

Assessment of the efficacy of four medicinal plants as fumigants against *Varroa destructor* in Algeria

Koumad, S.[®] and Berkani, M.L.

¹Animal Production Department. Agronomic National superior school. El-Harrach. Algeria.

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Correspondencia a los autores/Contact e-mail:
s.koumad@st.ensa.dz

INTRODUCTION

The mite *Varroa destructor* has been identified as the only responsible for a large number of cases of colony losses worldwide (Genersch et al. 2010, p. 344; Vanengelsdorp et al.2011, p.9; Martin et al.2012, p. 1306). In Algeria, *Varroa* was firstly described in 1981

SUMMARY

Varroa destructor is the most harmful honey bee (*Apis mellifera*) parasites and is one of the major threats to apiculture worldwide. Nowadays, several chemical and natural products have been used. The present study aims to evaluate the effect of the smoke of four medicinal plants (*Rosmarinus officinalis*, *Thymus palleescens*, *Mentha viridis* and *Laurus nobilis*) against the mite *varroa* on twenty five Algerian honeybee colonies. 50 g of each dried plant was administered using bee smoker were applied during one month. The results have showed that all plants showed fumigant activity in varying degrees against the mite *varroa*. The highest rate of efficacy was obtained by laurel and rosemary which achieved more than 80% of mortality followed by thyme and spearmint. (ANOVA) results showed a significant difference between treatments ($P < 0.05$). The use of those local natural plants reduced the infestation rates of bee colonies. Rosemary and laurel were the most effective; however, spearmint exhibited the lowest acaracidal activity against *Varroa destructor*. We suggest that the tested plants screened in our study seem to have a promising potential as an alternative to use chemicals to control *varroa* mites and that they may be useful in maintaining lower mite infestation rates in beehives. This method might be used by individual beekeeper as a practical treatment. Therefore, more field and laboratory studies are needed by testing essential oils of those in order to determine their components for better evaluation.

Evaluación de la eficacia de cuatro plantas medicinales como fumigantes contra *Varroa destructor* en Argelia

RESUMEN

Varroa destructor es el parásito más dañino de la abeja melífera (*Apis mellifera*) y es una de las mayores amenazas para la apicultura en todo el mundo. En la actualidad se han utilizado diversos productos químicos y naturales. El objetivo del presente estudio es evaluar el efecto del humo de cuatro plantas medicinales (*Rosmarinus officinalis*, *Thymus palleescens*, *Mentha viridis* y *Laurus nobilis*) contra el ácaro *varroa* en veinticinco colonias de abejas melíferas argelinas. 50 g de cada planta seca se administró durante un mes utilizando ahumador de abejas. Los resultados han mostrado que todas las plantas mostraron actividad fumigante en diferentes grados contra el ácaro *varroa*. La mayor tasa de eficacia se obtuvo con el laurel y el romero, que alcanzaron más del 80% de la mortalidad, seguidos por el tomillo y la hierbabuena. El ANOVA mostró una diferencia significativa entre los tratamientos ($P < 0,05$). El uso de esas plantas naturales locales redujo las tasas de infestación de las colonias de abejas. El romero y el laurel fueron los más efectivos; sin embargo, la menta verde exhibió la menor actividad acaracida contra el *Varroa destructor*. Sugerimos que las plantas examinadas en nuestro estudio parecen tener un potencial prometedor como alternativa al uso de productos químicos para controlar los ácaros de la *varroa* y que pueden ser útiles para mantener tasas más bajas de infestación de ácaros en las colmenas. Este método puede ser utilizado por apicultores individuales como tratamiento práctico. Por lo tanto, se necesitan más estudios de campo y de laboratorio probando los aceites esenciales de estos para determinar sus componentes para una mejor evaluación.

in the apiary "OumTheboul" El Kala (East Algeria) (Belaïd & Doumandji 2010, p. 83). That year, the damage to Algerian apiculture was extensive and the disease remains unsolved causing serious problems. Parasitism can decrease up to 25% of adult weight (Duay et al. 2003, p.63), deformations of the wing (Nordstrom et al. 2003, p.300) and reduce longevity of worker honey

bees (Amdam et al. 2004, p.745). Therefore, the mites could be the carriers of virus diseases in honeybees (Sanpa et al.2009, p.118).

However, the wide spread use and the over use of these chemicals resulted in the rapid development of chemical resistance in numerous mite population (Rosenkranz et al.2010, p.108), alteration of the taste and quality of hive products by the presence of residues (Bogdanov 2006, p.7; Martel et al. 2007, p.543). As an alternative to traditional acaricides, natural products present some advantages such as high toxicity to mites, low toxicity to bees, few residues in bee products and no drug resistance (Ruffinengo et al. 2005, p. 651). In a similar way, smoke from plants has produced mixed success in increasing varroa reduction (Cakmak et al. 2006, p.479).

