

<https://doi.org/10.48047/AFJBS.6.15.2024.14420-14429>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

USE OF AQUEOUS EXTRACTS OF *ADHATODA VASICA* AND *INULA VISCOSA*) AGAINST WHEAT *FRIES SITOBION AVENAE*

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Volume 6, Issue 15, Nov 2024

Received: 15 Sep 2024

Accepted: 25 Oct 2024

Published: 21 Nov 2024

doi: [10.48047/AFJBS.6.15.2024.14420-14429](https://doi.org/10.48047/AFJBS.6.15.2024.14420-14429)

Summary

This study concerns a field trial using aqueous extracts of two plants, malabar nut (*Adhatoda vasica*) and slimy inula (*Inula viscosa*), on wheat ears against the aphid *Sitobion avenae*. This study aims to evaluate the insecticidal activity of two aqueous extracts derived simply from insect-repellent plant extracts on wheat aphids in open fields.

In the laboratory, we realized Phytochemical screening of *A. vasica* and *I. viscosa*. Preparation of infusion doses. Spray 40 ml of each dose of infusate on two ears of wheat in the field. After artificial infestation by *S. avenae*, the ears are wrapped in a tulle bag; the number of individuals is counted every 24 hours.

Phytochemical screening results show a high level of secondary metabolites. The results of the bio-test against *S. avenae*, show the insecticidal effect to be too high depending on the doses and from 72h. in natural conditions. Bio-testing of insecticides applied via aqueous extracts of *slimy I.* and *A. vasica* may provide a promising alternative for aphid control, especially as this experiment has shown efficacy under real field conditions.

Key words: Bio-testing, Phytochemical screening, *Sitobion avena*, *Adhatoda vasica*, *Inula viscosa*.

INTRODUCTION

Plants have always been known as an essential source of medicine: The Egyptians used the opium poppy (*Papaver Somniferum* L.) for pain relief (Anaesthetic). Many other diseases continue to find cures thanks to the planet's floristic diversity [1]. Algerian flora is characterized by its great diversity, as a Mediterranean, Saharan and paleo-tropical shoot, estimated at over 3,000 species belonging to several botanical families [2]. This has given traditional pharmacopoeia an inestimable wealth. In recent years, due to the harmfulness of human health and the degradation of soil microfauna, research has been encouraged to perceive biological solutions. Finding alternatives to chemical molecules is as simple as looking at the metabolites of repellent plants as bioaggressors. Moreover, many studies have been highlighted on the effectiveness of plant extracts on several bacterial, cryptogamic and insect diseases. Among these studies, we can cite those of [3], who showed a very interesting nematicide effect with the extract of *Urtica dioica*. Those of [4], who found a biological solution to the biting of culicidae in Cameroon, using aqueous extracts of *Azadirachta indica* leaves by fumigation. Among the Mediterranean plants most tested on the insecticide and bactericide effect, *Inula viscosa*. The aqueous extracts of its leaves are used as a bio-insecticide, it has a remarkable toxicity on the larvae of the pest of stored data *Rhyzopertha dominica* [5] on the other hand the malabar nut is a plant that has been well exploited in conventional and Western medicine, with its anti-inflammatory, antioxidant, anti-microbial, anti-venom, anti-tuberculosis, anti-tumor, hepatoprotective, anti-cancer virtues [6], [7]. But it is not exploited for the insecticidal effect. As part of this study we will see the biocidal effect of *Adhatoda vasica* on insects, as an example we took the wheat ear aphid. At the heart of this experiment two plants attracted our attention: *Inula viscosa* and *Adhatoda vasica*. The use of *Inula* is very popular in Algeria. However, the virtues of the plant *Adhatoda vasica* or the Malabar nut are not known in Algeria. This study aims to prove the insecticidal effect of these two plants on the cereal aphid *Sitobion avenae*.

