

The Effectiveness of Zodia Leaves (*Evodia Suaveolens* Scheff) Oil as *Aedes aegypti* L Mosquito Repellent in Papua

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ABSTRACT

Dengue hemorrhagic fever (DHF) is a disease caused by infection with the dengue virus. It is controlled by using repellents that protect humans from mosquito bites. One of the repellents used by the community includes DEET chemicals but natural repellents used against mosquitoes such as the zodia plant are also needed. Zodia leaves (*Evodia suaveolens* Scheff) contain linalool and -pinene compounds as well as evodiamine and rutacarpine. Linalool functions by disrupting the nervous system in mosquitoes thereby causing convulsion and death. Therefore, this study aims to formulate and evaluate the zodia oil and test its effectiveness as a mosquito repellent (*Aedes aegypti* L) with repelling power method. The formulations were prepared with various concentrations of 25 %, 50 %, and 75 %. Based on the results, the protective power of Formula I (25 %) for 0, 1st, 2nd, and 3rd hours, respectively, was 100%, 100 %, 89.28 %, and 92.85 %. Furthermore, Formula II (50 % concentration) showed a protective power of 100 %, 100 %, 90.90 % and 91.66 %, while the third Formula (75 % concentration) showed a protection power of 100 %, 100 %, 96.15% and 93.33%. Therefore, it was concluded that the three zodia oil formulas are effective as a repellent against *Aedes aegypti* L mosquitoes.

Keywords: *Aedes aegypti* L., Dengue hemorrhagic fever, *Evodia suaveolens* Scheff, Repellent, Zodia oil.

I. INTRODUCTION

Dengue hemorrhagic fever (DHF) is a disease caused by infection with the dengue virus which is transmitted into the human body through the *Aedes aegypti* L mosquito. All regions in Indonesia are at risk for contracting this disease because both the pathogen and the vector are widespread in residential and public areas except in places with an altitude of 100 m above sea level. Therefore, this disease is still a public health problem and is endemic in some districts/cities in Indonesia [1]. The use of drugs and chemicals such as DEET (N, N-Diethyl-metaltoluamide) to eradicate *Aedes aegypti* L mosquitoes often

causes side effects hence, this increased the public interest in using medicinal plants that have long been used by the predecessors such as zodia [2].

Zodia (*Evodia suaveolens* Scheff) is an endemic plant to Papua used traditionally by the local people [3], it is used as a mosquito repellent, body odor remover, as well as wound and toothache medicine [2]. Furthermore, zodia belongs to the Rutaceae family which contains evodiamine. According to the results of a gas chromatographic analysis conducted at the Research Institute for Spices and Medicinal Plants (Balitro), the oil distilled from the leaves of this plant contains linalool (46%) and a-pinene (13.26 %) where linalool is well known as a mosquito repellent [4-6].

Previous studies on zodia related to the pharmaceutical preparations have been carried out, such as the potential of zodia leaf essential oil (*Evodia suaveolens* Scheff) as an

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insecticide for the *Aedes aegypti* L mosquito using the electric method [7], the effectiveness of zodia leaf essential oil (*Evodia suaveolens* Scheff) lotion repellent against *Aedes aegypti* L mosquito [8], and zodia soap as a repellent against *Aedes aegypti* L mosquitoes [9]. The studies also include the protective power of liquid soap repellent mosquito of zodia leaf essential oil (*Evodia suaveolens* Scheff) against *Aedes aegypti* L mosquito [10] and the effectiveness of repellent lotion preparations with a combination of zodia leaves (*Evodia suaveolens* Scheff) and lemongrass essential oil (*Cymbopogon citratus*) against *Aedes aegypti* L mosquitoes [11]. However, products in the form of oil have not been made.

II. MATERIAL AND METHODS

Material preparation

Zodia leaf samples (*Evodia suaveolens* Scheff) were obtained from a plant garden on Kertosari Street, Sabronsari Village, West Sentani District, Jayapura, Papua. The already dark green plants were prepared at the age of 1 and then separated from the stems using a knife. The zodia leaves were then cleaned using running water, dried in the open air, and not exposed to direct sunlight for 1 day to 2 days to reduce the water content in the samples [12].