Many studies demonstrated the acaricidal effect of various plants and their extracts of natural essential oil against varroa like rosemary, mint, thyme, camphor, marjoram, laurel, clove and eucalyptus (Imdorf et al.1999, p.223; Ariana et al. 2002, p.321; Damiani 2014, pp.702). In view of its bioclimatic diversity, Bouira is known for its wealth of medicinal plants, in particular the four aromatic herb species of thyme (*Thymus*

pallescens); rosemary (*Rosmarinus officinalis*); spearmint (*Mentha viridis*) and laurel (*Laurus nobilis*) which have been the purpose of the study. In addition to their therapeutic properties, their pure components have a high acaricidal activity. Thus, the trial was conducted to evaluate their effect by smoke against *Varroa destructor* in *Apis mellifera* Algerian honey bee colonies.

MATERIAL AND METHODS

PLANT MATERIAL

The aerial parts leaves of (*Rosmarinus officinalis*, *Mentha viridis*, *Thymus pallescens*, and *Laurus nobilis*) during the blooming phase, simultaneously a significant accumulation of bioactive substances were harvested early in the morning in April 2013, in the in Bouira, Algeria (36°22'N; 3°53'E).

Once the plants were identified in the Department of Botany of the National Institute of Agronomy of Algiers (Algeria), the leaves of the plants were cleaned and dried separately in open air and in the shade (**Figure 1**). Subsequently, the samples were stored in paper bags sheltered from light and moisture for sub-

