Biotechnological Communication

The Larvicidal Efficiency of three Different Extracts of *Rhanterium epapposum* Leaves Against the Dengue Fever Mosquito Vector Larvae, *Aedes aegypti*

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ABSTRACT

Dengue fever, which is transmitted by *Aedes aegypti* L. (Diptera: Culicidae), is one of the most important diseases affecting human health and the economy in the western part of Saudi Arabia. This study has aimed to investigate the biological activities of three different extracts (ethanol, acetone and water) from leaves of *Rhanterium epapposum* Oliv. (Asteraceae) (commonly known as Arfaj). Results obtained indicate that the tested extracts had significant varying effects on *A. aegypti* larvae. The larvicidal potency of the ethanolic extract at a high concentration of 50000 ppm was up to 98% after 24 hours of application. In contrast, the acetonic and aqueous extracts caused 72% and 30% mortalities of the larvae, respectively, at the same concentration and exposure time. After 48 hours of exposure, the mortalities of the larvae increased by 99%, 86% and 35%, respectively, for the ethanolic, acetonic and aqueous extracts. Moreover, this study has illustrated that the ethanolic extract had a higher larvicidal effect with a lower LC50 value (168.15 ppm) after 24 hours. This effect was also higher than those of the acetonic (847.75 ppm) and aqueous (2278.22 ppm) extracts. Further investigations have revealed that the chemical composition of the essential oils, such as alkaloids, flavonoids, triterpenes, cumarins and tannins, in the ethanolic leaf extracts of *R. epapposum* could be responsible for their higher toxicity against the larvae of the dengue fever mosquito vector. Thus, conducting studies on the chemical constituents and bioactive chemical ingredients constancy of *R. epapposum* ethanolic extract would give good insights for developing a sustainable mosquito control method.

KEY WORDS: RHANTERIUM EPAPPOSUM (ARFAJ), DENGUE FEVER VECTOR, BOTANICAL PESTICIDES, ETHANOLIC EXTRACT, MOSQUITO CONTROL.

INTRODUCTION

Worldwide, the transmission of several diseases to humans usually occurs by the propagation of mosquito-borne diseases (Alghamdi, 2021; Benelli et al., 2021, Zuharah et al., 2021). Indeed, malaria, dengue fever, West Nile fever, and lymphatic filariasis are examples of the most dangerous diseases that some mosquitoes species can transmit (Alghamdi, 2021, Zuharah et al., 2021). In addition, mosquitoes can cause harassment to vocational, entertaining and public events by a permanent stinging on human skin. From more than 300 different species of mosquitoes that have been identified, only a few species are known to be medically important to human health. Universally, the *Anopheles gambiae Giles, Culex pipiens* L. and *Aedes aegypti* L. are the most medically important species of mosquitoes (Gubler, 1997; Miyagi and Toma, 2000; ICMR Bulletin. 2003; Peffers et al., 2021). In tropical, subtropical, and temperate zones, Dengue Fever and Dengue Hemorrhagic fever, transmitted by mosquitos, exist in more than 100 countries (Monath, 1994; Murray et al., 2013; Zuharah et al., 2021).

These diseases cause public health risks to more than 2.5 billion people globally, with about 80 million people infected annually, amounting to a 4% infection rate (Monath, 1994; Murray et al., 2013; Zuharah et al., 2021). In the western part of Saudi Arabia, especially in Jeddah Province, the second-largest city and the fundamental ingress point to the country, the significant impacts of Dengue Fever on human health and the economy are widely felt (Alshehri, 2013; Alwafi et al., 2013; Alkhaldy and Barnett, 2021).