Zodia leaf essential oil

Zodia leaf essential oil was distilled using the steam distillation method for 4 hours, the leaves (1 kg) were dried in the open air and not exposed to direct sunlight. The essential oil was separated from the water using a separating funnel and then anhydrous Natriumsulphate (Na_2SO_4). The final product was obtained and stored in a closed vial bottle in a refrigerator [12].

Zodia leaves contain linalool and alpha-pinene up to 46% and 13.26% respectively. Linalool (3,7-dimethyl-1,6-octadien-3-ol) is a contact poison that increases sensory nerve activity in insects, furthermore, it causes motor nerve stimulation, causing seizures and paralysis in some insects [4]. After the wet zodia leaf samples were taken,

the results obtained after aeration was 6 kg (40%) of the dry samples.

Characterization of zodia leaf essential oil

The zodia plant contains essential oils that have the characteristics of linalool compounds which was obtained using preparative Thin Layer Chromatography (TLC) (silica Gel 60F₂₅₄) with toluene: ethyl acetate (93: 7) as eluent, detection using 10 % Sulfuric acid (H_2SO_4) reagent, and lavender essential oil as a comparison. A positive test result is indicated when the sample produces a blue color and the Rf value = 0.3 [13].

Preparation of zodia oil

Zodia oil formula was divided into three concentrations, namely 25%, 50%, and 75%, while one formula was used as a placebo. The selection of different concentrations was carried out to determine the significant difference between the three concentrations of zodia essential oil as a mosquito repellent. In contrast, the positive control used mosquito repellent sold in the market while hands with no test sample were used as a negative control.

Table 1: Zodia oil formula composition

Material	Formula (%) (v/v)		
	I	II	III
Zodia oil (mL)	25	50	75
Coconut oil(mL)	75	50	25
Total	100	100	100

The zodia oil preparations were made by mixing materials, for example, coconut oil as a carrier or solvent into a volumetric flask and then added with zodia leaf essential oil. The mixture was homogenized, poured into a 20 mL container and the composition of each oil in the container (zodia essential oil: coconut oil) is 1: 3, 2: 2, and 3: 1 (Table 1). Coconut oil functions as a carrier oil or solvent [14].

Zodia leaves (Figure 1-a) contain linalool in the essential oil which kills mosquitoes due to its function in increasing

sensory nerve activities and stimulation of motor nerves, thereby causing mosquitoes to experience paralysis and death. The essential oil was extracted using the steam distillation method due to its high volatility, such that when exposed to hot steam, it produces a distillate. Furthermore, the essential oil was obtained and separated from the water using a separatory funnel, and then with anhydrous Na_2SO_4

to bind and separate the water from the essential oil. From the sample, ± 6 kg produced 17.18 mL of essential oil hence, the average essential oil obtained was 2.86 mL/kg. The results of the organoleptic examination were in the form of a liquid-like oil, yellow with a characteristic zodia odor, and a pH of 6 (Figure 1-b).

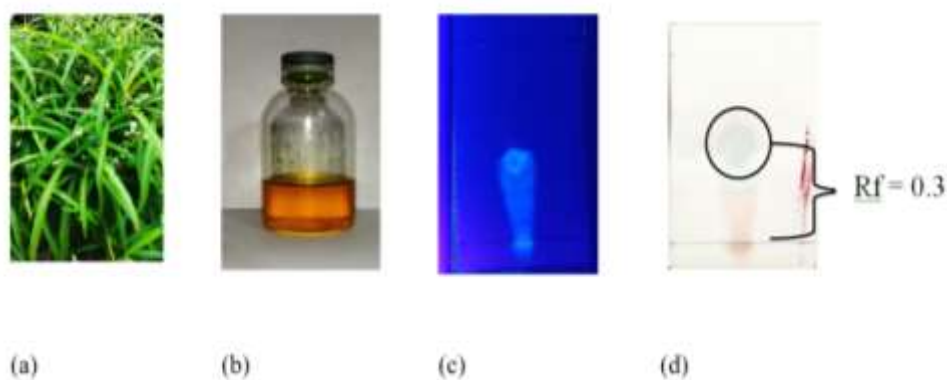


Figure 1: Zodia leaves (a); zodia essential oil from steam distillation (b); TLC test results of zodia leaf essential oil with eluent toluene: ethyl acetate 97:3), before spraying H_2SO_4 10% TLC under UV lamp 254 (c), after spraying H_2SO_4 10% (d)

The TLC test was carried out using a plate containing 60F₂₅₄ silica gel to determine the presence of linalool contained in the essential oil of zodia leaves. Furthermore, the solvent used was toluene: ethyl acetate (97: 3 v/v) while 93% toluene was used as the eluent, given that it is a non-polar solvent and was adjusted to the character of the nonpolar zodia leaf essential oil. Meanwhile, 3% ethyl acetate was added to reduce the level of the polarity of the eluent. Based on the calculated Rf value and the color of the stain compared with the data in the literature, a positive test for Linalool is indicated by a blue color and Rf value = 0.34 (Figure 1-c, d).

