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Insecticidal Effect of Two Chemotypes of the Essential Oil of *Eucalyptus camaldulensis* Dehnh against *Aphis fabae* Scopoli

M. Mehani[1,2], A. Rezzag[2], A. Chelgui[2], Ladjel Segni[1]

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ABSTRACT

Background: The objective of this study was to study the insecticidal and repellent effect of the essential oil of the plant *Eucalyptus camaldulensis* Red *Dehnh* extracted by hydrodistillation method planted in the Ouargla region against the black aphid.

Methods: The insecticidal effect of essential oils against aphid mortality by the introduction of bean leaflets in the different oily extracts with different concentrations and the count of dead individuals after 3, 6 and 24 h at the physiology laboratory in Ghardaïa University.

Result: Through this study, the leaves treated with the major compounds of *Eucalyptus camaldulensis* essential oil such as Benzene,1-methyl-4-(1-methylethyl) at 35 per cent concentration after 24 hours, a 65 per cent aphid mortality was recorded which is more effective compared to Sabinene and essential oils of *Eucalyptus camaldulensis* at different concentrations (5; 35%; and 50 per cent). As for the repellent effect is 87 per cent after 6 hours for the pure essential oil of *E. camaldulensis*.

Key words: Aphid repellent effect, *Aphis fabae* scopoli, Bean, Chemotype, Essential oil, Insecticidal.

INTRODUCTION

The broad bean, *Vicia faba* L. is a legume that has been part of agrarian systems for a very long time, its global area is estimated as 3 million hectares of which more than 50per cent are located in China, 20 per cent in North Africa and less by 10 per cent in Europe (Abu Amer *et al*., 2011).

In Algeria, the bean alone is the most important among food legumes, occupying 58,000 hectares, or 44.3 per cent of the total area reserved for this category of crop (Boussad and Doumandji, 2004). The bean occupies the first place among legumes in Algeria due to its high nutritional value and its various uses. It is mainly cultivated in the plains and the sub-coastal regions and has an important role in the national economy and in agricultural production (Aouar-Sadli *et al*., 2008).

The crop is attacked by various pests, insects occupy an important place, many of which are harmful. Among those the aphids (*Aphis fabae* L.) is very important and it is a phloem feeder, in other words, it absorbs the sap produced by plants, diverting part of the nutrients necessary for their growth to their advantage. In addition, during their food intake, they inject saliva that is often toxic to the plant and it is responsible to transmit viruses that cause serious diseases. The pest is responsible for significant yield losses (Dedryver, 2010).

Fir the control of this pest, different chemicals are used which are responsible for health problems in producers and consumers, development of resistance in pest, resurgence of other insect pests and destruction of natural enemies as well as ill effect on environment (Tata, 2011; Ryckewaert and Fabre, 2001; Bakroune, 2012). There is a need of new alternative methods of pest control (Goucen-Khelfane, 2014). Plant products are very good source of insecticides and the plant origin pesticides are biodegradablem safe to producers, consumers, natural enemies and environment. Considering the importance of plant products in plant protection, the present studies were carried out to test the nsecticidal and repellent effect of the essential oil of the plant *E. camaldulensis* extracted against *A. fabae*.

[1]Laboratory of Process Engineering, Faculty of Applied Sciences, Ouargla University, Ouargla, Algeria.
[2]Faculty of Natural and Life Sciences, University of Ghardaia, Ghardaia 47000, Algeria.

Corresponding Author: M. Mehani, Laboratory of Process Engineering, Faculty of Applied Sciences, Ouargla University, Ouargla, Algeria. Email: mounameh@gmail.com

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MATERIALS AND METHODS

Study area

The study was carried out at the physiology laboratory of the Department of Life and Nature Sciences at the University of Ghardaïa during the year 2020. To ensure that the experimental tests run smoothly, the tests are repeated several times.