MATERIALS AND METHODS

1. Choice of biological materials

Two plants were chosen: slimy inula (*Inula viscosa*) and Malabar nut (*Adhatoda vasica*), and the animal pest was the ear aphid (*Sitobion avenae*).



Fig. 1: *Inula viscosa* (original Boumerdes, 2018).



Fig. 2 - *Adhatoda vasica* (original, Baraki, Mahdi Boualem, 2018)

2. Work methodology

The study focuses on :

- Preparation of aqueous extract of *Inula viscosa* and *Adhatoda vasica*,
- A phytochemical screening of the leaf powder of two plants,
- Evaluation of the insecticidal activity of the two aqueous extracts.

3. Leaf harvesting

Dittrichia viscosa leaves were harvested in May 2018 in the Souk El Had region (Boumerdes). As for *Adhatoda vasica*, the leaves were plucked from a shrub at the Mahdi-Boualem Baraki station (INRAA).

4. Drying and grinding

Once the leaves have been picked, the dust covering them is washed off with clean water. After the washing water has evaporated in the open air, the leaves are dried in an oven set at 35°C for 7 days. The dried leaves are ground to a fine powder using an electric grinder. The resulting powder is stored in hermetically sealed glass bottles, protected from light and humidity.

5. Phytochemical screening

In order to detect the richness of bioactive substances in the leaves of the two plants, phytochemical screening was carried out using the method of [8]. [9].

6. Preparation of bio-test doses

Dilutions are prepared from the initial 100% aqueous extract, the doses prepared being D1 (75%), D2 (50%), D3 (25%) and the control (distilled water).

7. Application of bio-testing in the field

After preparing the doses, we collected 40 ml of each infusion to spray onto two aphid-free ears. Then spraying each dose, ten adult *Sitobion avenae* aphids were introduced onto the ears. At the end of the operation, the infested ears are wrapped in a fine-mesh tulle bag, which is tightly tied to the stem to prevent the aphids from escaping. Individuals are counted every 24 hours.

RESULTS

1-Results of phytochemical screening.

The results of this study are shown in the table below.

Table 1- Results of phytochemical tests carried out on leaf powder from the three plants.

Substances	Positive results	<i>Inula viscosa</i>	<i>Adhatoda Vasica</i>
Gallic tannin	Blue Black	 (+++)	 (+++)
Catechic tannin	Red Orange	 (++)	 (+++)

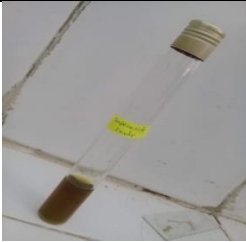
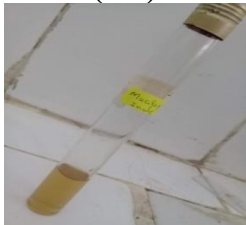
Leucoanthocyanins Red coloring



(++)



(+)

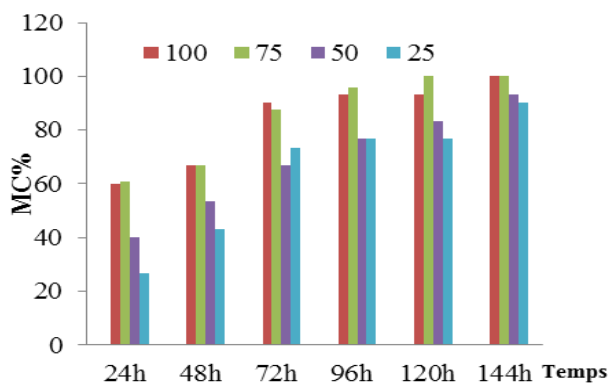
Saponosides	White precipitate		
		(++)	(+)
Flavonoids	Red Orange		
		(+++)	(+++)
Mucilage	Precipitation Flaky		
		(+)	(++)
Irridiodes	Blue coloring		
		(-)	(-)
Tannin Totals	Blue Black		
		(+++)	(+++)
Starch	Violet blue coloring		
		(-)	(-)
Glucosides	Brick Red Color		
		(++)	(+)

(-): No substance. (+): Low substance content. (++) : Medium substance content, (+++) : High substance content.

