 70 for both formulations (Table 5). On day 75 a slight reduction in mortality of temephos alone is observed, which decreases to 77% on day 80 and subsequently drops to levels below 40% from day 85 onwards, whereas in the association TSAEO there was a slightly better performance, where 100% larval mortality lasted for 75 days and dropped to 68 and 58% on days 80 and 85, respectively, reaching 0% at day 95.

The mean mortality percentage between temephos and TSAEO groups using water renewal were not statistically different as the MR 95% CI crosses the value 1.00 (Table 6). Similarly, mortality ratios between temephos and TSAEO groups without water renewal were not statistically different (Table 6).

Table 6: Mortality ratio means for temephos and TSAEO.

Water renewal?	**Average mortality (%)**		**Mortality Ratio (95% CI[2]) a/b**
	TSAEO[a]	**Temephos[1b]**	
Yes	86.53	85.09	1.01 (0.90–1.11)
No	87.95	89.92	0.97 (0.86 –1.07)

[1]: Formulated 1% temephos in sand; adding 100 mg/L (1 ppm pure temephos equivalent). [2]: 95% CI = Mortality Ratio 95% Confidence Interval. Source: Authors (2022).

Historically, temephos has been used as a means of controlling mosquitoes due to its long residual effect (Adetoro et al., 2022). Although temephos has been replaced by other larvicides it is still an option for pesticide management since *Ae. aegypti* resistance to this larvicide is decreasing (Palomino et al., 2022). According to Pontes et al. (2010), the residual effect of temephos under laboratory conditions exhibited 100% mortality for up to 85 days without water renewal. On the other hand, the 100% residual effect decreased to 60 days if 80% of the water is renewed every 5 days. In the field, water in the containers is constantly being renewed. In the present study, the maximum period of residual effect with water renewal was 45 days for TSAEO (Table 5).

By comparing mortalities in the experiments with TCSEO and TSAEO using water renewal, there was no difference in larval mortality (100% mortality for both experiments, Tables 3 and 5), until day 30. Then, larval mortality was higher in the experiment with the TSAEO mixture, where there was 100% mortality up to 45 days, and 88% after 50 days of exposure, while in TCSEO the mortality values dropped to below 30% starting from 45 days. Although in the experiment without water renewal TSAEO showed higher mortality percentages from day 75 to 90, the differences are negligible considering a real mosquito control scenario.

The formulation containing TSAEO with water renewal has average mortality percentage of 86.53%, which is higher than TCSEO (68.03%). Calculated MR = 0.78 (0.60-0.96) proves the mortality results for both formulations are different (Table 7). On the other hand, TCSEO and TSAEO formulations without water renewal exhibited average mortality of 87.80 and 87.95%, respectively (MR = 0.99 (0.98–1.01)) proving the mortality results for both formulations are not different.

Table 7: Mortality ratio means for TCSEO and TSAEO.

Water renewal?	**Average mortality (%)**		**Mortality Ratio (95% CI[1]) a/b**
	TCSEO[a]	**TSAEO[b]**	
Yes	68.03	86.53	0.78 (0.60–0.96)
No	87.80	87.95	0.99 (0.98–1.01)

[1]: 95% CI = Mortality Ratio 95% Confidence Interval. Source: Authors (2022).

The better performance of TSAEO when compared to the results of TCSEO is probably due to the higher volatility of D-limonene (the main constituent of the EO of *C. sinensis*) as compared to eugenol (the major component of the EO of *S. aromaticum*). Thus, loss of volatile D-limonene during the experiment results in mortalities similar to that of temephos alone (Araujo et al., 2016).