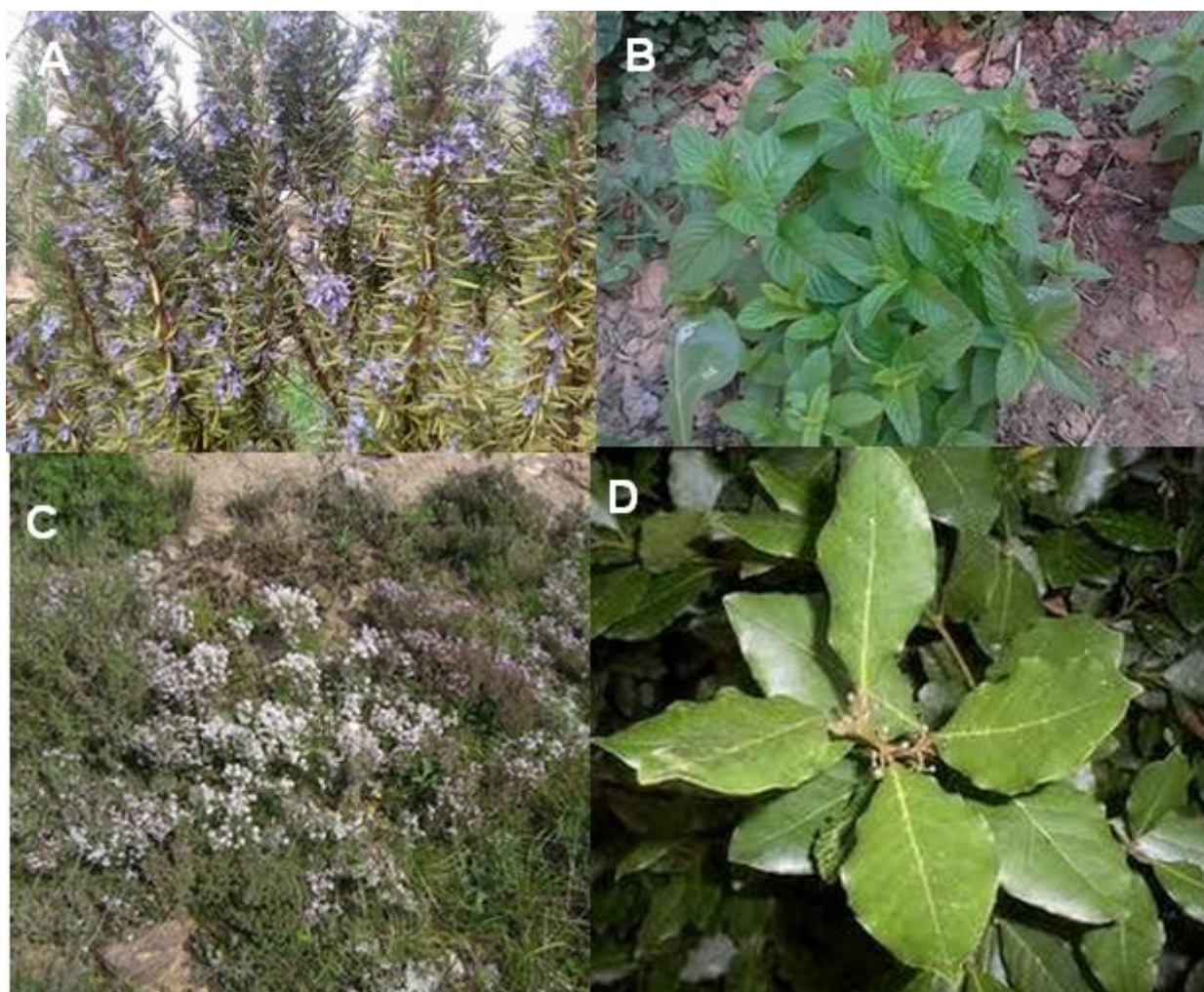


Figure 1. Plants tested: Rosemary (*Rosmarinus officinalis*) (A); Spearmint (*Mentha viridis*) (B); Thyme (*Thymus pallescens*) (C) and Laurel (*Laurus nobilis*) (D) (Plantas probadas: Romero (*Rosmarinus officinalis*) (A); menta verde (*Mentha viridis*) (B); Tomillo (*Thymus pallescens*) (C) y Laurel (*Laurus nobilis*) (D).

sequent use in the trial in order that they preserve their molecules as far as possible.

STUDY AREA

The experiment was carried out in spring 15/05/2013 on twenty five heavily infested adults-*Apis mellifera* honeybees colonies have been chosen with the same population size and were kept on the Langstroth hives from 'Bouira' in centern Algeria (36° 22' N; 3° 53' E; altitude: 555 m), characterized by a Mediterranean climate with a warm and dry summer and a cold and rain in winter, (average annual rainfall 415±500 mm) with the coldest month is February with an average of 7°C and dry in summer (average annual temperatures 37 ±18) with a the hottest month is August and an average temperature 37. The average temperature and relative humidity (RH) recorded during this period in Bouira were 18 to 29 °C and 75 to 80%, respectively.

FIELD TRIALS

Five groups (treatments and control) of five hives each were monitored. All colonies were infested by varroand had not received any treatment before the trial.

Group I colonies received smoke from laurel, Group II colonies received smoke from rosemary, Group III colonies received smoke from spearmint, Group IV: colonies received smoke from thyme and

Group V which served as a controls and did not received smoke.

50 g of leaves were placed in a smoker with burlap and puffs were blown during ten minutes (because excessive exposure can harm the bee) inside the hive through the entrance which was blocked thereafter for a few minutes and the hive was completely closed from all sides to allow dissipate of the smoke in the colony as reported by (Eischen and Wilson 1997, p.122).

COUNTING MITES

Over the field trial period, dead mite fallen were collected and counted each three days in all the groups before and after treatments. Each hive was equipped with sliding board composed of metal sheet fixed standard coated with a layer of grease (Vaseline) and was changed all the three days after counting the mites. The sticky bottom board was covered by a wire screen to prevent the bees from coming into contact with the debris. Then, the infestation, mortality and efficacy rates were recorded continuously during the pretreatments, treatments and control. Also, we visually estimated the number of bees observing frame sections covered by honey bees as proposed by (Delaplane et al. 2013, p.6).

-The efficacy of all the treatments was calculated by using the following formula:

$$\text{Efficacy (\%)} = (\text{No. of mites fallen for each treatment} / \text{Total number of fallen mites}) \times 100$$