Article Information:*Corresponding Author: kasiry@kau.edu.sa
Received 07/08/2021 Accepted after revision 29/09/2021
Published: 30th September 2021 Pp- 1226-1232

Available at: https://bbrc.in/
Article DOI: http://dx.doi.org/10.21786/bbrc/14.3.47

Commonly, most mosquitoes control programs rely on applying insecticides to mosquitoes' breeding locations targeting their immature phases (larval and pupal stages). However, the application of insecticides can be sometimes impractical and cause several environmental concerns such as water pollution that negatively impacts human households, thus threatening human existence in provincial and municipal regions (Lacey, 1990, Alghamdi, 2021). In a long term insecticides application trial, it has been shown that mosquito populations can adapt to these insecticides by developing resistance against the applied insecticides (Brogdon, 1998; Lui, 2015; WHO, 2009; Navridis et al., 2018; Al Nazawi et al., 2021).

Therefore, researchers have sought alternative methods to decrease the reliance on synthetic pesticides for mosquito control. In recent years, botanical pesticides have attracted more attention and interest as an alternative to synthetic pesticides (Asiry, 2015; Alghamdi, 2021). Naturally, plants possess several bioactive substances as secondary metabolic constituents, including alkaloids, cucurbitacin, flavonoids, glycosides, terpenoids and other compounds that play a central role in controlling mosquitos (Ghosh et al., 2012; Kour and Riat; 2021). Several studies have reported mosquito larvicides from many natural plant extractions, such as Neem, *Azadirachta indica* A. Juss (Coria et al., 2008; Howard et al., 2011; Chandramohan et al., 20016; Rasool et al., 2020), *Euphorbia hirta* Linn.

(Family: Euphorbiaceae) (Panneerselvam et al., 2013), Acacia nilotica (Vivekanandhan et al., 2018), *Citrullus colocynthis* (Rasool et al., 2020), *Artemisia annua* (Masotti et al., 2012), and other plants. However, plant varieties, parts of the plant used, age of plant parts (immature, mature or senescent) and solvent utilized throughout extraction are factors that significantly affect the effectiveness of botanical compounds that act as insecticides against mosquito larvae (Ghosh et al., 2012).In the Arabian Peninsula, Arfaj – *Rhanterium epapposum* – is an indigenous and persistent overshadow shrub traditionally used for medicinal purposes, e.g., gastrointestinal disorders and skin infections. Moreover, citizens in this area have used it as a grazing parsley for their sheep, goats and camels (Yaghmai and Kolbadipour, 1987; El-shanawany, 2016; Phondani et al., 2016, Mohammed et al., 2019; Rajendrasozhan et al., 2021).

This plant usually grows naturally without being cultivated and it is highly adapted to the arid land environment and extensively circulated in deserts, villages, havens, foothills, gorges and lowlands (Petrie, 2007; Kala et al., 2009). This plant has a wide distribution and can be found in North Africa and the deserts of Saudi Arabia, Kuwait and the United Arab Emirates (Aspinall, 2005; Petrie, 2007; Omar and Bhat, 2008). Chemically, more than 107 volatile elements, predominantly terpenoids, have been detected in the essential oil of *R. epapposum* (Yaghmai and Kolbadipour, 1987). In addition, bioactive compounds such as alkaloids, flavonoids, triterpenes, cumarins, and tannins have been reported in ethanolic extracts of aerial parts of *R. epapposum* (Eltahir and Dahab, 2019). However, studies on applying mosquito larvicides from *R. epapposum* seem to be, to some extent, rare. Consequently, under laboratory conditions, this study aimed to investigate the larvicidal activities of three different *R. epapposum* leave extracts against the 4th larval instars of the Dengue Fever mosquito vector, *A. aegypti*.

MATERIAL AND METHODS

Preparation of plant extracts: Arial parts of *R. epapposum* were obtained from Al-Nafud Desert, north of Hail region, in the northern central part of Saudi Arabia. These parts were carefully washed, and all pooled crusts such as wildflowers, dust atoms and other unnecessary substances were eliminated. Afterwards, the leaves dried under dark conditions at room temperature for four weeks. Then, the dried leaves were ground using an electric blender, and 50 g of the milled leaves were standardized with 100 ml of three pure solvents: ethanol, acetone and water. The three preparations were left in the shaker for 24 hours, at room temperature, after which they were centrifuged at 4000 rpm for 20 minutes. The floating solution, consisting of the target extract, was transferred to a 250 ml glass beaker. The solvent within the extract evaporated when heated to 60°C, and the rigid material was weighed and dissolved in recognized volumes of distilled water to obtain final concentrations of 5, 50, 500, 5000 and 50000 ppm.