Test insects

The test insects is composed of colonies of the bean aphid, taken from *V. fabae* which has already been planted in the greenhouse of the University of Ghardaïa, from this culture

samples of the aphid colonies on the plants at random the most attacked were placed in the Petri dishes.

Extraction of essential oil from the leaves of *E. camaldulensis*

In the present study the hydrodistillation method for the extraction of essential oil from the leaves of the *E. camaldulensis* plant planted in the southern Algerian wilaya of Ouargla were used and the major essential oil compounds such as: Sabinene, Benzene, 1-methyl-4-(1-methylethyl) which were separated from essential oils of *E. camaldulensis* at CREA laboratory in Italy by the GC-MS method MRN were tested against the black aphid (*A. fabae*).

Evaluation of the insecticidal effect of essential oil of *E. camaldulensis* and its major compounds

The various tests were carried out under laboratory conditions to study the insecticidal effect of the essential oils of *E. camaldulensis* and its major compounds against individuals of black aphids (*A. fabae*). Individuals of 3rd and 4th instar nymphs of *A. fabae* were used in the study.

Total 55 Petri dishes, with 5 repetitions for each treatment were prepared. Each box contains 1 leaflet of the bean, treated with a different extract. A total of 11 treatments were tested: 10 with the essential oil of *E. camaldulensis* and its major compounds at different concentrations (5%, 35%, 50%) and 1 for distilled water (control).

Each leaflet is introduced into the container containing the corresponding treatment so that the leaflet is well soaked for a few seconds. Then 10 individuals (adults) of aphid were put in each box (placed on the leaflets). Individuals who died 3, 6 and 24 hours after artificial infestation were counted for each box. This test was carried out as follows:

- **1st part (right half-sphere) (A)**

Contains a leaf of the bean treated with essential oils of *E. camaldulensis*.

- **2nd part (left half-sphere) (B)**

Contains a leaf of the bean not treated with essential oils of *E. camaldulensis*.

- **In the center of each box**

We placed 10 3rd and 4th instar larvae of aphids which were selected from the infested bean plants (Fig 1).

After 3, 6, 12 and 24 hours were counted for each box nymphs present on the box portion whose leaves are treated with the essential oil of *E. camaldulensis* (zone A) and the number of those present on the untreated part (zone B) and also the dead nymphs for each part.

Mortality rate

According to ABOTT (1925), the Mortality Rate are calculated by the following formula:

$$(Mc)\ \% = \frac{Mo - Mt}{100 - Mt} \times 100$$

(Mc) %: The corrected percentage for mortality.
Mo: The number of dead individuals in the boxes treated with the extracts.
Mt: Number of dead individuals in the witness boxes.

Statistical analysis

In order to compare the averages of the mortalities, the orientation of aphids towards the treated leaves of each Petri dish, as well as the repellency rate of the aphids. We used one-way analysis of variance (ANOVA). When there is a significant difference, a Student-Newman-Keuls test is used to identify homogeneous groups. These analyzes were performed using SPSS software.

RESULTS AND DISCUSSION

Evaluation of the insecticidal effect of different essential oils on aphid mortality

ANOVA statistical analysis revealed the significant differences in aphid mortality rates on leaves treated with the different essential oils of the *E. camaldulensis* plant (Table 1).

From Table 1 it is noticed that the highest mortality rate of *A. fabae* was recorded on leaflets treated with Benzene, 1-methyl-4- (1-methylethyl) with the concentration (35%). It reaches 65 per cent of mortalities after 24 hours of treatment.