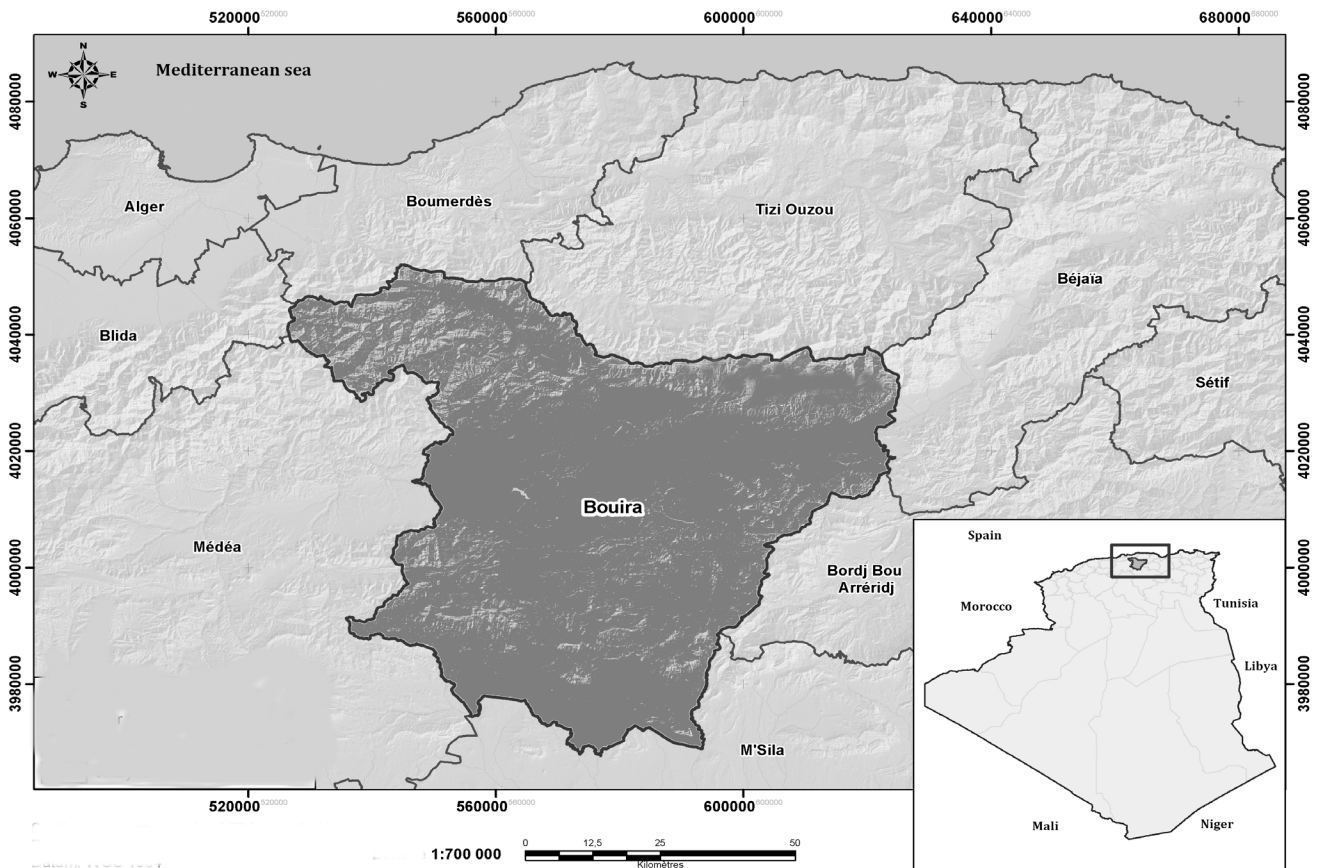


Figure 2. Localization of the study area on the map of Algeria, Bouira) (Localización del área de estudio en el mapa de Argelia, Bouira).

Table I. Number of fallen mites recorded under the effect of plants smoke (Número de ácaros caídos registrados bajo el efecto del humo de las plantas).

| Plants | Total varroa | Dead varroa | Rates of dead varroa |
|-----------|--------------|-------------|----------------------|
| Rosemary | 17129 | 14045 | 82 % |
| Laurel | 22553 | 18826 | 83.47 % |
| Spearmint | 20690 | 8882 | 42.93 % |
| Thyme | 16838 | 10172 | 60.41 % |
| Control | 17516 | 4589 | 26.20 % |

-The infestation levels were calculated with the following equation:

Infestation rate (%) = (number of mites/number of bees) × 100

STATISTICAL ANALYSIS

Data were subjected to one-way analysis of variance (ANOVA) at the significance level of 5 %) by using SPSS software (22.1 Version).

Statistical analysis of ANOVA was performed using the following mathematical model:

Firstly, if is no difference at all between the groups; that is, that the group means are really the same and any observed difference is a chance event. An accurate model would then be

$$x_{ij} = \mu + \epsilon_{ij}$$

If, on the other hand, it is assumed that there is a difference between the groups then the mathematical model was represented by:

$$y_{ij} = \mu + \alpha_j + \epsilon_{ij}$$

Where

y_{ij} = Dependent variable;

μ = overall mean;

α_j = Deviation due to level j of the factor

ϵ_{ij} = Deviation due to the observation (residual)

ϵ_{ij} = Statistical error.

The null and alternative hypotheses of one-way ANOVA can be expressed as:

H_0 : $\mu_1 = \mu_2 = \mu_3 = \dots = \mu_k$ ("all k population means are equal")

H_1 : At least one μ_i different ("at least one of the k population means is not equal to the others")

where

μ_i is the population mean of the i^{th} group ($i = 1, 2, \dots, k$)

In order to determine if the infestation rate is related to the bees number in the colonies, a linear regression was realized. The mathematical equation for the general linear model using population parameters is:

$$Y = ax + b$$

Where "y" is the dependent variable, "a" is the y intercept, "b" is the slope of the regression line, and "x" is the independent variable.