Vectors rearing: Eggs of the Dengue Fever vector *A. aegypti* were obtained from the Laboratory of Public Health Pests, Jeddah Governorate (Al Amana), Saudi Arabia. Enamel and plastic trays, filled with tap water, were used for rearing the new larvae of *A. aegypti*. The reared larvae were fed on aquarium fish foods containing overall nutrients and essential vitamins. Daily, small bowls containing clean water were used for transferring pupae, which were kept in a 50×50×50 cm cage wrapped in fine mesh awaiting the emergence of the adults. Sugar solution (5%, W/V) soaked in cotton in a small conical bottle was placed inside the cage and used for male adults feeding. 1-week-old chicks were used for feeding adult females.

Bioassay and mosquito mortality: In this study, the world health organization (WHO) standard procedure (WHO, 2005) was followed to determine larvicidal actions of the leave extracts of R. epapposum. Twenty-five 4th instars larvae of A. aegypti were transferred using a dropper to test cups (250 ml), each containing 100 ml tap water, to which five known concentrations were included. The tested cups were randomly distributed into three treatments. Five concentrations with four replicates of each concentration across each solvent extract of R. epapposum were utilized, resulting in a total of 60 tested cups (three extracts × five concentrations × four replicates) being investigated in this study. A control cup treatment including only tap water was also included to make a total of 16 concentrations of solvents. The mortality of A. aegypti larvae was assessed by observing no movement and no reaction to gentle prodding and counting the dead larvae in the tested cups after 24 hours and 48 hours. The ratio of A. aegypti larvae mortality was calculated using the following equation:

$$\% \text{ of larvae mortality} = \frac{Mean\ larvae\ mortality}{Total\ of\ introduced\ larvae} \times 100$$

Statistical data analyses: Percentages of larval mortality of *A. aegypti* were subjected to the Generalized Linear Model (GLM) within SAS version 9.2 (SAS, 2009). This model was inclusive of the following factors: the concentrations of solvents (16 levels), observation time (2 levels) and their interaction as a fixed effect. At the same time, the block was included in this model as a covariate random effect. The Least Significance Difference (LSD) was applied to identify differences in treatment means where significant differences were detected. Additionally, the percentages of larvae mortality of *A. aegypti* were calculated and plotted with consistent concentrations on logarithmic probability paper to complete the corresponding log-concentration probit lines. The regression lines were produced to determine the lethal concentrations of 50% on *A. aegypti* larvae, and the probit and toxicity index (LC_{50}) were computed according to Abbot (1925), Finney (1936) and Busvine (1971).

RESULTS AND DISCUSSION

The three tested extracts, ethanol, acetone and water, of *R. epapposum* leaves varied significantly in their larval mortality percentages of *A. aegypti*. The mortality of *A. aegypti* was also affected significantly with the time of exposure of these extracts (Table 1). Moreover, the percentages of *A. aegypti* larval mortality were likewise significantly influenced by the interaction between the tested extracts and the exposure time. At the same time, there was no effect of the arrangement of experimental cups on the larval mortality of *A. aegypti* since the experiment was conducted in an homogenous environment (Table 1).

Table 1. The Generalized Linear Model (GLM) showing the analysis of variance table for the overall effects of the solvents' concentrations (C), observed time (T), their interaction (C x T) and blocking on the larvae mortality of *A. aegypti*.

