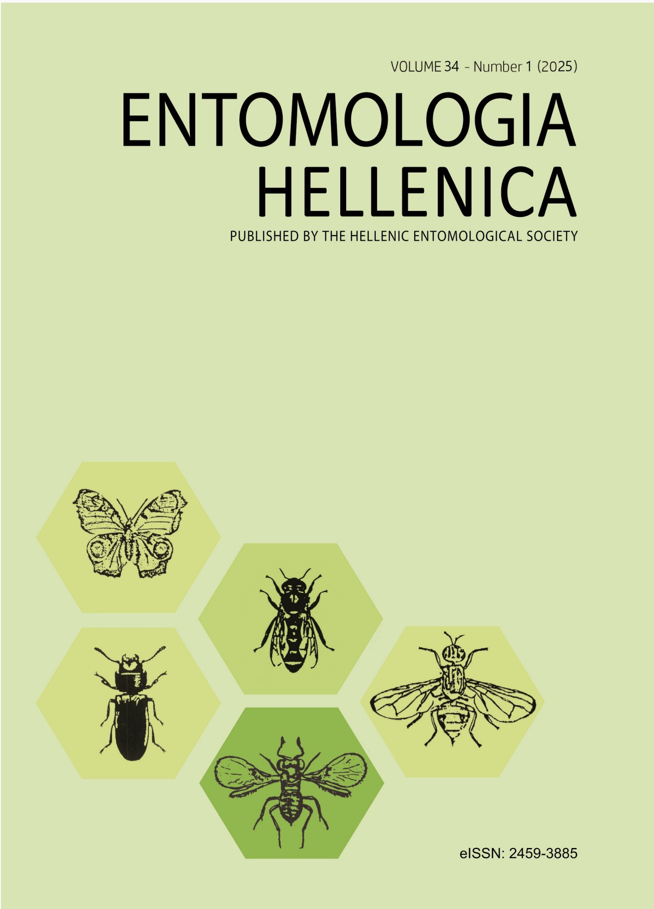
ENTOMOLOGIA HELLENICA

Vol 34, No 1 (2025)

Entomologia hellenica 34(1)



Insecticidal effects of aqueous extracts from Artemisia herba-alba Asso and Rosmarinus officinalis L against Drosophila melanogaster (Diptera: Drosophilidae)

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doi: [10.12681/eh.39805](https://doi.org/10.12681/eh.39805)

ENTOMOLOGIA HELLENICA **34** (2025): 100-109

Received 20 December 2024 Accepted 21 May 2025 Available online 01 June 2025

Insecticidal effects of aqueous extracts from *Artemisia herba-alba* Asso and *Rosmarinus officinalis* L against *Drosophila melanogaster* (Diptera: Drosophilidae)

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ABSTRACT

Synthetic insecticides are frequently used to control or prevent agricultural insect pests. However, their excessive use is generally associated with environmental pollution, loss of biodiversity, health risks to humans, and adverse effects on various living beings. Considering the harmful consequences of chemical pesticides on the environment and public health, this highlights the necessity for target-specific, biodegradable, and environmentally friendly products, such as those extracted from plants. Hence, in the present study, the bio-efficacy of two botanical plant extracts obtained from the leaves of *Artemisia herba-alba* Asso and *Rosmarinus officinalis* L. by the maceration method was investigated on *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) a widely used biological model for assessing insecticidal effects. The aqueous extracts were applied topically through different doses varying from 1 to 20 µg per larva for *A. herba-alba*, and 1 to 35 µg per larva for *R. officinalis*, on the third instar larvae, and the inhibition doses of adult emergence (ID_{25} and ID_{50}) were determined. Our results indicate that the aqueous extracts of *A. herba-alba* showed very promising insecticide activity against *D. melanogaster* compared to *R. officinalis* extract. Topical toxicity recorded ID_{25} and ID_{50} values of 1.35 and 3.17 µg per larva for *A. herba-alba*, followed by 2.03 and 6.42 µg per larva for *R. officinalis*, respectively. These findings reflect clearly that the aqueous plant extracts of *A. herba-alba* and *R. officinalis* have great potential to develop new botanical insecticides as safe alternatives for insect-pest control.

KEY WORDS: *botanical insecticides, aqueous extracts, toxicity*.