The correlation is determined by a regression line whose mathematical function is:

$y = -731.91x + 40059$ with $R^2 = 0.412$. The values of x represent the infestation rate and those of y correspond to the number of bees in the colony

RESULTS

MITES FALLEN

Among the four plants tested, the most promising were laurel and the rosemary. Results have showed that all smoke plants achieved 50 percent mites knockdown and were significantly effective against Varroa in treated colonies compared to the untreated ones. The number of the fallen dead mites during the first few days of was significantly different in each colony ($p < 0.05$) throughout the four treatments, the highest number of mite drop per colony was recorded by the laurel (18826) and rosemary (14045), followed by thyme (10172), spearmint (8882) which offered the lowest toxicity against varroa and the control (4589) (Table I). Overall, the plants killed 83.47%, 82%, 60.41%, 42.93% and 26.20% respectively.

Table II. Colonies infestation rates (Means \pm SD), in %, before and after smoking of plants tested (Tasas de infestación de colonias (media \pm SD), en %, antes y después del ahumado de las plantas sometidas a ensayo).

| Plants | Before smoke | After smoke | Reduction rates |
|-----------|----------------|---------------|-----------------|
| Rosemary | 17.49 \pm 12 | 14.28 \pm 9 | 3.21 |
| Laurel | 15.21 \pm 3 | 10.72 \pm 6 | 4.49 |
| Thyme | 29.16 \pm 11 | 26.81 \pm 2 | 2.35 |
| Spearmint | 14.99 \pm 16 | 13.18 \pm 3 | 1.81 |
| Control | 19.32 \pm 6 | 21.02 \pm 2 | +1.70 |

INFESTATION RATE

Moreover, the degree of infestation is variable for the different hives composing the four experimental batches. Mainly due to differences in the hygienic behavior of the colonies (Castagnino, et al. 2016, p.553). We recorded as follows: All the hives have recorded an infestation rate higher than 5%, the batch of thyme has the highest infestation rate. Besides, the colonies which were treated by rosemary; laurel, spearmint and control have an average infestation rate either. Also, over the trial, no bee mortality was recorded.

The infestation rates after and before the smokes of the four tested plants are reported in (Table II).

Generally the effect of smokes of the four dry plants was effective against varroa during the trial. We noted that peak of the fallen mites appeared during the first hours preceding the treatments application. Furthermore, the infestation rates recorded after the application of plants smokes and ranged from 14.28%, 10.72%, 26.81%, 13.18% and 21.02% for rosemary, laurel, thyme, spearmint and the control respectively, comparing to the initial rates (17.49%, 15.21%, 29.16%, 14.99% and 19.32%) in the tested colonies.

In addition, the lowest reduction rate (1.81%) was recorded by the spearmint and the highest rate (4.49%) by the laurel. Thus, the smoke of the four plants reduced the infestation rate of 3.21%, 4.49%, 2.35%, 1.81% and + 1.70 % for rosemary, laurel, spearmint, thyme and control respectively. Then, the spearmint recorded the lowest reduction infestation rate compared to the laurel which recorded the highest reduction rate, which

means that the smoke plants act differently on the varroa mortality, the less reduction can be explain by:

- It is possible that we did not apply sufficient smoke.
- The infestation of the batches was different from the beginning of the trial.
- Group of thyme was more infested.
- Number of varroa could be reproduced during the treatment period, in batch 4 (the mint did not prevent the multiplication of varroa in the brood, it only acts on the phoretic varroa).
- The active material that was presented in each plant leaves has a different acaricidal effect.

Therefore, the correlation coefficient obtained is $r = 0.64$. We conclude that there is a significant correlation between the rate of infestation and the number of bees. However, the ANOVA showed a highest significant difference ($p < 0.05$, $F = 0, 96$).

EFFICACY OF SMOKED PLANTS

The average efficacy rates of the four plants used by fumigation on varroa were shown in (Figure 3).