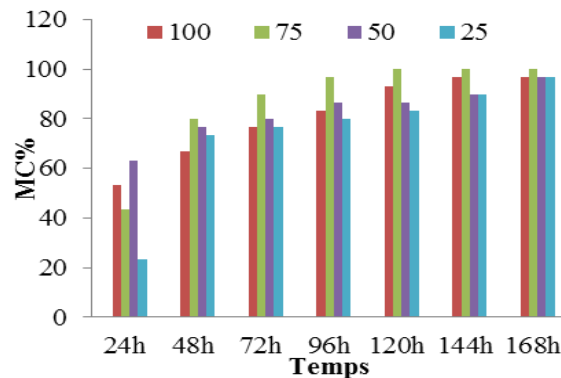
2. Effects of aqueous plant extracts on aphids

2.1.- Adjusted mortality

Adjusted mortality is calculated according to the formula in [10]: $M_c = (M - M_{100} / 100 - M_{211}) * 100$



Evaluation of corrected mortality in aphids treated with different doses of the aqueous extract of *Inula viscosa*.



Evaluation of corrected mortality in aphids treated with different doses of the aqueous extract of *Adhatoda vasica*.

1.2.- Evaluation of the lethal concentration (CL) the lethal dose DL_{50}

1.2.1 *Inula viscous*

Table 2 - Summary of analyses of the effect of tested doses on mortality rates.

Time	Equation of the regression line	LD50 (mg/ml)	R	$\leq R \geq$
24h	$Y = 1.793x + 1.692$	69.98	0.97	$0 \leq R \leq 1$
48h	$Y = 1.139x + 3.071$	49.38	0.87	$0 \leq R \leq 1$
72h	$Y = 1.683x + 3.117$	13.15	0.78	$0 \leq R \leq 1$
96h	$Y = 4.112x + 0.881$	10.04	0.61	$0 \leq R \leq 1$
120h	$Y = 0.911x + 4.143$	8.72	0.61	$0 \leq R \leq 1$

R: Coefficient of determination. LD₅₀: Lethal dose to kill 50% of individuals.

1.2.2.- *Adhatoda vasica*:

Table 3- Summary of analyses of the effect of tested doses on mortality rates

Heurs	Equation of the regression line	LD50 (mg/ml)	R	$\leq R \geq$
24h	$Y = 1.430x + 232$	74.84	0.64	$0 \leq R \leq 1$
48h	$Y = 2.816x + 0.490$	39.95	0.95	$0 \leq R \leq 1$
72h	$Y = 1.714x + 2.876$	17.35	0.98	$0 \leq R \leq 1$
96h	$Y = 3.385x + 1.005$	15.14	0.78	$0 \leq R \leq 1$
120h	$Y = 4.066x + 0.338$	14.01	0.83	$0 \leq R \leq 1$
144h	$Y = 2.048x + 2.725$	12.91	0.92	$0 \leq R \leq 1$
168h	$Y = 2.263x + 3.353$	4.46	0.78	$0 \leq R \leq 1$
192h	$Y = 0.787x + 4.586$	3.35	0.66	$0 \leq R \leq 1$

1. R: coefficient of determination, LD₅₀: The lethal dose to kill 50% of individuals.

1.3 - ANOVA statistical test:

Table 4 - Analysis of variance for doses of aqueous extract D1, D2, D3 and powder of *Inula viscosa* on aphid adults *Sitobion avenae*

Source	DDL	SCE	Mc	F	P
Model	15	261268,8889	17417,9259	38,3485	< 0,0001
Error	164	74488,8889	454,2005		
Corrected total	179	335757,7778			

DDL: Degrees of freedom, SCE: Sum of squares, Mc: Mean of squares: Fischer F-value, P: Probability.

Table 5 - Analysis of variance of D1, D2, D3 aqueous extract and *Adhatoda vasica* powder on *Sitobion avenae* aphid adults.

