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SCREENING OF *THAPSIA GARGANICA* FOR CONTROL OF *CULEX PIPIENS* MOSQUITO LARVAE

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ABSTRACT

In this study, the presence of some secondary metabolites was tested in the aerial parts of the medicinal plant *Thapsia garganica* L. by screening method, then the effectiveness of its powder using by infusion was examined at different concentrations (15, 30, 45 and 60g/ l) on newly moulted fourth-instar larvae (L_4) of *Culex pipiens* L. mosquito. The obtained results revealed that the used plant contains all cited components and exhibits a toxic effect on fourth-instar larvae. This toxicity was evident by an increase in larval mortality with respect to the concentration compared to the control group. The lethal concentrations LC_{50} and LC_{90} were estimated at 23.27 g/ l and 69.47 g/ l respectively.

Key words: Secondary metabolites, LC_{50} and LC_{90}, screening, toxicity, Culicidae, hematophageous insects, pathogens, neurotoxic insecticides, biopesticides, medicinal plants, Algeria

The class of insects encompasses numerous orders, such as Diptera, specifically the family Culicidae. This family is divided into three subfamilies: Toxorhynchitinae, Anophelinae, and Culicinae, and comprises approximately 4500 species. Commonly known as mosquitoes (Kendie, 2020), Culicidae are hematophagous insects and pose significant concerns in terms of economic development and public health. In addition to their nuisance, mosquitoes act as vectors for several pathogens that they transmit to humans. Mosquitoes and insects in general are typically controlled using conventional neurotoxic insecticides, such as organochlorines, organophosphates, carbamates, and synthetic pyrethrinoids. However, these chemicals have shown long-term adverse effects on humans and non-target species (Costa et al., 2008). As a result, researchers have been motivated to propose more selective alternative methods that do not pose any toxic effects. The advent of the biological method has introduced a new, safer, more selective, and biodegradable approach to combat mosquitoes, inducing toxic effects against various Dipteran species. This method involves the use of natural enemies, such as larvivorous fish; *Gambusia affinis* (Biard and Girard) (Bendali et al., 2001) and hydracarids; *Piona uncata* (Koenike) (Gacem et al., 2022), as well as microbiological insecticides as *Bacillus thuringiensis* (Vectobac WDG) (Boudjelida et al., 2008; Bouaziz et al., 2023), botanical biopesticides like spinosad, azadirachtin (Rehimi et al., 2011; Maiza et al., 2013) and medicinal plants (Amira et al., 2018; Djeghader et al., 2018, 2019; Dasenaki et al., 2022; Belkhiri et al., 2023). The purpose of this study is a screening for larvicidal activity of the medicinal plant *Thapsia garganica* L. (Apiaceae) against fourth-instar larvae (L_4) of the domestic mosquito *Culex pipiens* L. (Diptera-Culicidae).

MATERIALS AND METHODS

Thapsia is a genus of flowering plants in the Apiaceae family, which includes twenty species native to the Mediterranean region. Some species have uses in traditional medicine (Ladjel et al., 2011; Athmouni et al., 2015). Among them is *T. garganica*, which contains a molecule called thapsigargin that acts as a potent non-competitive inhibitor of ubiquituous enzymes (calcium/ ATPases). By doing so, it increases the calcium levels in the endoplasmic reticulum, leading to cellular apoptosis (Makunga et al., 2006). The *T. garganica* plant was collected in April, 2022 from Kheiri, Oued Adjoul (36° 51' 20.39" N,6° 08' 18.00" E) in Jijel, a coastal province situated in the northeastern region of Algeria.

The province boasts a coastline stretching 121.2 km and covers an area of 62.38 km^2. The aerial parts of the cited plant were cleaned, washed with tap water to remove any foreign matter such as soil, dried for 15 days in a dry place protected from sunlight to preserve the integrity of the molecules as much as possible and ground into a fine powder using an electric grinder. Using the obtained fine powder, phytochemical tests were evaluated to highlight the presence of certain bioactive compounds, after preparing Aqueous Extract with adding 5g of plant powder to 100ml of boiling water, which is then left to infuse for 15 minutes. Afterward, it is filtered and each test was prepared with a specific test as follows: Alkaloids using Wagner's test (Raaman, 2006), Tannins using Ferric-chloride test (EL-Haoud et al., 2018), Flavonoids using Alkaline reagent test (Singh and Kumar, 2017) and finally saponins and foam index (I) according to EL-Haoud et al., (2018). In the laboratory, the species *Cx. pipiens* was bred according to Amira et al. (2013). The powdered aerial parts of the medicinal plant *T. garganica*, were weighed in grams according to each concentration, then placed in 1 liter of hot water (infusion) and covered until the color stops changing; 15, 30, 45 and 60g/ l. After it cools down, 25 newly molted fourth-instar larvae of *Cx. pipiens* were placed in testing plastic cups containing 300 ml of each concentration, with three replicates for each one. After 24 hr exposure period, the water was changed and food was provided. The deaths were recorded daily until the end of the instar (Amira et al., 2013). For statistical analysis, The ANOVA test was utilized to assess significance between control and treated series, if there is a difference the Tukey test is employed to determine the existence of potential variations among the series themselves. The statistical assessments were executed using MINITAB and R software.