According to our results, all the plants showed to possess strong acaricidal activity against varroa, laurel and rosemary was found to be the most effective. The two plants averaged the high fallen number of varroa and recorded a high efficacy of 92.77% and 88.96% against Varroa destructor; however, the results obtained showed a low efficacy of 64.29% recorded by the

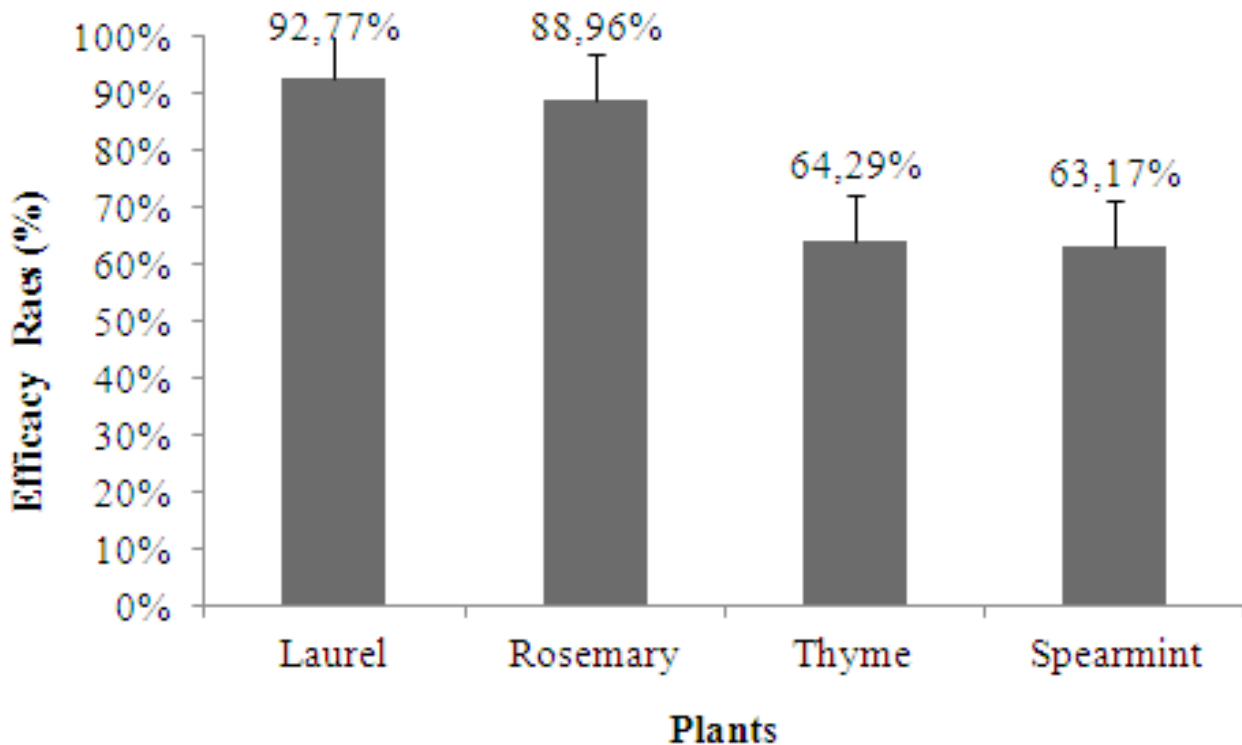


Figure 3. Efficacy rates of smoke of the four plants tested, Significant difference between the means of the infestation rates ($P < 0,05$) (Tasas de eficacia del humo de las cuatro plantas sometidas a prueba, Diferencia significativa entre los medios de las tasas de infestación ($P < 0,05$))

thyme and 63.174% for the spearmint. The ANOVA of the smoke effect of each plant tested against varroa was statistically significant ($p < 0.05$, $F=11.722$) and efficacy varied from one treatment to another and from one hive to another.

MORTALITY RATE

The mortality of the varroa vary from one hive to another, the table below shows that whatever the hive the mortality rate is more than 50%. Rosemary and laurel which have recorded a higher mortality rate compared to the spearmint which recorded a low mortality rate after the smoke of 30.65% (Figure 4).

The mortalities rates of varroa recorded in the treated colonies with the laurel, rosemary, thyme, spearmint and control were: 90.02%, 83.38%, and 79.20% 30.65% and 79.56% respectively.

The One Way ANOVA statistical analyzes and Multiple Comparison of Means showed high significant of the mortalities rates between treatments of varroa under the effect of the four smoke plants was highly significant ($p < 0.05$, $F=20.882$).

DISCUSSION

In this study, acaracidal effect of plants from laurel (*Laurus nobilis*), rosemary (*Rosmarinus officinalis*) thyme (*Thymus palleescens*) and spearmint (*Mentha viridis*) against the mite varroa was evaluated by fumigation. It is clear that the smoke treatment caused a significant mortality during the first application in spring this period coincided with the emergence or the hatching of the young bees of their cells, thus the release of the varroa which were fixed on their bodies

and their exposure to smoke treatment which neutralized them.