Comparison of aphid mortality rate between different treatments at 5 per cent

It is evident from the Fig 2 that the mortality rate of aphids resulting from the effect of essential oil of *E. camaldulensis*, Sabinene and Benzene, 1-methyl-4- (1-methylethyl) at 5 per cent concentration, the mortality rate of aphids resulting from the effect of different essential oils increases with time. The mortality rate of aphids recorded by the effect of essential oil of Sabinene is the highest which is: 8 per cent at 3 hours, 19.26 per cent at 6 hours, 33.95 per cent at 12 hours and 53, 47 per cent at 24 hours compared to other effects of essential oils on aphid mortality rate. While the effect of Benzene, 1-methyl-4- (1-methylethyl)

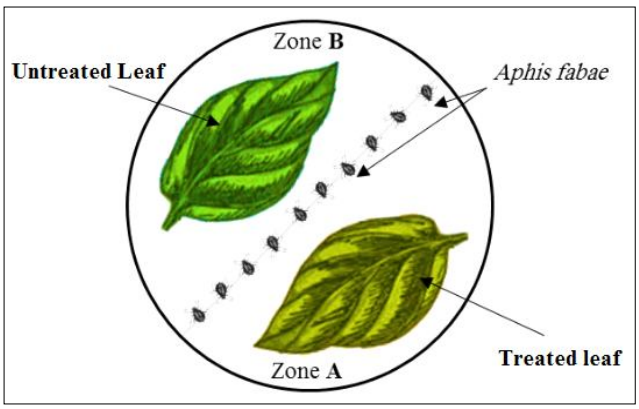

Fig 1: Arrangement of leaflets treated in the aphid orientation test.

and *E. camaldulensis* essential oil on aphid mortality rate begins at 6 h.

Comparison of aphid mortality rate between different treatments at 35%

According to Fig 3, which represents the aphid mortality rate under the effect of various treatments, there is an increase in the aphid mortality rate as a function of time to 35 per cent, including the aphid mortality rate obtained by benzene, 1-methyl-4-(1-methylethyl) becomes more important from 6h with a value of 26.26 per cent, compared to other treatments namely the essential oil of *E. camaldulensis* and Sabinene.

Comparison of mortality rates between different treatments at 50 per cent

According to Fig 4, the mortality rate of aphids under the effect of different treatments at 50 per cent increases with time. The same black aphid mortality rate obtained by the effect of essential oil of E. *camaldulensis* and Benzene, 1-methyl-4-(1-methylethyl) as a function of time whose values are 55% and 52 per cent, respectively after 24 hours.

Comparison of aphid mortality rates resulting from different treatments with different concentrations

Fig 5, shows the mortality rate of black bean aphids obtained by different treatments with different concentrations at 5, 35 and 50 per cent as a function of time, The death (mortality) rate of aphides is important between 3 h and 6 h with the leaves treated with pure essential oil of *E. camaldulensis* with values of: 16 and 30.37 per cent, respectively. On the other hand, the leaves treated with the Benzene, 1-methyl-4- (1-methylethyl) at 35 per cent concentration for 12 h and 24 h have a higher mortality rate compared to other essential oils with a value of 44.36 and 65 per cent, respectively.

Comparison of mortality rates between Sabinene and Benzene, 1-methyl-4- (1-methylethyl) with different concentrations

It is evident from the Fig 6 that the effect of Sabinene and Benzene, 1-methyl-4- (1-methylethyl) on the aphid mortality rate that the effect of two treatments with different concentrations (5, 35 and 50%) on the black bean aphid mortality rate increases with time and concentrations. The

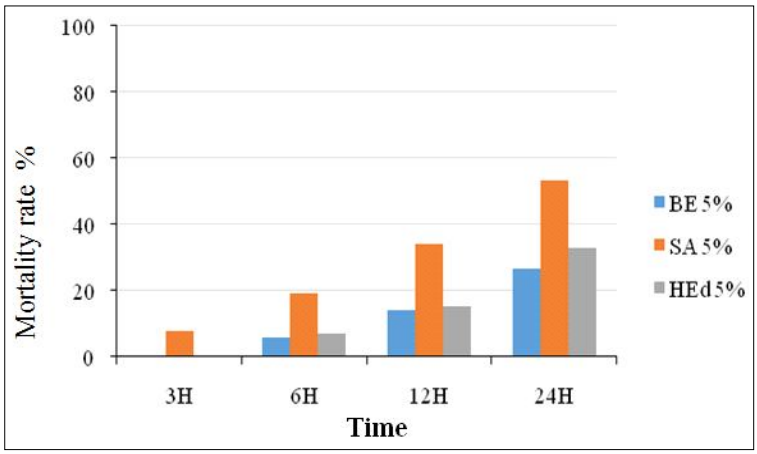

Fig 2: Mortality rate of aphids resulting from different treatments at 5%.