Factor	DF	Sum of Squares	Mean Square	F Value	Pr > F
Concentrations of solvents (C)	15	74892	4993	243.9	0.000
Time (T)	1	1741	1741	85.04	0.000
C × T	15	904	60.23	2.94	0.001
Block	3	48.5	16.17	0.790	0.503
Error	93	1904	20.47		
Total	127	79484			

Table 2. Toxicity of acetone, ethanol and water extracts of *R. epapposum* leaves against the 4th instars larvae of the Dengue Fever mosquito vector after 24 hours of exposure.

Solvent	LC_{50} (ppm)*	95%FL	Slope±SE	Toxicity Index	χ2	DF	Sig.
Ethanol	168.15	18.35-883.81	0.46±0.04	100.00	7.68	3	0.053
Acetone	847.75	ND	0.27±0.02	19.83	13.06	3	0.004
Water	2278.22	ND	0.47±0.03	7.38	84.26	3	0.000

*Concentrations are expressed in µL/ml, FL: fiducial limits, Toxicity index = [(LC50 of the most toxic tested compound/LC50 of the tested compound)100]. ND: non-detectable

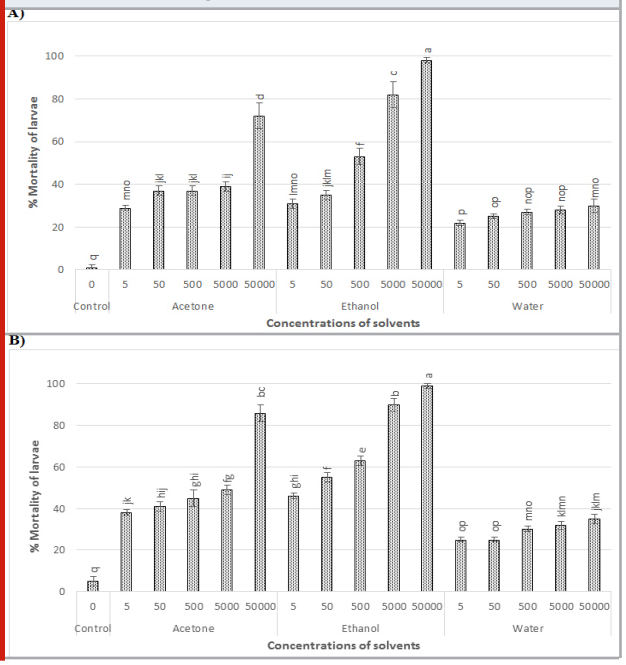
Table 3. Toxicity of acetone, ethanol and water leaf extracts of *R. epapposum* against the 4th instars larvae of the Dengue Fever mosquito vector after 48 hours of exposure.

Solvent	LC_{50} (ppm)*	95%FL	Slope±SE	Toxicity Index	χ2	DF	Sig.
Ethanol	27.39	0.12-201.71	0.49 ±0.05	100.00	13.12	3	0.003
Acetone	568.43	ND	0.29 ±0.04	4.82	22.09	3	0.004
Water	2233.25	ND	0.38± 0.03	1.22	33.31	3	0.000

*Concentrations are expressed in µL/ml, FL: fiducial limits, Toxicity index = [(LC_{50} of the most toxic tested compound/LC50 of the tested compound) 100]. ND: non-detectable

The ethanolic extract of R. epapposum was the best extract that caused higher larval mortality of *A. aegypti* in terms of both the used concentrations and time of exposure compared with acetone and water extracts. In contrast, the aqueous extract had the lowest effect on *A. aegypti* larvae mortality (Fig. 1). At the lowest concentration of 5 ppm, the percentages of larval mortality of *A. aegypti* were found to be 31%, 29% and 22% for ethanol, acetone and water extracts, respectively, after 24 hours of exposure (Fig. 1A). In this time of exposure, the ethanolic extract of *R. epapposum* was able to cause 98% mortality of the larvae at a higher concentration (50000 ppm). In comparison, the acetonic and aqueous extracts caused 72% and 30% mortalities of the larvae, respectively, at the same concentration (5 ppm) and exposure time (24 h) (Fig. 1A). After 48 hours of exposure at 5 ppm, the effect of R. epapposum leave extracts on the percentages of larval mortality of *A. aegypti* increased and was more efficient. This increment was in the order of 46%, 38% and 25% mortalities for ethanol, acetone and water extracts, respectively (Fig. 1B). Similarly, at the same exposure time (48 h), *R. epapposum* leave extracts at the highest concentration of 50000 ppm increased the mortality of *A. aegypti* larvae by 99%, 86% and 35% for the ethanolic, acetonic and aqueous extracts, respectively (Fig. 1B).