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Introduction

Chemical pesticides are widely employed in agriculture to preserve crop production against insect pests, weeds, and fungal diseases (Sharma et al., 2020; Tang et al., 2021; Barathi et al., 2024). Their global use has increased rapidly; globally, nearly 3 billion kg of pesticides are used annually, with a budget of around 40 billion USD (Sharma et al., 2020). These results curb pests, reduce yield losses, and therefore guarantee food availability (Sharma et al., 2020).

However, if chemical pesticides have played a crucial role in pest management and raising agricultural productivity, most of them are non-specific, toxic, and pose significant hazards to the environment, which leads to detrimental consequences on non-target ecosystems, such as environmental pollution, as well as on non-target organisms (Bilal et al., 2019; Rajmohan et al., 2020; Sharma et al., 2020; Barathi et al., 2024). Additionally, their repetitive and inappropriate use in the last century resulted in higher resistance levels in target insects (Jan et al., 2015; Hawkins et al., 2019).

Considering the diverse and significant hazards of chemical pesticides on the environment and public health, it becomes necessary to seek alternative methods that can be eco-friendly, more accessible, and effective for the development of sustainable agriculture compared to traditional chemical intervention (Senthil-Nathan, 2020; Corzo-Gómez et al., 2024).

Natural compounds, such as those extracted from plants, are known for being environmentally friendly, low persistence, and high biodegradability, making them safer and more effective alternatives to synthetic insecticides (Isman, 2020; Boulahbel et al., 2022; Henagamage et al., 2023). Many plants, through their derivatives, such as powders, essential oils, crude extracts, and semi-purified or purified compounds, provide valuable secondary plant metabolism products that could be used in agriculture for pest control (Isman & Akhtar, 2007; Pavela, 2016; Lamsal et al., 2020; Henagamage et al., 2023).

In addition, these specific active ingredients in extracts and essential oils have received growing interest in recent years, primarily owing to their varied mechanisms of action on target pests, including repellent properties, toxicity, antifeedant, neurotoxic and growth regulation effects (Isman & Akhtar, 2007; Souto et al., 2021; Corzo-Gómez et al., 2024).

Moreover, they are target-specific, which ensures the safety of non-target organisms, especially beneficial organisms such as pollinator bees and predators, and they are locally and readily available in the environment (Lengai et al., 2020; Henagamage et al., 2023).

Considering these effects of plant derivatives against various insects, an effort was made to explore the aqueous extracts of medicinal plants as an eco-friendly strategy for controlling pest populations.

Consequently, the contact toxicity of aqueous extracts from *Artemisia herba-alba* Asso and *Rosmarinus officinalis* L., obtained from the Algerian flora, was evaluated in the present study to assess their potential insecticidal activity against third instar larvae of *D. melanogaster*.

Materials and Methods

Insect rearing: *D. melanogaster* flies (Canton-S strain) were reared in plastic vials on a standard Drosophila medium comprising cornmeal, agar, yeast and an antifungal agent. The flies were maintained in a breeding room at a constant temperature of 25 ± 2°C and relative humidity of 70% with a 12h:12h light/dark photoperiod (Boulahbel et al., 2022). The flies were relocated to a new standard medium every three days to prevent larval competition and ensure an adequate supply of offspring for testing.

Plant aqueous extract preparation: The aqueous extracts of *A. herba-alba* and *R. officinalis* leaves were processed according to the maceration method described by Handa (2008), with slight modifications. 10 g of each powdered material of *A. herba-alba* and *R. officinalis* were macerated in 200 mL of boiling distilled water and stirred using a magnetic stirrer at 500 rpm with periodic shaking for 72 h. Then, the suspension was filtered through a Buchner funnel and filtered through a muslin cloth filter, followed by filtration using Whatman No. 1. The crude extract was obtained into a sterilized conical flask of 100 mL. The extracts were poured into clean glass dishes and placed in an electric oven at 40 °C for 4 days to obtain dried powder. The obtained residues of dried extract for *A. herba-alba* and *R. officinalis* were stored in the refrigerator at 4 °C until use.