Table 1: Analysis of variance and classification of homogeneous groups of aphid mortality rates.

Treatments	3H	6H	12H	24H
HEp	16 b	30.37 b	40.26 ab	60 a
HEd 5%	0 a	7 a	15.32 a	32.84 a
HEd 35%	6 ab	23.26 ab	40.26 ab	62.95 a
HEd 50%	8 ab	17.05 ab	29.05 ab	51.9 a
SA 5%	8 ab	19.26 ab	33.95 ab	53.47 a
SA 35%	9 ab	21 ab	37.69 ab	62.26 a
SA 50%	3 a	8.05 a	13.53 a	26.95 a
BE 5%	0 a	6 a	14.26 a	26.63
BE 35%	8 ab	26.26 b	44.37 b	65 a
BE 50%	7 ab	15 ab	27.95 ab	50.84 a
Signification	0,00	0.00	0.00	0.01

*Significant if P<0.05; 3H; 6H; 12H; 24H very significant; HEp: Pure essential oil of *Eucalyptus camaldulensis*; SA: Sabinene; BE: Benzene, 1-methyl-4-(1-methylethyl); HEd: Essential oil dilute of *Eucalyptus camaldulensis*.

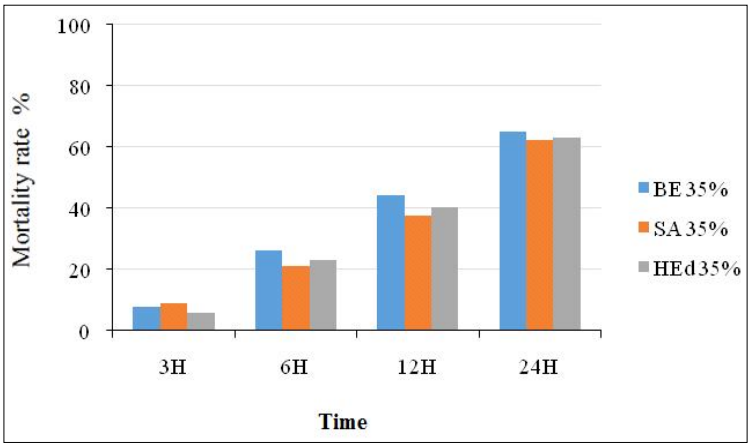

Fig 3: Mortality rate of aphids results by different treatments at 35%.

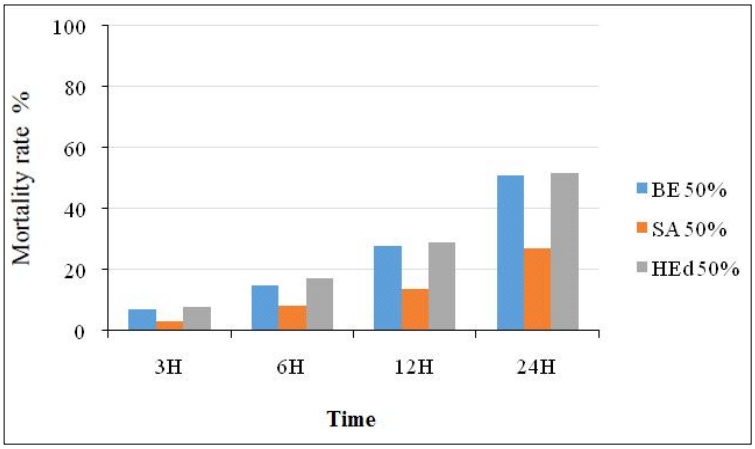

Fig 4: Mortality rate of aphids results by different treatments at 50%.