Figure 1: Percentages of larval mortality of *A. aegypti* affected by used concentrations of solvents of leaf extracts of *R. epapposum* after A) 24 hours and B) 48 hours. Means in each bar followed by the same letter are not significantly different, according to the LSD test.



The toxicity of the ethanolic extract of *R. epapposum* against the larvae of *A. aegypti* was higher, demonstrating a lower LC_{50} value (168.15 ppm) after 24 hours in which this plant extract displayed more efficacy than both the acetonic (847.75 ppm) and aqueous (2278.22 ppm) extracts that had large values of LC_{50} (Table 2). Similarly, the toxicity effect of ethanolic extract increased after 48 hours showing the lowest LC_{50} value (27.39 ppm) compared to acetonic (568.43 ppm) and aqueous (2233.25 ppm) extracts (Table 3). The slope rate of the toxicity lines of the ethanolic, acetonic and aqueous extracts of *R. epapposum* leaves were 0.46, 0.27 and 0.47; 0.49, 0.29 and 0.38 for 24 and 48 hours, respectively (Table 2 and 3).

Controlling disease vectors (mosquitoes in particular) by applying synthetic insecticides could sometimes be inefficient due to the resistance developed by disease vectors to these insecticides and their adverse effects on human health and the environment (Alghamdi, 2021; Al Nazawi et al., 2021; Zuharah et al., 2021). Therefore, scientists have sought a safe and sustainable control method such as botanical insecticides that can naturally grow and be collected from different areas worldwide (Zuharah et al., 2021).

In this study, the larvicidal efficiency of various extracts from *R. epapposum* showed solvent concentration and time dependent actions. The ethanolic extract showed higher toxicity and potentially caused 98% mortality of the larvae of the Dengue Fever mosquito vectors after 24 hours at a high concentration (50000 ppm). This effect, however, was moderate and weak when the larvae of Dengue Fever mosquito vectors were treated with the acetonic and aqueous extracts, respectively. The toxicity effect increased by increasing the exposure time of used extracts of *R. epapposum* at low concentrations (≤5000 ppm). Although studies on testing larvicidal efficiency of *R. epapposum* extracts against mosquitoes are rare, the results of this study supported by the comparative study conducted by Asiry et al. (2017), who found the ethanolic extracts from both *R. epapposum* and sweet wormwood, *Artemisia annua*, were the best extracts for controlling the 4th instars larvae of the Dengue Fever mosquito vectors compared with bitter apple, *Citrullus colocynthis*, and Fattaka, *Pergularia tomentosa*. In addition, Demirci et al. (2017) found that the essential oil of the aerial parts of *R. epapposum* had a repellent effect against the Dengue Fever mosquito vectors compared with positive control.

The chemical configuration of the vital oils, such as Alkaloids, Flavonoids, Triterpenes, Cumarins and Tannins in the ethanolic leave extract of *R. epapposum*, could be responsible for its higher toxicity against the larvae of Dengue Fever mosquito vectors. Kala et al. (2009) reported that the evaporative oil structure of aerial parts of *R. adpressum* has the rich source of sesquiterpenoid combinations such as Bicyclo [4.4.0] dec-1-ene, 2-isopropyl-5methyl-9-methylene, β-Cadinol, α-Cadinol, α-Eudesmol, β-Eudesmol, Myristicin, Spathulenol and other vital compounds. Awad and Abdelwahab (2016) reported that limonene, sabinene, a-pinene and b-myrcene were the dominant compounds in leaves essential oil extracted from *R. epapposum*. In addition, Demirci et al. (2017) identified about forty-five constituents from the essential oil of the aerial parts of *R. epapposum* with higher abundances of camphene, myrcene, limonene and a-pinene. Eltahir and Dahab (2019), in their study on the phytochemical analysis of two medicinal plants, indicated that the ethanolic extract of *R. epapposum* seemed to have more abundant compounds of Alkaloids and Flavonoids, and it had larvicidal properties