Treatment and bioassay: Aqueous extracts of two medicinal plants, *A. herba-alba* and *R. officinalis,* were assessed for contact toxicity using the topical treatment following the method described by Bensebaa et al. (2015) on third instar larvae of *D. melanogaster*. Serial testing doses ranging from 1 to 20 µg per larva for *A. herba-alba* and 1 to 35 µg per larva for *R. officinalis* were diluted in distilled water, with 1 µL topically applied to the dorsal thoracic region of third-instar larvae of D. melanogaster using a microsyringe. Control groups were exposed only to 1 µL of acetone, and all larvae were maintained under the same laboratory conditions as The results showed that the mortality rate increased with increasing doses of the plant extracts, indicating that the effect of the *A. herba-alba* and *R. officinalis* extracts was dose-dependent (Fig. 1).

previously described. Each dose was tested on three replicates of 30 larvae. The observed mortality was checked daily until the complete emergence of adults and assessed considering the cumulative mortality of immature stages (dead larvae, dead pupae, adults who could not emerge from pupae and partially emerged adults). The adult emergence inhibition was corrected using Abbott's (1925) formula. The inhibition doses (ID_{10}, ID_{25}, and ID_{50}) that caused 10, 25, and 50% inhibition in adult emergence were determined with the corresponding 95% fiducial limits (95% FL) and the Hill slope.

Data analysis: Data are presented as mean ± SEM. The toxicity data were examined using non-linear sigmoid curve fitting, and the treatment's activity was determined based on a dose-dependent response. The significance of differences was performed by one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD), and results with $P<0.05$ were considered statistically significant. All statistical analyses were performed by GraphPad Prism Software version 6.01.

Results

This study showed that the aqueous extracts from *A. herba-alba* and *R. officinalis* were effective in causing mortality of D. melanogaster larvae, pupae and inhibited adult emergence. Various types of malformations and deformities were observed in larvae, pupae, and adult flies that formed from treated larvae, such as burned larvae, dead adults inside pupae and partially emerged adults from the puparium followed by death at each stage of development of *D. melanogaster*.

Indeed, our data demonstrated that the corrected inhibition values ranged from 24.07±0.37% for the lowest dose (1 µg/larva) to 94.11±2.95% for the highest tested dose (20 µg/larva) with the aqueous extracts of *A. herba-alba* (Table 1).

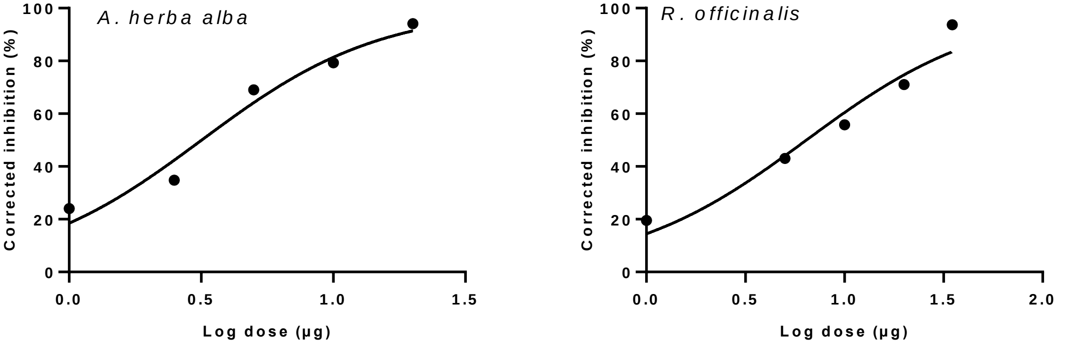

FIG. 1: Effect of aqueous extract of *A. herba-alba* and *R. officinalis*, topically applied on the third instar larvae of *D. melanogaster*: sigmoidal dose dependent response curve of corrected inhibitions (%) of adult emergence as a function of the logarithm of the doses.

TABLE 1. Effect of aqueous extract of *A. herba-alba and R. officinalis (µg)*, topically applied on the third instar larvae of *D. melanogaster* : Corrected inhibitions (%) of adult emergence as a function of the doses. (mean ± SEM; n = 3 replicates containing each 30 larvae). Control mortality: 9.33 ± 0.33 %.

Treatment	**Doses µg/larva**	**Mean corrected inhibition (%)**
A. herba-alba	1	24.07 ± 0.37
	2.5	34.81 ± 1.48
	5	69.00 ± 0.11
	10	79.26 ± 0.74
	20	94.11 ± 2.95
R. officinalis	1	19.57 ± 0.89
	5	43.07 ± 0.75
	10	55.81 ± 0.74
	20	71.01 ± 1.01
	35	93.70 ± 3.29

The same pattern was observed with the aqueous extracts of *R. officinalis* during the mortality test (Table 1). Corrected inhibition percentages were as follows: 19.57±0.89% for the lowest dose (1 µg/larva) and 93.70±3.29% for the highest dose (35 µg/larva). The inhibition percentage recorded in untreated control animals was 9.33±0.33%.