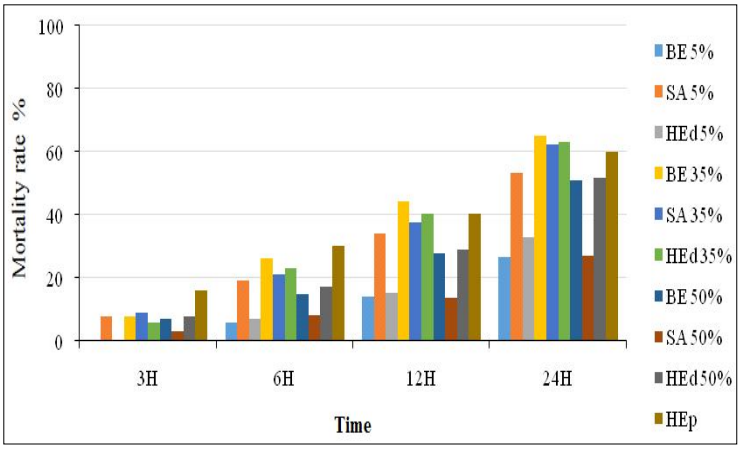

Fig 5: Mortality rate of aphids resulting from different treatments with different concentrations.

highest mortality rate was recorded by leaves treated with Benzene, 1-methyl-4- (1-methylethyl) compared to leaves treated with Sabinene from 6 h with the concentration of 35 per cent with a value by 26.26 per cent. At 24 almost the same mortality rate was noticed by the effect of two treatments at 35 per cent as a function of time whose values are 66 and 68 per cent, respectively.

Comparison of mortality rates of aphids resulting from Benzene, 1-methyl-4- (1-methylethyl) with different concentrations

According to Fig 7 the mortality rate of aphids under the effect of Benzene, 1-methyl-4- (1-methylethyl) with different concentrations increases with time. In addition, at 35 per cent concentration of Benzene, 1-methyl-4-(1-methylethyl) records a higher black aphid mortality rate as a function of time compared to other concentrations, which was 65 per cent mortality from 24 h.

Comparison of aphid mortality rates resulting from Sabinene with different concentrations

Fig 8 showed that the black aphid mortality rate under the effect of Sabinene with different concentrations increases with time. However, at 35 per cent of Sabinene concentration, the mortality rate is greater as a function of time compared to other concentrations which is: 9 per cent at 3 h, 21 per cent at 6 h, 37.69 per cent at 12 h and 65 per cent at 24 h.

Comparison of mortality rate of aphids resulting from essential oil of *E. camaldulensis* with different concentrations

Fig 9 showed that the mortality rate resulting from *E. camaldulensis* essential oil with different concentrations increases with time. While at 35 per cent concentration of essential oil of *E. camaldulensis* records a higher mortality rate from 24 hours which is 62.95 per cent.

Pesticides can also have a negative effect on biodiversity by affecting wild flora and fauna and reducing species diversity (Isenring, 2010). In addition, the intensive use of pesticides causes another problem: resistance and the selection of new, more powerful individuals, insects or weeds (FAO, 2013).

Botanical insecticides are a major weapon in the farmer's arsenal against crop pests. Botanical insecticides offer a more natural, "ecological" approach to pest control than synthetic insecticides do (Audrey Leatemia *et al*., 2004).