Source	DDL	SCE	Mc	F	P
Model	15	252657,2222	16843,8148	34,9836	< 0,0001
Error	164	78962,2222	481,4770		
Corrected total	179	331619,4444			

The results of the analysis of variance revealed a highly significant difference ($P < 0.0001$) between the doses of the two extracts used against *Sitobion avenae* adults and the aphid mortality averages. It was noted that the higher doses (100% and 75%) were more effective than the lower doses (50% and 25%).

DISCUSSION

1. - Phytochemical screening

Phytochemical test results reveal the presence of several substances. *Inula viscosa* has a high flavonoid and total tannin content, with a medium content of gall tannins, free quinone and saponosides. Similarly, the screening of *Adhatoda vasica* shows high levels of all tannins and flavonoids, and low levels of saponosides. These results confirm those found by [11], who worked in the same region and on the same *Dittrichia viscosa* plant. No studies have been carried out on *Adhatoda vasica* in Algeria. However, the richness of this plant in flavonoids was confirmed by studies carried out by [12] and [13]. However, the levels of secondary metabolites in medicinal and aromatic plants, such as total phenolics, flavonoids, catechins and anthocyanins, vary according to geographical and climatic conditions [14]. These differences can also be explained by the age of the plants at harvest. Indeed, [15], confirm that tannins frequently accumulate in older organs. Harvesting season and soil type can also influence the bioactive substance content of plants.

2. - Effects of aqueous plant extracts on aphids

2.1 Corrected mortality

The results obtained after processing the data from the two extracts used show an increase in the corrected aphid mortality rate as a function of dose variation with their probit. It should be noted that after 24 h treatment with the 100% (powder) and 75% doses, aphid adult mortality reached over 50%. However, after 72 hours and with the same doses, mortality barely reached 100%. On the other hand, the 50% and 25% treatment doses showed 90% mortality after 144 hours. To this end, the use of *Inula viscosa* aqueous extract as an insecticide against *Sitobion avenae* adult aphids is highly effective. Before it was studied by researchers as a plant with insecticidal activity, this plant was already an ecological plant of excellence. In its area of distribution, *Dittrichia viscosa* plays a role as a host plant for many parasitoids [16]. The flowers of *D. viscosa* are often infested by *Myopites stylata* Fabricius, a dipteran that causes gall formation [17]; and whose larvae are parasitized by the generalist parasitoid *Eupelmus urozonus*, which also feeds on olive fly and thus helps protect olive production [18]. Predators can also be found in the aerial part of *D. viscosa*, such as predatory *Macrolophus* bugs [19]. The results of this study are in line with those of [20], who worked on aqueous extracts of the three spontaneous plants

Inula viscosa, *Salvia officinalis* and *Urtica urens*. These authors show the efficacy of *Inula viscosa* leaf extracts on *Tuta absoluta* larvae compared with the other plants. However, when they applied half doses of aqueous solutions, the percentages of mobile forms of leafminer tended to increase for all applications except that of inula leaf extract, whose effect remained stable, similar to that of the 100% dose. Furthermore, the conclusion of their study shows that the efficacy of the different extracts only begins to become effective at the 4th day. In this study, efficacy appeared after 72 hours. This can be explained by the effect of contact time: aphids were sprayed directly onto the ears, whereas leafminer larvae were found under the parenchyma of tomato leaves. It should be noted that aqueous extracts of *Adhatoda vasica* applied at different doses resulted in mortality exceeding 50% after 24 hours, at doses of 100% and 50%. On the other hand, after the same time, the 75% dose gave a mortality slightly exceeding 40%. However, 100% mortality was achieved 120 h after application of the highest doses. On the other hand, with the lowest doses of 25% and 50%, 100% mortality was achieved after a time exceeding 144h. The results of this study show an insecticidal effect on cereal aphids. In India, this plant is widely used for medicinal purposes. It is highly regarded as a remedy for all diseases of the respiratory organs [21]. However, many plant metabolites act as insecticides or repellents, such as pyrethrum, nicotine and rotenone, which have already been used as insect control agents [22]. Furthermore, the richness of *Adhatoda* leaf extract in secondary metabolites such as tannins and flavonoids according to the present study, and its richness in the main alkaloids: vasicine, vasicinone and vasicinol, enables the plant to have a defensive power against bioaggressors [23]. The latest authors have shown that the three alkaloids exert powerful sterilizing effects against *Dysdercus koenigii* and *Tribolium castaneum*, by stopping oocytes on their way to the oviduct. Also, they have an anti-appetizing effect on insects.

