AF-783
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RESEARCH ARTICLE Indian Journal of Agricultural Research

Chemical Composition and Insecticidal Activity of *Thymus algeriensis* Boiss and Reut. Essential Oil against *Aphis fabae* Scopoli1763

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10.18805/IJARe.AF-783

ABSTRACT

Background: The aim of the present work was to study the chemical composition and evaluate the insecticidal activity of *Thymus algeriensis* Boiss and Reut. essential oil (TAEO) against *Aphis fabae* Scopoli is a polyphafous which desap many cultivated plants, under shelter and in the open field.

Methods: The extraction of essential oil from the aerial parts of *T. algeriensis* was performed by hydro-distillation (yield: 2.7% w/w). The extracted essential oil was analyzed by gas chromatography coupled with mass spectrometry (GC/MS) and by gas chromatography (GC).

Result: From this essential oil 22 compounds representing 92.27% of the oil were identified. The main constituents were carvacrol (61.5%), γ-terpinene (8.81%) and p-cymene (10.83%). The experiment was carried out in random blocks (05), to study the behavior of *Aphis fabae* and its host, namely *Vicia fabae*. Thus, twenty-five bean plants infested with black aphids were chosen and distributed at random. The plants were treated by spraying at different doses of (HETA) (control, acetone; 0.1; 0.05 and 0.01%). The results obtained showed that TAEO at 0.1% results in a decrease of infestation rate of *A. fabae*, leading a mortality rate of 75.51%.The results of the treatment carried out at the beginning of spring are very promising; oil could thus be used as bio-insecticide.

Key words: *Aphis fabae*, Essential oils, Insecticidal activity, *Thymus algeriensis*, *Vicia fabae*.

INTRODUCTION

Broad bean (*Vicia faba* L.) is a large seeded food crop, the most cultivated in Algeria with nearly 60,000 hectares (fresh pods and dry seeds), intended for human and animal consumption. It plays an important role in the development of the national economy. Its high levels of protein (25-35%) and carbohydrates (53%) give it a very high nutritional value (Larralde and Martinez, 1991). As a result, it has good nutritional value, replacing certain conventional protein sources (Tewatia and Virk, 1996). Broad bean is a good crop rotation for cereal crops and it is the legume which fixes the greatest quantity of atmospheric nitrogen/ha for poor soils in the arid regions of Algeria. Its national annual average production is 500,000 quintals in 2017 and an average yield of 8.56 quintals/ha. The development of this crop in Algeria is hampered by socio-economic constraints (Absence of a certified seed production program, High cost or absence of labor, Unattractive production price), abiotic problems (winter cold, spring frosts, heat and salinity) and biotic (diseases Botrytis (chocolate spot), rust, parasitic plants Orobanche and pests black aphid, weevil), remain a real obstacle, preventing the increase in yields (Maatougui, 1996).

Among the pests, insects have an important place, in particular, aphids. One of the original characteristics of aphids is their ability to produce, in the same colony, winged and wingless individuals, which perform different ecological functions.

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How to cite this article: Kouache, B., Kaci, Z., Brada, M. and Fauconnier, M.L. (2023). Chemical Composition and Insecticidal Activity of *Thymus algeriensis* Boiss and Reut. Essential Oil against *Aphis fabae* Scopoli1763. Indian Journal of Agricultural Research. doi:10.18805/IJARe.AF-783.

Submitted: 26-02-2023 **Accepted:** 31-05-2023 **Online:** 10-06-2023

As for the impacts of the black bean aphid, *A. fabae*, they can be reflected either directly by food intake causing the weakening of the plant and the disruption of cell multiplication processes by provoking the formation of galls, serving as a source nutrients (Dedryver *et al.*, 2010) or indirectly, through the transmission of plant viruses and saprophytic fungi responsible for sooty molds Hogenhout *et al*. (2008).

In order to thwart the harmful effects of *A*. *fabae* described above, several authors have looked into the extension of the insect *A. fabae* with broad beans, which prompted them to carry out numerous the majority of which focused on aspects of controlling the aphid pest mainly by chemical means. In addition to high costs, these molecules have negative effects on the environment (water pollution, *etc*.) and human health (residues) Nordey *et al*. (2021).

Furthermore, they are involved in the reduction of biological potential (destruction of beneficial insects; pollinators, parasitoids and predators) (Ruchika and Kumar, 2012).