Associations between temephos and EOs of *C. sinensis* and *S. aromaticum* have been evaluated for their effects on *Ae.*

aegypti larvae resistant populations. Combinations of *C. sinensis* and *S. aromaticum* with temephos evaluated in field-collected populations exhibited LC95 different from that of Rockefeller strain and resistance profile similar to the resistance of temephos alone. Therefore, the authors do not recommend these associations for use as larvicides. In this study a Rockefeller strain was used and no significant differences between temephos alone and in association with the same EOs were found. In contrast, eugenol caused no resistance in mosquitoes exposed for up to 30 generations (Adhikari et al., 2022). These results suggest *S. aromaticum* essential oil would effectively help reduce future resistance over generations. However, additional research in this subject is required.

In this study two formulations were used, namely, associations between technical grade temephos and essential oils homogenized in hydrogel impregnated in ceramic supports and commercial temephos was used in the granulated formulation. This difference between the formulations may have affected the results.

4. Conclusion

The residual effect of commercial temephos lasts approximately three months without water renewal, which gives sufficient time for planning larvicide application four times a year. Similar results were found in this study when associations between temephos and EO were used. However, TSAEO showed a slightly longer residual effect than temephos. Although associations between temephos and the EOs of *C. sinensis* and *S. aromaticum* does not affect significantly the residual effect of temephos, future research may encounter other essential oils exhibiting different residual effect profiles.

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References

Adams, R. P. (2007). *Identification of Essential Oil Components By Gas Chromatography/Mass Spectrometry* (4th edition). Allured Pub Corp.

Adetoro, F. A., Anikwe, J. C., Makanjuola, W. A., Omotayo, A. I., & Awolola, S. T. (2022). Comparative Evaluation of Larvicides for Larval Source Management of Mosquitoes in Lagos, Nigeria. *Egyptian Academic Journal of Biological Sciences. A, Entomology*, *15*(1), 33–46. https://doi.org/10.21608/eajbsa.2022.221971

Adhikari, K., Khanikor, B., & Sarma, R. (2022). Persistent susceptibility of *Aedes aegypti* to eugenol. *Scientific Reports*, *12*(1), 2277. https://doi.org/10.1038/s41598-022-06302-8

Araujo, A. F. O., Ribeiro-Paes, J. T., Deus, J. T., Cavalcanti, S. C. H., Nunes, R. S., Alves, P. B., & Macoris, M. L. da G. (2016). Larvicidal activity of *Syzygium aromaticum* (L.) Merr and *Citrus sinensis* (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of *Aedes aegypti*. *Memórias Do Instituto Oswaldo Cruz*, *111*, 443–449. https://doi.org/10.1590/0074-02760160075

Clevenger, J. F. (1928). Apparatus for the determination of volatile oil. *Journal of the American Pharmaceutical Association*, *17*(4), 345–349. https://doi.org/10.1002/jps.3080170407

Elliott, M., Janes, N. F., & Potter, C. (1978). The Future of Pyrethroids in Insect Control. *Annual Review of Entomology*, *23*(1), 443–469. https://doi.org/10.1146/annurev.en.23.010178.002303

Ferhat, M. A., Meklati, B. Y., Smadja, J., & Chemat, F. (2006). An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. *Journal of Chromatography A*, *1112*(1), 121–126. https://doi.org/10.1016/j.chroma.2005.12.030

Hendrichs, J., Pereira, R., & Vreysen, M. J. B. (Eds.). (2021). *Area-Wide Integrated Pest Management: Development and Field Application*. CRC Press. https://doi.org/10.1201/9781003169239

Junkum, A., Intirach, J., Chansang, A., Champakaew, D., Chaithong, U., Jitpakdi, A., Riyong, D., Somboon, P., & Pitasawat, B. (2021). Enhancement of Temephos and Deltamethrin Toxicity by *Petroselinum crispum* Oil and its Main Constituents Against *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, *58*(3), 1298–1315. https://doi.org/10.1093/jme/tjab008

Lesmana, S. D., Maryanti, E., Susanty, E., Afandi, D., Harmas, W., Octaviani, D. N., Zulkarnain, I., Pratama, M. A. B., & Mislindawati, M. (2022). Organophosphate Resistance in *Aedes aegypti*: Study from Dengue Hemorrhagic Fever Endemic Subdistrict in Riau, Indonesia. *Reports of Biochemistry & Molecular Biology*, *10*(4), 589–596. https://doi.org/10.52547/rbmb.10.4.589