Collection of *Aedes aegypti* L mosquitoes

The mosquitoes were collected at 08.00-10.00 by volunteers wearing black clothes and recheck under the microscope to ensure the mosquito was *Aedes aegypti*. When a mosquito approaches a volunteer, it is caught with

a suction device that has been specially prepared and placed into a glass cage.

Research Design

The research design used is the evaluation of zodia oil preparations.

- Organoleptic test includes appearance including shape, color, and the smell of zodia oil (Yuniarsih, 2010).
- The Power of Hydrogen (pH) Acidity Test was carried out by weighing 1 gram of zodia oil and then dipping the pH paper into the solution. The degree of acidity (pH) obtained was observed and recorded [15].
- Patch Test
- The safety test was carried out on volunteers for 15 minutes, the reaction was then observed to determine the occurrence of irritation/allergy [15].
- Test of Protection as Repellent

This research was conducted and involved ethical number 01/KEPK-JYP/VII/2020. The lower left arm was smeared with repellent material, namely zodia oil, while the lower right arm was used as a negative control [16]. Hands that have been sprayed with zodia oil were left for 5 minutes, placed in the mosquito cage for 15 minutes, and then removed and placed back after 1, 2, 3, 4, 5, and 6 hours with an observation period of 15 minutes every hour to determine the protection power.

The effectiveness test of Zodia oil was carried out in a mosquito cage measuring 10 x 10 x 10 cm, the walls were made of glass and covered with nylon gauze. 3 cages were provided for testing negative control (-), positive control (+), and placebo [4-6, 10]. Each was placed in a sample of 15 *Aedes aegypti* L mosquitoes which has not sucked blood at all. The arm was sprayed with Zodia oil of each test formula as well as for the control (-) and (+).

The power of protection against mosquito disturbances was determined by the formula:

$$Pp = \frac{C-T}{C} \times 100 \%$$

Where:

Pp: Protective power

C: Numbers perched on control arm (does not contain zodia leaf oil)

T: Figures perched on arms smeared with zodia leaf oil

III. RESULTS AND DISCUSSION

Zodia oil preparation

In the formulation of zodia oil preparations, the results showed three variations namely coconut oil F I (25%: 75%), FII (50%: 50%), and FIII (75%: 25%) (Figure 2) which indicates that the formula was well formulated and homogeneous. Both of the materials were mixed and then shaken until homogeneous as observed from the absence of color separation in the two oils. Also, the volatile oil which was previously yellow became faded because it has been mixed with the coconut oil base.

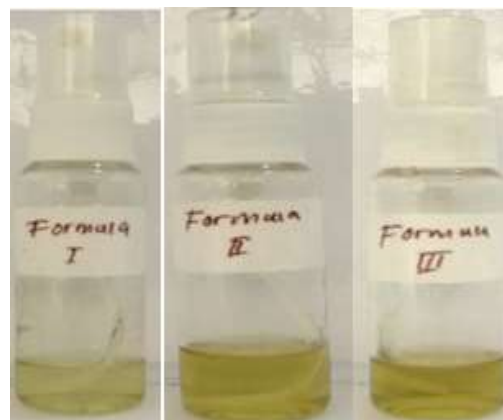


Figure 2: Preparation of zodia oil concentration of 25% (a), 50% (b), 75% (c).

Evaluation of Zodia oil Preparations

The organoleptic examination showed that F I had a faint yellow color and a characteristic smell of zodia oil, while F II and III had a faded yellow color and a very pungent smell of zodia oil due to variations in concentrations of 50% and 75% (Table 2).