Faced with such a situation, the search for natural substances of botanical origin, with an insecticidal effect, would be a good alternative for the control of aphids Chermenskaya *et al*. (2010).

The Algerian flora deserves to be explored further. We were interested in a species of the Lamiaceae family which develops there naturally such as *T. algeriensis* and deserves to be prospected within the framework of an integrated fight against *A. fabae*.

T. *algeriensis*, is an endemic plant and considered as the most widespread North African species. The plant is largely used as a culinary herb and as a traditional medicine to treat digestive and respiratory infections (Guesmi *et al*., 2016 and Ziani *et al*., 2019).

It is in this context that this work fits, which aims to study the effect of extracted essential oils, known for their insecticidal properties (Mersha *et al*., 2014; Hridayesh *et al*., 2014; Shiwakoti *et al*., 2016; Song *et al*., 2016; Karahacane and kaci, 2021), rich in carvacrol (Popović *et al*., 2013 and Aissaoui *et al*., 2018).

MATERIAL AND METHODS

The study was conducted from June to December 2021 at Laboratory of Natural Substances Valorization, Faculty of Nature and Life Sciences and Earth Sciences, University Djillali Bounaama, Khemis Miliana, Ain Defla, Algeria.

Plant material and essential oil hydrodistillation

Aerial parts (leaves and flowers) of wild *T*. *algeriensis* were collected during the month of June of the year 2020-2021 around nine o'clock in the morning at the locality of Mekhatria in the province of Ain-Defla located in northern Algeria (at 140 Km Northwest of Algiers - latitude: 36°25′N; longitude: 2°21′7″ E; Altitude: 365 m). For essential oils extraction, the collected plant material is spread out, dried, in the open air, for eight days to guarantee on the one hand a good conservation of its physicochemical parameters and on the other hand to prevent the bacterial proliferation. The aerial parts subject to experimentation are kept in paper bags. A specimen was deposited in the Herbarium of Agronomic Sciences Department of Djilali Bounaama University of Khemis Miliana (Fig 1). The botanical identification was made on the basis of a herbarium specimen made by Mr Belouad, Mr Hazzit of botany department of ENSA El Harrach, Algiers.

The extraction of essential oils is carried out by Clevenger hydrodistillation, a quantity of 50 g of air dried plants was added to 600 ml of distilled water in a 2-liter fask with 600 ml. The set was placed in a mantle heater connected to a refrigerator to ensure the condensation of the essential oils for 2 hours. Two phases are observed, an aqueous phase (aromatic water) and an organic phase (essential oil). The obtained essential oil was collected, dried over anhydrous sodium sulfate and stored in sealed bottles in the dark at 4°C until use.

Gas chromatographic analysis coupled with mass spectrometry (CG/MS) of the essential oil

Ten mg of essential oil was dissolved in 5 ml of diethyl ether. The essential oils were analyzed by gas chromatography coupled to a flame ionization detector (GC-FID) and by gas chromatography coupled to a mass spectrometer (GC-MS).

GC-FID analysis: The analysis of the oil was carried out by means of an Agilent technology HP GC 6890 system

Vegetative stage

Flowering stage

Fig 1: *Thymus algeriensis* Boiss and Reut.

with a flame ionization detector (FID), using a capillary column coated with 5% phenyl methyl siloxane (30 m × 0.25 mm × 0.25 μm film thickness Agilent Technologies, Hewlett-Packard, CA, USA). The temperature program was as follows: 40°Cduring1 min, then raised in a first ramp to 200°C at 6°C/min, followed by a second ramp to 280°C at 30°C/min and finally kept at 280°C during 2 min.

Injection was realized in split less mode at 280°C; the volume injected was 1 μl of diluted oil (10 mg of oil/5 ml diethyl ether). Detector temperature was fixed at 300°C; Carrier gas was helium at 1 ml/min.