Statistical analysis indicated that all plant extracts were toxic with a significant effect of doses ($F_{(4, 10)}$=114.4; P<0.0001 for *A. herba-alba* followed by $F_{(4, 10)}$= 94.85 ; P<0.0001 for *R. officinalis*). Tukey's HSD test indicates a significant relationship between the different tested doses (Fig. 2).

The inhibition doses (ID) of adult emergence were estimated with ID_{25} and ID_{50} values of 1.35 and 3.17 µg per larva for *A. herba-alba*, followed by 2.03 and 6.42 µg per larva for *R. officinalis*, respectively (Table 2).

These results indicate that aqueous extracts of *A. herba-alba* gave very promising contact toxicity against D. melanogaster over the *R. officinalis* extract (Table 2). The non-linear regression shows a Hill Slope of 1.28 for *A. herba-alba* and 0.95 for *R. officinalis* (Table 2).

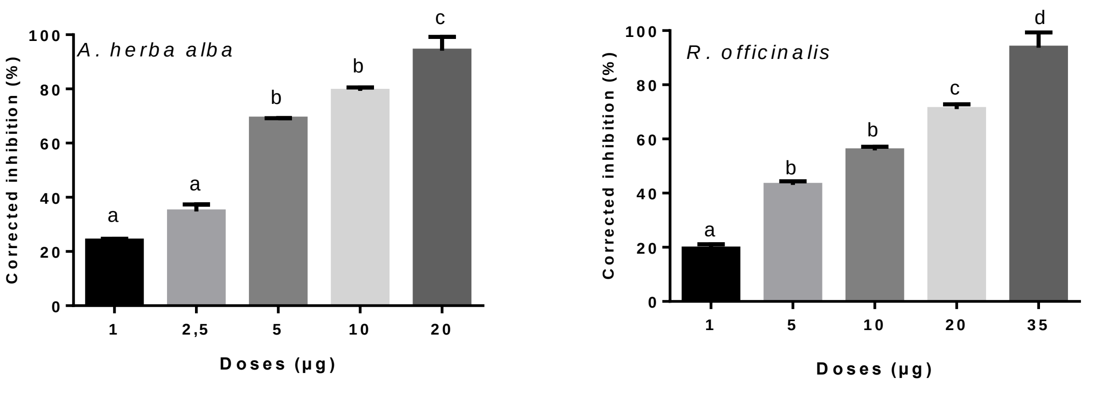

FIG. 2. Effect of aqueous extract of *A. herba-alba* and *R. officinalis* (ug), topically applied to third instar larvae of *D. melanogaster* on the adult exuviation inhibition (%) (mean ± SEM; n = 3 replicates containing each 30 larvae). The mean values followed by different letters are significantly different for each treatment according to Tukey's test at P<0.05.

Discussion

Botanical insecticides are naturally occurring insecticides extracted from plants that could serve as suitable alternatives to the indiscriminate use of synthetic chemical insecticides (Gupta et al., 2021; Ngegba et al., 2022; Corzo-Gómez et al., 2024).

Furthermore, numerous plant extracts provide potential sources of bioactive compounds including alkaloids, terpenoids, flavonoids, phenolic compounds, and quinones, which have the potential to exhibit acute toxicity, repellency, antifeedant properties, and insect growth regulation against various agricultural pests (Isman & Akhtar, 2007; Pavela, 2016; Khan et al., 2017; Souto et al., 2021; Gupta et al., 2021; Ngegba et al., 2022).

TABLE 2. Toxicity activity of aqueous extract of *A. herba-alba and R. officinalis*, topically applied on the third instar larvae of *D. melanogaster*.

Treatment	Doses µg/larva	Value	Fiducial Limits (95 %)	R^2
A. herba-alba	ID_{10}	0.57	[0.20-1.61]	0.96
	ID_{25}	1.35	[0.70- 2.57]	
	ID_{50}	3.17	[2.13-4.72]	
	HillSlope	1.28	[0.61-1.95]	
R. officinalis	ID_{10}	0.64	[0.11- 3.67]	0.94
	ID_{25}	2.03	[0.69-5.97]	
	ID_{50}	6.42	[3.51-11.72]	
	Hill slope	0.95	[0.34-1.56]	

The data are expressed in terms of the inhibition doses (ID_{10} ID_{25} and ID_{50} Values) that caused 10, 25, and 50 % inhibition in adult emergence and their 95% fiducial limits (95% FL). R^2: coefficient of determination.