All treatments caused a fall mites in the tested colonies over trial and the natural products used in this work seemed to have no adverse effects on bees. In addition, the high number of fallen mites indicates the high infestation level of colonies. As claimed by (Flores et al.2015, p.163) that, estimating the mite population in beehives in a reliable manner is an important factor in Varroa control.

Hence, our results show that varroa showed more sensitivity towards laurel and rosemary. In general, in agreement with literature records relative to (Damiani et al. 2014, p.707; Maggi et al. 2011, p.400) and less for the spearmint (Cakmak 2006, p.479) which reported that the group treated with the smoke of spearmint (*Mentha aquatica*) had recorded decrease in the average number of mites.

Bensghir (2014, p.100) Found that colonies bees placed beneath a laurel showed a higher number of varroa fallen. Author suggested that the laurel caused changes in the hemolymph of the bees and thus increased the number of varroa fallen.

However, the mortality established during the diagnosis was higher, because in this period of high heat the mites become more vulnerable. After the smoke treatment, the mortality rate of mites was average except for the spearmint which had the lowest mortality rate. The treatment by fumigation has therefore reduced the rate especially for the laurel and rosemary which had the highest rate infestation reduction, followed by the thyme.

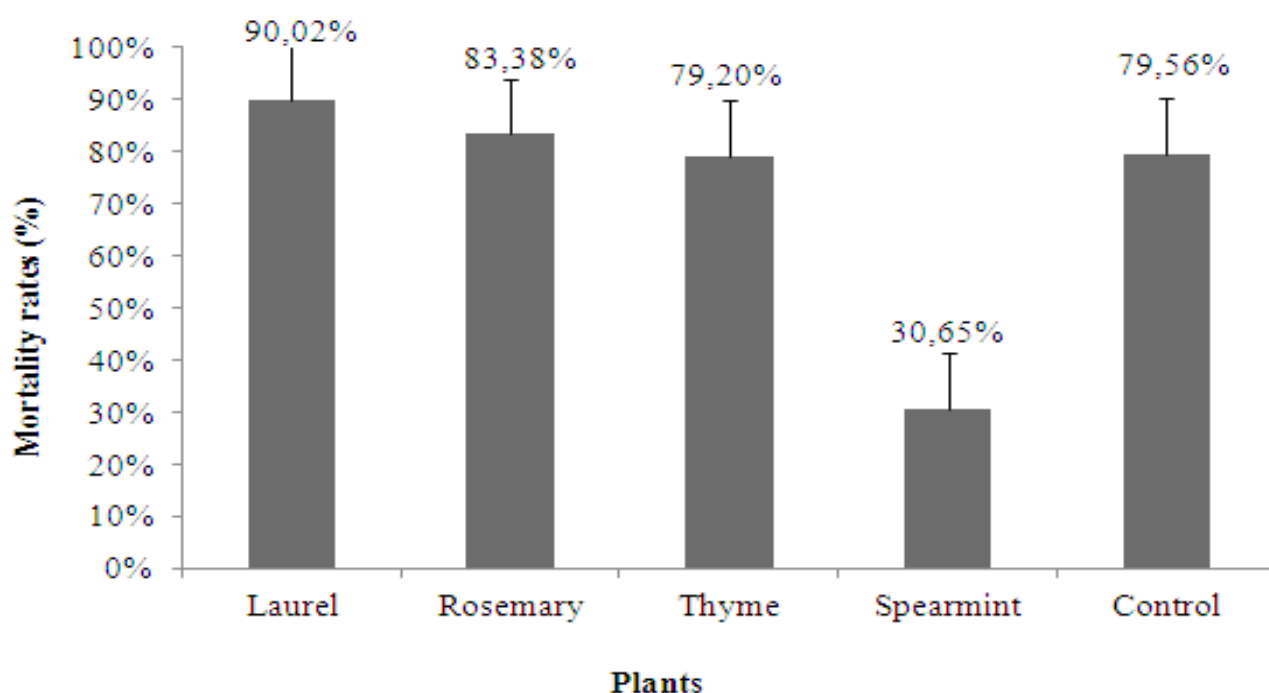


Figure 4. Mortality rates of four smoke of the four plants tested, Significant difference between the means of the infestation rates ($P < 0,05$) (Tasas de mortalidad del humo de las cuatro plantas sometidas a prueba, Diferencia significativa entre los medios de las tasas de infestación ($P < 0,05$).