GC-MS analysis: GC/MS was performed with an Agilent HP 6890 GC system coupled with an Agilent HP 5973 Network Mass Selective Detector operated by HP Enhanced Chem Station software. Analytical conditions were fixed as follows: Agilent HP- 5MS capillary column (30 m × 0.25 mm, df = 0.25 μm), a split splitless injector at 250°C (splitless mode), temperature program: from 40°-250°C at 6°C/min, mobile phase: carrier gas helium at 1 ml/min. The mass spectra were recorded in EI mode (70 eV), scanned mass range: from 35 to 500 amu. Source and quadrupole temperatures were fixed at 230°C and 150°C, respectively. The identification of the components was performed on the basis of chromatographic retention indices and by comparison of the recorded spectra with computed spectral library (Wiley 275. L) Adam (2007). For sesquiterpene hydrocarbons, further confirmations were obtained by comparing the mass spectra with data from the literature (Adams, 2007). Retention indices (RI) were calculated by means of a mixture of homologue n-alkanes (C7-C30) analyzed under the same chromatographic conditions used for the analysis of essential oils (Adams, 2007).The identification of the various components is based on the comparison of the retention times of each component, their mass spectra and their Kovats indices (KI) given by the literature with those of the standard compounds of the library of computerized data (Wiley 275, L), those described by Adams (2007) and also the database developed by the General and Organic Chemistry Unit, Gembloux Agro Bio Tec, University of Liège (Belgium).

RESULTS AND DISCUSSION

Extraction and composition of *T. algeriensis* essential oil

The essential oil from dried *T. algeriensis* aerial parts was isolated by steam distillation in a Clevenger-type. The essential oil of *T. algeriensis* (EOTA) yield harvested during the month of June was of 2.7±0.3% (w/w) (n= 05). Freshly isolated essential oil was a yellow liquid with an intense odor. This plant can be assigned to one of the oil-rich species of the Lamiaceae family. Twenty two components were

Table 1: Chemical composition of *T. algeriensis* Boiss and Reut essential oil from Northern Algeria (mean of triplicates).

N°	Compouds	Tr	Concentration (%)
1	α-thujene	13.50	0.90
2	α-pinene	13.92	1.50
3	Camphene	14.75	0,07
4	Sabinene	16.70	0,32
5	β-pinene	16.45	0,16
6	Myrcene	17.48	1.60
7	α-phellandrene	18.27	0,17
8	Carene	18.67	0,07
9	α-terpinene	19.16	1.37
10	P-cymene	19.96	10.83
11	Limonene	20.21	0,58
12	γ-terpinene	22.33	8.81
13	Linalol	25.25	1.87
14	Camphre	14.75	Trace
15	Borneol	29.70	0,34
16	Menthol	30.53	0.15
17	α-terpeneol	31.56	0.36
18	Thymol	39.00	0.91
19	Carvacrol	40.55	61.5
20	Nerol	42.94	021
21	Geranyl acetate	43.65	0,015
22	β-caryophellene	54.92	0,53
Oxygenated monoterpenes			65.36
Monoterpene			26.38
Sesquiterpenes			0,35
Total compound identified			92.27

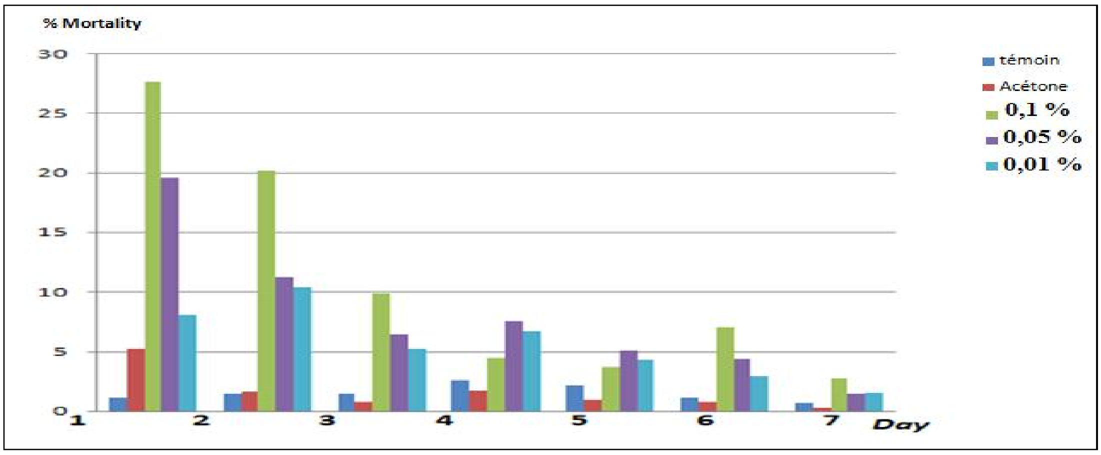

Fig 2: Average mortality of Black bean aphids (*A.fabae*) during the spraying period by *T. algeriensis* essential oils (TAEO) at different doses. Lot: control, Acetone, TAEO (0.1%), TAEO (0.05%) and TAEO (0.01%).

identified accounting for 92.27% of the total oil (Table 1). The oxygenated monoterpenes was the predominant chemical group (65.36%), followed by the monoterpenes (26.38%), whereas the sesquiterpenoids (0.53%) were very low. Chemical profiling of TAEO sample revealed that it could belongs to the carvacrol chemotype (61.5%). Thus, p-cymene (10.83%) and γ-terpinene (8.81%) were reported as the major components for *T. algeriensis* (Coskun *et al*. (2008) and Kouache *et al*. (2017).