Table 2: Organoleptic Results of Zodia Oils

Formula	Organoleptic Observation		
	Form	Color	Smell
Formula I	Liquid	Fade Yellow	Zodia
Formula II	Liquid	Yellow	Typical Zodia Sting
Formula III	Liquid	Yellow	Typical Zodia Sting

The average pH for formulas I, II, and III was 5, 5, and 5.3 due to several factors such as the concentration of zodia oil of 75% where the pH was 6-7 [7], and the 25% coconut oil concentration with pH of 5 hence, the pH in the first test in formula III was 6, meanwhile, in the second and third tests, the pH was 5 (Table 3).

Table 3: Zodia oil pH test results

Formula	Average of pH
Formula I	5
Formula II	5
Formula III	5.3
Placebo	5
Positive control	5

Note: Formula I concentration of zodia essential oil (25%), Formula II concentration of zodia essential oil (50%), Formula III concentration of zodia essential oil (75%), and Placebo (coconut oil).

The placebo and the positive control both had a pH of 5. The pH value of the three zodia oil formulas is

applicable because it is in line with the physiological pH range of the skin namely 4.5-6.5. When pH is too alkaline, it causes scaly skin, meanwhile, when the pH is too acidic, it causes skin irritation [16].

The safety of the preparations was tested using a patch test on 20 volunteers sprayed with zodia oil and a placebo on the back of the hand for 15 minutes. Two volunteers in formulas II and III experienced allergies in the form of itching on the hands along with redness but there was no swelling which is presumably caused by different skin types in each volunteer (Table 4). Previous studies showed that there are different types of skin namely normal, dry, and oily [16-18]. When the pH is too alkaline, it leads to dry and sensitive skin, in contrast, when the pH is too acidic it causes skin irritation.

Table 4: Zodia oil safety test

Formula	Criteria			Total panels
	No irritating	Slightly irritating	Irritating	
Formula I	20	-	-	20
Formula II	19	1	-	20
Formula III	19	1	-	20
Placebo	20	-	-	20

Note:

- No irritating Does not cause redness, does not itch and does not swell
- Slightly irritating Redness, itching, and no swelling
- Irritating Redness, itching, and swelling

Test of zodia oil protection power as a repellent for *Aedes aegypti* L. mosquitoes

The mosquitoes were collected between 08.00-10.00 given that the blood-sucking behavior of female *Aedes aegypti* L mosquitoes occurs every two to three days in the morning until the afternoon, namely 08.00-12.00 and 15.00-17.00. Female mosquitoes often bite more than one person (multiple bitter) to get enough blood. Furthermore, disease transmission occurs because every time a mosquito sucks blood, it transfers the saliva through its proboscis[19].

Adult *Aedes aegypti* has a medium size with brownish-black body color. The body and legs are covered with scales with silvery-white stripes, meanwhile, on the posterior part of the body, there are two vertically curved lines, namely the left and the right, which are characteristics of this species. In general, the scales fall out or fall off easily, hence, it is difficult to identify older mosquitoes. The size and color of *Aedes aegypti* differ between populations, depending on environmental conditions and also the nutrients obtained during the development period [20].

In this study, the essential oil was placed in a bottle and was applied by spraying [26]. The advantage of spray mosquito repellent compared to other types includes it is easier to manufacture and practical, does not cause air pollution, and saves electricity. In addition, mosquito repellent spray tends to reach hidden places such as under the bed, behind window curtains, and hung cloths [12].

The effectiveness of zodia oil as a repellent in this study was tested in three cages made of glass and covered with nylon gauze where each contained 15 *Aedes aegypti* L mosquitoes that had not sucked blood. This test was carried out every 15 minutes for 6 hours, observations were made on the protective power of each zodia oil formula, placebo, and positive control against *Aedes aegypti* L mosquitoes.