Norris, E. J., Gross, A. D., Bartholomay, L. C., & Coats, J. R. (2019). Plant essential oils synergize various pyrethroid insecticides and antagonize malathion in *Aedes aegypti*. *Medical and Veterinary Entomology*, *33*(4), 453–466. https://doi.org/10.1111/mve.12380

Palomino, M., Pinto, J., Yañez, P., Cornelio, A., Dias, L., Amorim, Q., Martins, A. J., Lenhart, A., & Lima, J. B. P. (2022). First national-scale evaluation of temephos resistance in *Aedes aegypti* in Peru. *Parasites & Vectors*, *15*(1), 254. https://doi.org/10.1186/s13071-022-05310-x

Peng, S., Lin, J.-Y., Cheng, M.-H., Wu, C.-W., & Chu, I.-M. (2016). A cell-compatible PEO–PPO–PEO (Pluronic®)-based hydrogel stabilized through secondary structures. *Materials Science and Engineering: C*, *69*, 421–428. https://doi.org/10.1016/j.msec.2016.06.091

Pontes, R. J. S., Dantas Filho, F. F., Alencar, C. H. M., Regazzi, A. C. F., Cavalcanti, L. P. G., Ramos Jr, A. N., & Lima, J. W. O. (2010). Impact of water renewal on the residual effect of larvicides in the control of *Aedes aegypti*. *Memórias Do Instituto Oswaldo Cruz*, *105*(2), 220–224. https://doi.org/10.1590/S0074-02762010000200019

Santana, G. C., Mello, A. C. S., Valerio, M. E. G., & Macedo, Z. S. (2007). Scintillating properties of pure and doped BGO ceramics. *Journal of Materials Science*, *42*(7), 2231–2235. https://doi.org/10.1007/s10853-006-1319-6

Santos, G. P. C. dos, Assis, C. R. D., Oliveira, V. M., Cahu, T. B., Silva, V. L., Santos, J. F., Yogui, G. T., & Bezerra, R. S. (2022). Acetylcholinesterase from the charru mussel *Mytella charruana*: Kinetic characterization, physicochemical properties and potential as *in vitro* biomarker in environmental monitoring of mollusk extraction areas. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *252*, 109225. https://doi.org/10.1016/j.cbpc.2021.109225

Santos, L., Brandão, L., Costa, A., Martins, R., Rodrigues, A., & Almeida, S. (2022). The Potentiality of Plant Species from the Lamiaceae Family for the Development of Herbal Medicine in the Control of Diseases Transmitted by *Aedes aegypti*. *Pharmacognosy Reviews*, *16*(31), 40–44. https://doi.org/10.5530/phrev.2022.16.7

Schmolka, I., & Lundsted, I. (1986). *The Synthesis and Properties of Block Copolymer Polyol Surfactants. Block and Graft Copolymerization.* (1st ed.).

Selles, S. M. A., Kouidri, M., Belhamiti, B. T., & Ait Amrane, A. (2020). Chemical composition, *in vitro* antibacterial and antioxidant activities of *Syzygium aromaticum* essential oil. *Journal of Food Measurement and Characterization*, 1–7. https://doi.org/10.1007/s11694-020-00482-5

Şengül Demirak, M. Ş., & Canpolat, E. (2022). Plant-Based Bioinsecticides for Mosquito Control: Impact on Insecticide Resistance and Disease Transmission. *Insects*, *13*(2), 162. https://doi.org/10.3390/insects13020162

Sharma, N., & Tripathi, A. (2008). Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiological Research*, *163*(3), 337–344. https://doi.org/10.1016/j.micres.2006.06.009

WHO. (2005). *Guidelines for laboratory and field testing of mosquito larvicides*. https://apps.who.int/iris/handle/10665/69101 (Accessed 27 Jul 2022)