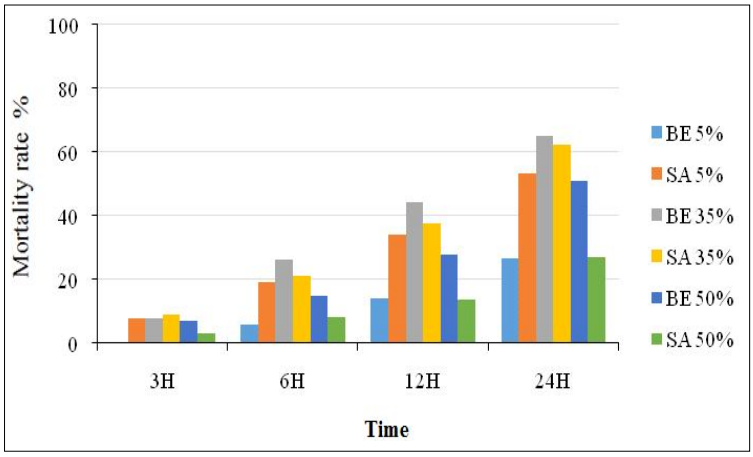

Fig 6: Mortality rate of aphids resulting from different concentrations of Sabinene and Benzene, 1-methyl-4- (1-methylethyl).

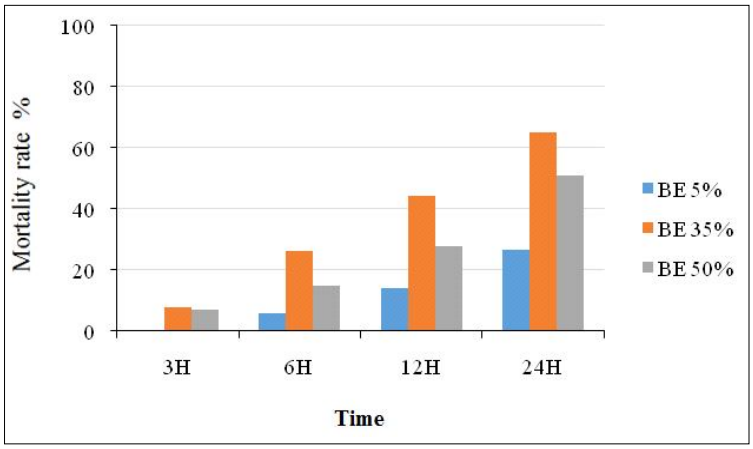

Fig 7: Mortality rate of aphids resulting from Benzene, 1-methyl-4- (1-methylethyl) with different concentrations.

Many studies have revealed an aphicidal effect of plant extracts, such as Neem (*Azadirachta indica*) and plants based on pyrethrum (Cross *et al*., 2007).

For their part, aqueous extracts based on *Thymus algeriensis* caused a percentage of mortality reaching 22.5 per cent on adults of *A. fabae*. So a low result compared to our work on essential oils (Bourouba *et al*., 2019).

The essential oils of *E. camaldulensis* have a very remarkable insecticidal effect on adults of *A. fabae*, with a concentration of 35 per cent for Benzene, 1-methyl-4-(1-methylethyl) recording a value of 65 Per cent. The results obtained show a significant effect of the oily extract in inducing massive mortalities in adults of *A. fabae*. The present results on the effect of essential oils of *Eucalyptus* are almost in line with the work of Hedjazi and Tabti (2017) that are worked on the insecticidal activity of plant extracts (*Pistacia atlantica*, *Marrubium vulgare* and *Thymus algeriensis*) against the aphid black bean (*A. fabae*), its results shows a more efficient mortality rate which is 70 per cent after 24 hours. This toxic effect could depend on the chemical composition of the extracts tested and the level of sensitivity of the insects (Ndomo *et al*., 2009).

According to Abedjalil *et al*. (2015) who demonstrated the efficacy of sage extract against black bean aphid and are very encouraging about the possibility of using these compounds as a means of biological control against *A. fabae* in order to avoid any treatment by them conventional insecticides with harmful effects for humans and the environment.

In addition (Zahaf, 2016), showed a very important insecticidal effect varying according to the concentrations used and the time, the tested extract presented a toxic effect on black bean aphids, it allows to cause a mortality rate reaching 100% from the 2nd day of contact with a concentration of 50 per cent and 100 per cent.