The acaracidal effect is due to the camphor with principal compound for the *Rosmarinus officinalis* dried (Maggi et al, 2011,p.399) and (1.8 Cineole) (44.12%) for the *Laurus nobilis* (Yalçın et al.,2007,pp.717;Sangun et al; 2006,p.731), and (Carvicol and thymol) natural constituent of several species of thyme (El Bouzidi et al., 2013,p.452; Pirbalouti et al., 2013, p.45) which are widely used in honey bee colonies as a treatment against varroa (Imdorf et al. 1999, p.210; Lindberg et al. 2000,p. 196; Fassbinder et al.2002,p.85).

Tananaki et al. (2014, p.1254) applied the thymol powder, on the top of the brood frames, with an average efficacy of 64.5%. Also, according to the report of (Ariana et al. 2000, p.327-319; Emsen et al. 2011, p. 804; Rahimi et al. 2017, p. 182) thyme was one of the best method against varroa.

Nonetheless, Melathopoulos et al. (2010, p.6); Daher-Hjaij and Alburaki (2006, p.96) noted that thymol had positive activity on reducing varroa infestation rates in the colonies.

Our results showed outside the rosemary and the laurel which have been effective. The thyme and especially spearmint seem to act slowly on the mortality of varroa. Indeed, compared to other plants rosemary and laurel recorded a mortality close to 100% of varroa after treatment with 15mg by fumigation. In line with (Daher-Hjaij&Alburaki, 2006, p. 95), with another species of mint (*Mentha pelgium*) they recorded an efficacy of 44.9% using 10 g of this plant by smoking on bee colonies infested with varroa. On the other hand, the results of (Harouz-Cherifi&Habbi-Cherifi, 2015) on rosemary by fumigation revealed an average efficiency of 51.20%. Nevertheless, the thyme is the plant which recorded the lowest efficacy.

In the contrary, Ghomari et al. (2014, p.36) demonstrated that the effects of thyme (*Thymus vulgaris*) were positive against varroa at the dose of 15 mg.

Otherwise, Shaddel-Telli et al.(2008, p.329) reported 36.51% of the reduction of infestation rate of varroa by *Thymus kotshyonus* fumigation. Moreover, Kutukoğlu et al. (2012, p.556) founded an effectiveness of laurel of (76.7%) in the spring. Contradictorily, (Daher-Hjaij&Alburaki, 2006, pp. 95) have found a high efficiency of 74.4% of the laurel by fumigation on colonies bees. Al-Abbadi and Nazer (2003, p. 20) signaled that the effective time of application depends on the mite level of infestation and the life cycle of the mite.

Furthermore, Islam (2016, p.99) evaluated the insecticidal activity of aromatic plants of thyme (*Thymus linearis*), rosemary (*Rosmarinus officinalis*), and mint (*Mentha longifolia*) and reported that they were more effective against varroa.

In addition, the weakness of the efficiency of the treatment finds its origin in the presence of capped brood which protects varroa inside cells and so prevents the penetration of the smoke (Sammataro et al.2009, p. 257) because only phoretic varroa which were presented on the bees which are affected by the smoke but not those who were trapped in the cells of the brood. In other words, the varroa that was fixed to

the lower part of the body of the larva unfortunately escape from the effects of the treatment. So, it becomes imperative in our opinion for beekeepers to watch over the condition of the hive before the period of the slope of the eggs to avoid any contamination.

In the other hand, it is important to know that drying and conservation method of the plant material have an important goal to the acaracidal effect showing that the main components of the plants differ according to the geographical locations from which they were collected (Maggi et al, 2011, p.398), it was confirmed that the chemical composition of plants depends upon cultivation, climatic conditions, the variety, time and the way of harvesting (Imdorf et al.1999, p.210), drying and storage method (Verma& Chauhan 2011, p.72).

Notwithstanding the fact that the plants show specificity towards the mite, results of field test have been mixed, and some studies reported a success of those products and others reporting no effect as treatment towards mites. Therefore, Abd-El-Fattah et al. (2012, p. 34) suggested that to remove all mites from a hive by a smoke treatment is not a practical method for varroa control. This technique is not applicable at temperature below 12°C because the bees would form a very dense cluster to oppose the smoke entering, which would result in the suffocation of the queen.

CONCLUSION

Overall, the results of the presented work indicated the four plants without harmful effect against honeybees decreased percentage of varroa mites mortalities, especially for the *Rosmarinus officinalis* and *Laurus nobilis*.

However, it is clear from the findings of the presented study that the fumigant toxicity of the plants tested against the mite *Varroa destructor* varies noticeably with some factors such as temperature, the amount of smoke, environmental conditions storage method which influence the bees mortality. Therefore, laboratory trial by evaluating their essential oil could provide more accurate information on their potential with special regard to their exact chemical composition, mode of action in order to determine their effects on the varroa mite and honeybees colonies.

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