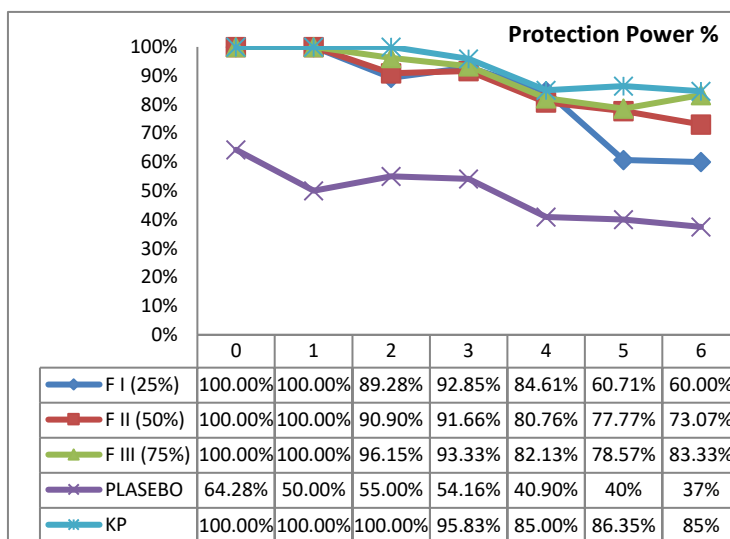
The graph in Table 5 shows that the higher the concentration of zodia oil, the greater the protective power against mosquitoes, and the higher the time, the lower the protective power. Furthermore, the protective power was observed to decrease in the 3rd to 6th hour but not as drastically in formulas II and III. The decrease in the

repellent effect was due to the zodia leaf essential oil in preparation. The sweat from the volunteers' hands removed the essential oil from the skin surface.

The mechanism of action of essential oils as a mosquito repellent is by releasing odors and repellent compounds. Meanwhile, the human skin secretes lactic acid and other excretory products that mosquitoes use to detect human odors and presence. Essential oils when applied to human skin are absorbed into the pores of the skin and evaporate in the presence of body heat, producing an odor that is detected by the mosquito's chemical receptors [21-22]

In the mosquito's anatomy, the antennae and palps function as chemical senses that are very sensitive and stimulated by chemical odors. When the essential oil evaporates, the odor released is detected by the chemoreceptors which then triggers nerve impulses. This smell confuses mosquitoes hence, the brain responds to avoid the smell. Essential oils also work by masking odors in humans, hence, the receptors on the senses are disturbed and mosquitoes are unable to detect chemical products from humans [23].

Table 5: Zodia oil protection test as a repellent for *Aedes aegypti* L. mosquitoes



F I: Formula I (Zodia leaf essential oil 25: 75 coconut oil)

F II: Formula II (Zodia leaf essential oil 50: 50 coconut oil)

F III: Formula III (Zodia leaf essential oil 75: 25 coconut oil)

Placebo: Base Control (coconut oil)

KP: Positive Control (repellent on the market)

Furthermore, the graph in Table 5, shows that the higher the concentration of the essential oil, the greater the protective power. Also, the higher the time, the smaller the protective power of the mosquito repellent. The repellency power decreased because essential oils are volatile hence, the effect was reduced gradually. The evaporation process is influenced by environmental and human factors. Environmental factors include room temperature, wind, and humidity, while human factors include body temperature and activities carried out by the volunteers during testing.

Based on the results, the repellency of zodia oil is effective, this is in line with a study where, several preparations were made and had >80% protection results such as the use of solid soap of zodia leaves essential oil as a repellent against *Aedes aegypti* L mosquitoes with a concentration of 1.5% which provided a protective power of 80.93% at 0 hours [9]. Another study also tested the effectiveness of repellent lotion of a combination of zodia leaves (*Evodia suaveolens* Scheff) and lemongrass essential oil (*Cymbopogon citratus*) against *Aedes aegypti* L mosquitoes [11] which had 100% protective power at 0 hours. Also, it was found that the effectiveness of 1.5% zodia leaf essential oil lotion against *Aedes aegypti* mosquitoes had a protection power of 88.07% at 0 hours [8]. Another study showed that the potential of zodia leaves essential oil (*Evodia suaveolens* Scheff) as an insecticide for *Aedes aegypti* L by the electric method [7] had protective power of 100% within 20-30 minutes.

Table 5 shows that the positive control had higher mosquito repellency than zodia oil at concentrations of 25%, 50%, and 75% because there was no chemical substance to maintain repellency at higher preparations. Meanwhile, the active chemical contained in the citrus peel scent is DEET with a more concentrated 130 g/L content which functions to inhibit mosquitoes for longer. Most of the mosquito repellents on the market contain the active ingredients diethyltoluamide (DEET), dichlorophenyl dimethyl phosphate (DDP), Malathion, and Parathion.

DEET works by inhibiting the chemical receptors of carbon dioxide and lactic acid in mosquitoes.