This work investigated the insecticidal efficacy of two plant extracts, derived from *A. herba-alba* and *R. officinalis*, via topical treatment on third-instar larvae of D. melanogaster to develop potential plant-based insecticides. Our study found that the leaf extracts of two plants prepared with distilled water showed significant contact toxicity against *D. melanogaster*. The mortality of the immature stage increases as the doses of two plant extracts are increased, and the pupae are the most critically affected stage of the *D. melanogaster* life cycle, resulting in insect death before reaching the adult stage.

Topical toxicity tests showed that *A. herba-alba* (ID_{50} = 3.17 µg per larva) and *R. officinalis* (ID_{50} = 6.42 µg per larva) have different levels of insecticidal effect against *D. melanogaster*.

Furthermore, the aqueous extract of *A. herba-alba* inhibited adult emergence more effectively than the *R. officinalis* extract.

The findings of this investigation are in agreement with those obtained by Aziz et al. (2018). The author indicated that the extract of *A. herba-alba* exhibited larvicidal activity against mosquitoes from both Indian and Saudi Arabian strains, such as *Anopheles stephensi* Liston, Culex quinquefasciatus Say and Culex pipiens Linnaeus. Similar findings were reported by El-Ashmouny et al. (2022) who showed that *A. herba-alba* plant extract has toxic effects on the development of larval stages for *Spodoptera littoralis* Boisduval species.

Moreover, the insecticidal activity of *A. herba-alba* essential oil has been proven on numerous insect pests such as Tribolium castaneum Herbst (Ben Slimane and Baouindi, 2016; Boukraa et al., 2022), Tineola bisselliella Hummel (Bouchikhi-Tani et al., 2018) and *Cydia pomonella* Linnaeus (Mahi et al., 2023).

According to the following literature, the biological test revealed that the ethanolic and aqueous extract from *R. officinalis* exhibited insecticidal properties and caused 100% mortality to 3rd instar larvae of *Spodoptera frugiperda* Smith (Kalinda & Rioba, 2020).

Furthermore, recent studies have shown that the ethanolic, aqueous and methanolic plant extracts contain certain bioactive compounds with insecticidal potential against insect pests (Akbar et al., 2022; Bini et al., 2023; Henagamage et al., 2023). For instance, *Mercurialis annua* L. and *Anacardium occidentale* L. have demonstrated insecticidal activity against larvae of *Tribolium confusum* Jaquelin Du Val and *Helicoverpa armigera* Hübner (Nasr et al., 2021; Bini et al., 2023).

Phytochemicals carried out on *A. herba-alba* and *R. officinalis* have allowed the isolation of many secondary metabolites, such as monoterpene and sesquiterpene constituents, which effectively control insect pests (Lengai et al., 2020; Mahi et al., 2023).

These bioactive compounds are known for inhibiting acetylcholinesterase activities, a crucial enzyme in the insect's central nervous system (Abdelgaleil et al., 2009; Chaubey, 2014), and their interference with the metabolism of the juvenile hormones and ecdysones (Tsao & Coats, 1995). This could explain the insecticidal effects of *A. herba-alba* and *R. officinalis* extracts on developing D. melanogaster

Conclusion

The results of this study suggest that the aqueous extracts of *A. herba-alba* and *R. officinalis* possessed significant insecticidal effects on *D. melanogaster*. Among the two extracts tested, the extract from *A. herba-alba* showed significantly higher efficacy, with ID25 and ID50 values approximately twice as potent as those observed for *R. officinalis*. These findings indicate that aqueous plant extracts have great potential for developing new botanical insecticides as sustainable alternatives for managing insect pests, including the harmful Drosophila pest, *Drosophila suzukii* Matsumura. Further research is required to identify the bioactive compounds in these aqueous plant extracts and to evaluate their sublethal effects on the development and reproduction of *D. melanogaster*.

Acknowledgements

This research was supported by DGRSDT (General Directorate of Scientific Research and Technological Development-Algeria), from the Ministry of Higher Education and Scientific Research (MESRS).

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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