This difference in percentage mortality rate may be due to a difference in the chemical that differs from plant to plant, the time of harvest of the plants and the method of extraction. According to Kim *et al.* (2003), the toxic effects

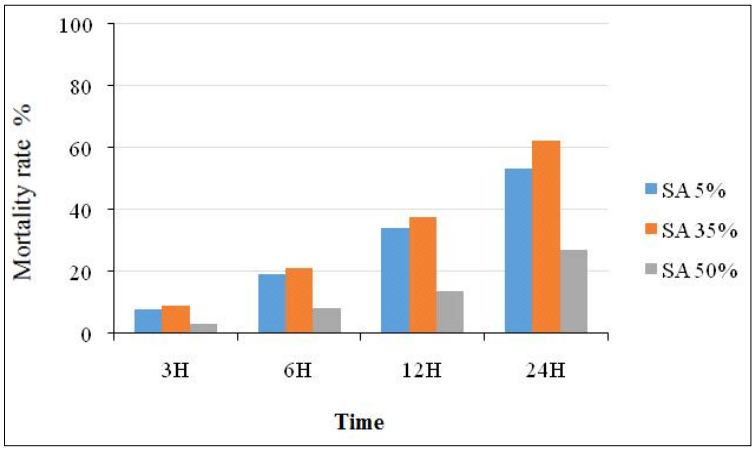

Fig 8: Mortality rate of aphids resulting from Sabinene with different concentrations.

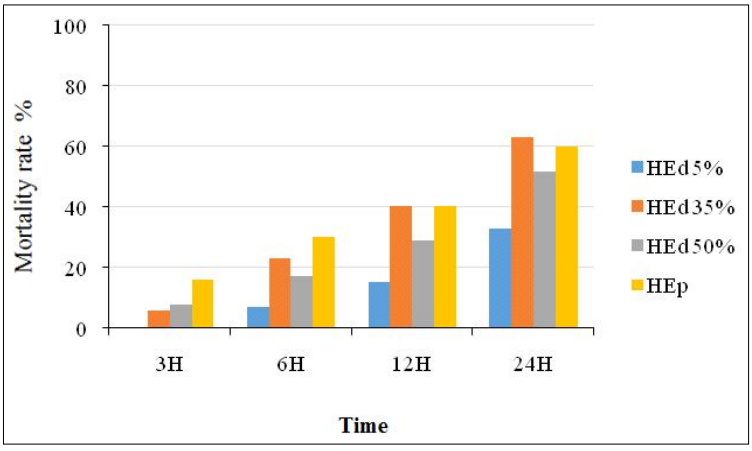

Fig 9: Mortality rate of aphids resulting from essential oil of *E. Camaldulensis* with different concentrations.

of essential oils depend on the species of insect, the plant and the time of exposure.

Raven *et al*. (2003), report that many of the terpenoids found in plant essential oils are poisons, which can cause heart attacks in insects. Used medicinally, cardiotonic terpenoids can slow down or stimulate the heartbeat of insects. Thus an imbalance of the hormonal balance can have considerable effects on the physiology and behavior of the insect and thus contributes to its poisoning (Moreteau, 1991).

CONCLUSION

Today, there is great concern about the danger presented by the chemicals used to control insect pests, due to their unwanted actions which cause the appearance of several diseases on the fauna and flora and even on the man, also pesticides play an important role in the destruction of ecosystems. This is why researchers are beginning to realize the importance of returning to nature. In this context, this work was carried out as part of the search for natural products that can substitute chemicals, used to protect plants against their enemies.

The main objective of the present study is to test the use of essential oil extracts from the medicinal plant *E. camaldulensis* with Benzene, 1-methyl-4- (1-methylethyl) chemotype against the bean pest, the aphid black, *A. fabae*. It was noted that the leaves treated with the essential oil of benzene, 1-methyl-4- (1-methylethyl) at 35per cent concentration after 24 h recorded a more effective aphid mortality rate with a value of 65 per cent.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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