Compared with the positive control, the zodia oil formulations showed a significant difference, while the positive control showed better results. The positive control used DEET (diethyl-meta toluamide), which is more effective in repelling mosquitoes than the administration of essential oil in zodia oil. DEET functions by manipulating the smell and taste produced from the skin and inhibiting the receptors on the mosquito antennae to prevent the detection of the skin. However, there are several side effects of the DEET, for example, it is not suitable for breastfeeding mothers and children below 2 months of age. Also, in high doses and for a long time, it causes skin irritation, erythema (redness of the skin), muscle cramps, and rashes. In addition, repeated use and prolonged absorption through the skin potentially lead to poisoning especially in children [24, 27].

The continuous use of these chemicals, in addition to hurting human health, also makes mosquitoes resistant [25]. Based on the government regulations through the Pesticide Commission of the Ministry of Agriculture (1995) a repellent preparation is said to be effective when it has a protective power of >90% and lasts for 6 hours of observation. The results of this study indicated that zodia oil with an essential oil concentration of 25% protects up to >90% at the 0 to 1 hour at 100% and the 3rd hour at 92.85%, while 50% protect up to >90% at 0 to 1 hour by 100%, and the 2nd hour by 90.90% and at the 3rd hour by 91.66%. Furthermore, the zodia oil with a concentration of 75% protects up to >90% at the 0 to 1 hour at 100%, 2nd hour at 96.15%, and the 3rd hour at 93.33% (Table 5). Formula III with a concentration of 75% at the 6th hour had higher protective power because this study involved living things which led to the occurrence of range errors and other factors, namely the condition of mosquitoes on the surface of volunteers' hands due to the effect of the repellent in the previous 5 hours. Based on these results, no concentration of zodia leaf essential oil lasted

effectively for 6 hours, hence, it was concluded that the zodia oil formula as a repellent has not been maximized to repel mosquitoes for more than 6 hours, due to the absence of active ingredients such as diethyltoluamide (DEET), dichlorovinyl dimethyl phosphate (DDP), malathion, and parathion in maintaining dissipation time.

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CONCLUSIONS

Zodia leaf essential oil is applicable as an effective mosquito repellent though the positive control is higher than the test sample. From the experiment, the three zodia oil formulas with concentrations of 25%, 50%, and 75% are effective as a repellent against the *Aedes aegypti* L mosquito.

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فعالية زيت أوراق الزوديا (Evodia Suaveolens Scheff) مثل *Aedes aegypti* L طارد البعوض في بابوا

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ملخص

حمى الضنك النزفية (DHF) هي مرض تسببه الإصابة بفيروس حمى الضنك. يتم التحكم فيه عن طريق استخدام المواد الطاردة للحشرات التي تحمي الإنسان من لدغات البعوض. أحد المواد الطاردة للحشرات التي يستخدمها المجتمع تشمل المواد الكيميائية DEET ولكن هناك حاجة أيضًا إلى المواد الطاردة الطبيعية المستخدمة ضد البعوض مثل نبات الزوديا. تحتوي أوراق Zodia (Evodia suaveolens Scheff) على مركبات linalool و pinene- بالإضافة إلى evodiamine و rutacarpine. يعمل اللينالول عن طريق تعطيل الجهاز العصبي في البعوض مما يسبب التشنج والموت. لذلك ، تهدف هذه الدراسة إلى صياغة وتقييم زيت الزوديا واختبار فعاليته كطارد للبعوض (*Aedes aegypti* L) مع قوة طاردة. تم تحضير المستحضرات بتركيزات مختلفة 25% ، 50% ، 75%. بناءً على النتائج ، كانت القوة الوقائية للصيغة I 25% للساعات 0 و 1 و 2 و 3 على التوالي 100% و 100% و 89.28% و 92.85%. علاوة على ذلك ، أظهرت الصيغة II تركيز 50% قوة وقائية بنسبة 100% و 100% و 90.90% و 91.66% ، بينما أظهرت الصيغة الثالثة (تركيز 75%) قوة حماية 100% و 100% و 96.15% و 93.33%. لذلك ، استنتج أن تركيبات زيت الزوديا الثلاثة فعالة كطارد ضد بعوض الزاعجة المصرية. الكلمات الدالة: *Aedes aegypti* L، حمى الضنك النزفية، Evodia suaveolens Scheff، طارد، زيت Zodia.

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