against Khapra beetle Trogoderma granarium (Coleoptera: Dermestidae).

More recently, Rajendrasozhan et al. (2021) reported several compounds within three extracts (methanol, 80% methanol and aqueous extracts) from aerial parts of *R. epapposum* such as 2-methoxy-4-vinylphenol in all three extracts, whereas ethanol, 2-methoxy-, acetate; n hexadecanoic acid; and 2,3-butanediol were existing in greater volume entirely in the methanol, 80% methanol and aqueous extracts, respectively.The current study focused on the possible application of botanical insecticides against the Dengue Fever mosquito vectors, especially essential oils that have been considered environmentally safe and alternatives to synthetic insecticides. It is thought that these botanical insecticides have broad-spectrum effectiveness, and prorated trait in their mode of action (Zuharah et al., 2021).

These botanical insecticides also contain adequate chemical constituents that could function as repellents, contact insecticides, fumigants, anti-feedings, or could disrupt and obstruct the energetic functions of disease-vectors (Shaalan et al., 2003; Tang and Yang, 2007; Rozman et al., 2007; Ghosh et al., 2012; Alghamdi, 2021; Zuharah et al., 2021). In the west of Saudi Arabia, the Dengue Fever vector A. aegypti is considered the most important mosquito species responsible for spreading Dengue Fever (Alshehri, 2013; Alkhaldy and Barnett, 2021). In the past few years, the number of cases of Dengue Fever disease has multiplied in Jeddah Province (AL-Ghamdi et al., 2009; Alkhaldy and Barnett, 2021).

These occurrences has led to the extensive application of insecticides to control the Dengue Fever vector in Jeddah. Indeed, insecticides have adverse side effects on humans health and the environment (Marshall et al., 2003; Pimentel, 2005; Palis et al., 2006; Schou et al., 2006; Winqvist et al., 2011; Alghamdi, 2021; Zuharah et al., 2021). The findings of the present study demonstrate an alternative method which can be used of synthesized insecticides for controlling the 4th instars of the Dengue Fever vector *A. aegypti* by observing larvicidal efficiencies of three extracts from *R. epapposum*. Consequently, more studies on the chemical constituents, larvicidal effectiveness and the bioactive chemical ingredients constancy of *R. epapposum* ethanolic extract are required for a sustainable control method for disease–causing mosquito vectors.

CONCLUSION

The data of this study illustrate that the ethanolic extract had a higher larvicidal effect with a lower LC_{50} value (168.15 ppm) after 24 hours. This effect was also higher than those of the acetonic (847.75 ppm) and aqueous (2278.22 ppm) extracts. Further investigations revealed that the chemical composition of the essential oils, such as alkaloids, flavonoids, triterpenes, cumarins and tannins, in the ethanolic leave extract of *R. epapposum* could be responsible for its higher toxicity against the larvae of the dengue fever mosquito vector. Thus, conducting studies on the chemical constituents and bioactive chemical ingredients constancy of *R. epapposum* ethanolic extract would give good insights for developing a sustainable mosquito control method.

ACKNOWLEDGEMENTS

The author would like to thank Mr. Fahad Al- Zahrani for accessing the Laboratory of Public Health Pests, Jeddah Governorate (Al Amana). Also, he is grateful to Dr. Nasir A. Ibrahim for helping with data collection.

Conflict of Interest: No conflict of interest.

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