2.2.- Evaluation of the lethal dose LD_{50}

The lethal concentration (CL) dose after which 50% of *Sitobion avenae* adults treated with *Inula viscosa* died differed according to the dose used. A high LD_{50} of 69.98 mg/ml was recorded after 24 h of treatment, while after 48 h it was 49.38 mg/ml; and the lowest LD_{50} was obtained after 120 h with a value of 8.72 mg/ml. These results are similar to those found by [20] who worked on aqueous extracts of woodruff using the root part of the plant. After 24 h treatment of tomato leafminer larvae, they noted an LD_{50} equal to 68.18mg/ml. However, their results differ from those found in this study using aqueous extracts from the aerial part of the same plant. They reported an LD_{50} equal to 0.996 mg/ml after 24h of treatment. The explanation for the fact that the results of the two studies are almost the same using two different parts of the inula is as follows: it is highly probable that the same quantities of metabolites are found in the root and aerial parts. It is important to note that our plant was harvested in May, while that of [20] was picked in April. The harvesting period and the phenological stage of the plant play an important role in the presence and quantity of metabolites found in the different parts of the plant. Moreover, according to the work of [19], many parameters enter into the metabolite content of plants, such as age, climatic conditions and sampling region. Treatment of aphids with the aqueous extract of *Adhatoda vasica* recorded a high LD_{50} after 24 hours, at 74.84 mg/ml, and 39.95 mg/ml after 48 hours; the lowest LD_{50} was obtained after 144 hours, at 12.91 mg/ml. Extracts of *Adhatoda vasica* for agronomic use are very little exploited. Many studies have been carried out on the virtues and pharmaceutical use of malabar nuts, especially in America and India. But rarely find the documentations that deal with the subject of biological control and the insecticide effect on bio-aggressors. However, our observations on the ground allowed us to see that despite the infestation of aphids on the different weed crops that surround the malabar nut, it remains unaffected.

Nevertheless, a study by [23], shows that the anti-appetizing function of the main alkaloids of *Adhatoda vasica* at rates of between 0.05 and 0.1% can be observed against *Aulacophora foveicollis* and *Epilachna vijintioctopunctata*. However, the low doses which did not give the desired toxicity on the pest can be explained by the low content of the targeted metabolites and/or the presence of other elements in the aqueous extracts. Similarly, according to [24], crude extracts may not give the desired results due to the presence of numerous other compounds. The main ingredient present in the plant must be extracted and used to combat the pest.

CONCLUSION

In this work, the aqueous extracts of two plants tested are very rich in secondary metabolites (tannins, leuco-anthocyanins, saponosides and flavonoids). The results of the insecticide bio-test applied by the two aqueous extracts of *Inula viscosa* and *Adhatoda vasica* in the field at the Institut Technique des Grandes Cultures (ITGC) proved the overly high insecticidal effect on the wheat aphid (*Sitobion avenae*). The results of the present study show that slimy inula and *Adhatoda vasica* can provide a promising alternative for aphid control, especially as this experiment has demonstrated efficacy under real field conditions. In this study, the various extracts applied only began to take effect after 72 h under natural conditions,

Thanks

The authors would like to express their sincere thanks to MRS ADOURI Mariem, BENAÏSSA Z'hor and RECHID Zineb for their contribution to this work.

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