RESULTS AND DISCUSSION

The results of phytochemical tests revealed that the aerial parts contain active compounds including alkaloids, tannins, flavonoids, and saponins. The alkaloids were inferred to be present based on the brown color produced upon interaction with Wagner reagent. Upon adding ferric chloride solution, a dark green color appeared, indicating the presence of catechic tannins. In flavonoids test, the appearance of yellow color after using alkaline reagent test indicates their presence, The results indicated the presence of abundant and stable foam in all tubes after shaking the aqueous extract, indicating the presence of saponins with foam index of 111.11. The plant *T. garganica* is a medicinal plant utilized in numerous traditional treatments, and this is attributed to its composition of these active compounds, with the most notable being Alkaloids, they can have diverse functions, including Antioxidant, Antimicrobial, Antifungal, CNS stimulant, Antiviral, Anticancer activities and as a Plant growth regulator (Dey et al., 2022), Tanins, which are a group of polyphenolic compounds, they can protect plants that contain them from herbivores, pathogens and also deter animals from eating it by imparting a bitter taste, also they have Antioxidant, Antiviral and Antiinflammatory properties (Tong et al., 2023), Flavonoids, they are known for their Antioxidant, Anti-cholinesterase, and Antiinflammatory activities (Panche et al., 2016), Saponins, they are generally recognized for their capacity to combat pathogens, deter pests, and repel herbivores because of their antimicrobial, antifungal, antiparasitic and insecticidal properties (Nguyen et al., 2020). These results are consistent with numerous studies on several parts of the same plant (Chibani et al., 2014; Athmouni et al., 2015; Bouimeja et al., 2018; Aici and Benmehdi, 2021).

The observed mortality of *Cx. pipiens* fourth instar larvae treated with *T. garganica* increases with the concentration used; for the lowest concentration (15g/ l), a rate of 41.33% is recorded, reaching 94.66% for the highest concentration (60g/ l) (Table 1). The results obtained from the one-way ANOVA test, reveal a highly significant difference (p=0.000) in mortalities among the various concentrations compared to the control. The differences between the concentrations themselves were analyzed using the Tukey test, which demonstrated distinct differences among them (a, b, bc, c and d) (Fig. 1). Furthermore, an angular transformation of the adjusted mortality averages into probits and the tested concentrations into decimal logarithms was carried out to evaluate the equation and plot the regression line, the determination coefficient of this line indicates

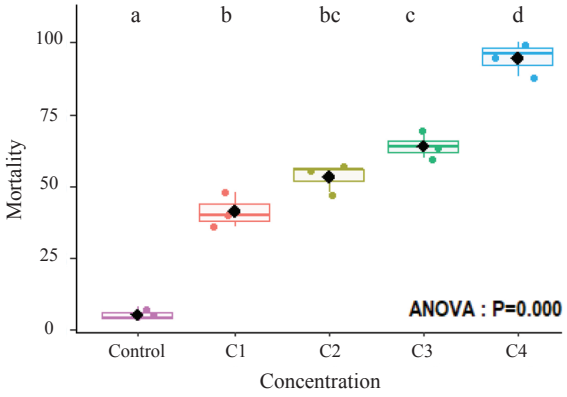

Fig. 1. One-way ANOVA and Tukey tests

Table 1. Efficacy of *T. garganica* against fourth instar larvae of *Cx. pipiens*

Mortality (%)

Concentrations	Observed mortality (%)	Corrected mortality (%)
Control	5.33± 2.30	/
C1= 15 g/l	41.33± 6.11	37.92± 7.70
C2= 30 g/l	52.0± 5.65	50.72± 4.35
C3= 45 g/l	64.0± 4.0	61.89± 5.09
C4= 60 g/l	94.66± 6.11	94.44± 6.36

Regression equation, LC_{50} and LC_{90} (g/ l) with Fiducial Limits (FL)

Treated stage	R^2	Regression equation	LC_{50} (FL)	LC_{90} (FL)
L_4	71.8	y=2.69851x+1.31148	23.27 (10.11-53.52)	69.47 (30.2-109.78)

a very strong positive relationship between probits and decimal logarithms, The lethal concentrations LC_{50} and LC_{90}, determined with their Fiducial Limits (FL) from the regression line, are 23.27 and 69.47 g/l respectively (Table 1), these results confirmed the insecticidal activity of the plant powder on the fourth instar larvae with all tested concentrations; which can be due to its components cited in phytochemical study. Additionally, creating effective natural pesticides to control mosquitoes, especially and insects in general, could be explored from plants because they have confirmed their effectiveness using one of their parts, their extracts, one of their active ingredients, or their essential oils have been utilized against various species of mosquitoes (Djeddar et al., 2021; Kharoubi et al., 2021; Kirouani et al., 2022; Belkhiri et al., 2023; Khaldi et al., 2023) and insects (Tabti et al., 2020; Dasenaki et al., 2022; Jmii et al., 2022; Moullamri et al., 2024).

AUTHOR CONTRIBUTION STATEMENT

KA and ND performed experiments. Data analysis was conducted by KA, ND and HG. The manuscript was written by KA. All authors reviewed and approved the manuscript.

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CONFLICT OF INTEREST

No conflict of interest.

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