This yield is higher than that obtained by Amarti *et al*. (2011) which is 0.3% ± 0.07 and 1.13 respectively and is close to those of Guesmi *et al*. (2014) with a yield of 2.3%.

Hudaib *et al*. (2002) underlined the importance of the choice of the thyme harvest period to obtain oil of quality and quantity, They found that the yield differs from one period to another, The best yield (3.16%) is obtained for the plant harvested at the end of June, at the beginning of July Kouache *et al*. (2017). Likewise, they showed age or the stage of development of the plantinfluence of the on the yield and the composition of the oil. The two-year-old plant gives a yield of 0.5% while that of five years gives a yield of 0.15%, the plant being picked at the same period Faleiro *et al*. (2003).

Effect of *T. algeriensis*. essential oil tested by spraying against the infestation of Black bean aphids (*A. fabae*).

The results of the treatments are represented in Fig 1. The blocks treated by TAEO (0.1%) represent the best mortality rates. As for the duration of the treatment, the difference is not significant (p= 0.4123) for the dose 0.05 and 0.01%. Mortality rates obtained at different concentrations 0.1%, 0.05% and 0.01%) were (75.51%), (55.48%) and (39.03%) respectively. Statistical analysis shows that treatment of block (0.01%) has a weak effect on black bean aphid mortality; it is then very significant for lots 0.1% and 0.05%, which shows the effectiveness of the treatment by the concentration of TAEO of 0.1% and 0.05% (Fig 2).

These results are consistent with those reported in the literature (Kouache *et al*., 2017 and Aissaoui *et al*., 2018). With respect to the factor treatment dose, there is a significant difference ($p<0.05$) between the three lots.

One possible explanation for this result is the presence of carvacrol (61.5%), as major component and synergic effect with other monoterpenes, such as p-cymene (10.83%) and γ-terpinene (8.81%). Essential oils are endowed with significant insecticidal properties. However, this toxicity is highly variable depending on the nature of the components of the essential oil, the dose used, the duration of treatment and the stage of the pest in question Sabraoui *et al*. (2016). Indeed, previous studies have found that carvacrol was acaricidal and larvicidal against several species while carvacrol and γ-terpinene acted as insectide and larvicides (Iori *et al*., 2005) carvacrol and the structurally related compounds like p-cymene are effective as larvicides (Isman, 2006); it has an insecticide activity against *A. fabae* but without negative effect on the growth of the fabae plant (Burley *et al*., 2008). γ-terpinene, another active substance of Thymus oil, has a very good larvicide effect in the form of pure substance (Kouache *et al*., 2017). On the other hand, the first application of TAEO (0.1%) presents the highest mortality rate (75.51%) after seven days of treatment. This result of aphid mortality by spraying thyme oils is consistent with the literature (Shiwakoti *et al*., 2016; Baba-Aissa *et al*., 2017 and Kouache *et al*., 2017).

CONCLUSION

The use of natural products as insecticides could represent an important alternative for the fight and control of the black bean aphid (*A. fabae*) because they are low toxicity sources, rich in biodegradable bioactive compounds. The present results demonstrate that the essential oil of *T. algeriensis* can be used as a natural insecticide. Carvacrol, p-cymene and γ-terpinene are the major compounds in the essential oil of *T. algeriensis* and may act synergistically to produce the insecticidal action observed against *A. fabae* not necessarily due to their majority constituents; it could be due to minority constituents. This effectiveness requires more studies to elucidate the action mechanism of the compounds of essential oils as well as to isolate each compound and to study the synergistic effect. Treatment with TAEO (0.1% concentration) shows the best results under laboratory conditions, it have to be checked in the field in order to assess their effectiveness in the natural environment in interaction with biotic and abiotic factors, to prepare its exploitation as biopesticides.

Conflict of intertest: None.

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