CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

GC/MS ANALYSIS OFVOLATILE CONSTITUENTS, TOXICITY AND ACARICIDAL ACTIVITY OF THE *EUCALYPTUS ROBUSTA* ESSENTIAL OIL TO FIGHT *VARROA DESTRUCTOR* OF BEES

Ghania Atmani-Merabet[*1,2,3], Abdelmalik. Belkhiri[1,2], Mohamed Abdeslem DEMS[4], Abdeldjallil Lalaouna[1], Zakaria Khalfaoui[5], Bouzid Mesbahi[6].

[1] Departments of Dental Surgery and Pharmacy, Laboratory of Pharmacognosy, Constantine3 University, Algeria

[2] Laboratory of Pharmacology and Toxicology, Constantine 1University, Algeria.

[3] Departments of Chemistry, Faculty of Sciences, Mentouri Constantine 1University, Algeria.[4] Biotechnology Research Centre (Crbt Constantine),Constantine, Algeria.

[5] Technical Institute of Breeding (ITE Hamma Bouziane), Constantine, Algeria.[6] Forest Conservation in Constantine, Algeria

Abstract:

The objective of this research was to determine the chemical composition, evaluate toxicity and acaricidal effect of essential oil of Eucalyptus robusta to control Varroa destructor of bees. Essential oilof Eucalyptus robusta (E. robusta) was extracted from fresh leaves by steam distillation using a Clevenger apparatus and was analysed by chromatography in gas phase coupled with mass spectrometry GC - MS, With an Agilent CGMSD Technology. The brine shrimp lethality test (BSLT) was used to evaluate the toxicity of the oil; the modified method of McLaughlin et al was employed in this study. The acaricidal features of the essential oil was evaluated usingbee hives infected by Varroa destructor, the method followed is the biological method "covers background". Results were analyzed by ANOVA followed by the post hoc tests.Fresh leaves of E. robusta by steam distillation yielded 0.83 % (v/w) of essential oil. Investigation of the oil onGC/MS resulted in the identification of 15 compounds, the most abundant constituent was 1,8-cineole (65,97 %), other notable compounds include p-cymene (7,83%), o-cymene (4,75%), Tetracosane (4,20%), alpha-pinene (1,89%) and 1-phellandrene (1,52 %) were also among the constituents identified. The acaricidal features of the essential oil was evaluated usingbee hives infected by Varroa destructor,a significant effect of oil was observed (p< 0.05). Cytotoxic effect was assayed using the brine shrimp lethality test, Probit's analysis of the result revealed an LC50 value of 9, 42 µg/ml.

Essential oil extracted from E. robusta showed the presence of 1,8- cineole coupled with high cytotoxic and acaricidal effects suggest it may not be suitable for medicinal purposes but can be used as insecticidal agents.

Keywords: *Eucalyptus robusta; leaf essential oil; toxicity; Chemical composition; Varroa destructor.*

Corresponding author:

G. Atmani-Merabet,

Departments of Dental Surgery and Pharmacy,
Laboratory of Pharmacognosy, Constantine3 University, Algeria
E mail id: mgachimie2014@hotmail.com

QR code

Please cite this article in press G. Atmani-Merabet., ***GC/MS Analysis of volatile Constituents, Toxicity and Acaricidal Activity of the Eucalyptus Robusta Essential Oil to Fight Varroa Destructor of Bee,*** *Indo Am. J. P. Sci, 2018; 05(04).*

INTRODUCTION:

Eucalyptus (family, Myrtaceae) is one of the world's most widely planted genera [1]. *Eucalyptus robusta* Smith, commonly referred to as Australian brown mahogany, is a fast growing, evergreen tree, bearing pendant leaves, native to Australia. This species has been established in many countries including Algeria.

Essential oils are natural products composed mainly of terpenes and terpene-derivatives in addition to some other non-terpene components [2]. The leaves of *E. robusta* contains up to 0.34 % w/w essential oil (Filomeno et al., 2016) and the main constituents are, alpha-pinene [3,4], p-cymene[3,5], 1,8-cineole [4]. However, other chemotypes such as alpha-phellandrene, p-cymene, β- pinene, myrtenal, among others have been documented [3,5]. Composition pattern of essential oils reflects their nutritional, cosmetic, pharmaceutical or medicinal values.

Many studies have reported the insecticidal activity of *Eucalyptus* essential oils against *V. destructor* an ectoparasite that contributes to the collapse of bee colonies, resulting in economic losses and ecological problems related to the role of bees as the most important pollinators on Earth [6-8].
A special practice of local beekeeping in the forest regions of Skikda (city by the sea, 471 km from Algiers) is to combine conventional treatments with aromatic plants for a better efficiency and lasting effect, *Eucalyptus* leaves are used fumigation near hives. The efficacy of this traditional practice is plausible given the many scientific studies supporting the insecticidal properties of *Eucalyptus* essential oils [9].

Synthetic pesticides have been widely used and pose potential risks of contamination of honey and other hive products with chemical residues [10]. There is also clear evidence for the evolution of resistance in *Varroa* mite populations to conventional pesticides [11]. Natural essential oils are excellent candidates to fight *Varroa* since they are safe for bee colonies and for humans. It is on this basis, we investigated the chemical composition, acaricidal and toxic effects of essential oil extracted from the leaves of *Eucalyptus robusta*. This study paves way for combating the resistance phenomenon of *Varroa* using natural, safe means for environment and bees.

MATERIAL AND METHODS:

Plant material and distillation

Leaves were collected in April 2014 at the herbarium of "Draa Naga", located at 15 km east of Constantine, Algeria.*Eucalyptus robusta* has been identified and its voucher specimen was deposited at the arboretum of the Forest Department of Constantine under the following reference number: 0065/47. Essential oil was extracted from fresh leaves by steam distillation using a Clevenger apparatus. Distilled oil was immediately dried over anhydrous sodium sulfate and was stored in screw-capped dark glass vials at 4 °C until further testing.

Chromatographic (GC/MS) analysis

Essential oil extracted of the leaves of *Eucalyptus robusta* was analysed by chromatography in gas phase coupled with Mass spectrometry GC/MS, With an Agilent CGMSD Technology apparatus in the light of the following experimental protocol: The ionisation energy was 70ev, the scan band: 45-400 u. The utilized column was; Capillary chromatographic column with a polar stationary phase [5% phenyl, 95% dimethyl polysiloxane], HP-5MS. The length of the column is 30 m, its diameter is 0.25 mm and the film thickness is 25 µ m. The column temperature was programmed from 50 to 200°C for 10°C/min. C. The interface is maintained at 230 ° C. and the ionization source at 150 ° C. The debit of gas vector (Helium) was fixed to 0.5 ml/min. The volume of injected specimen was 0.5 µL. The constituents of essential oils were identified by comparing their Kovats index (retention index), calculated to the retention time with those of the reference products, and Comparing it with chemical compounds collected by Adams (2007), and finally comparing their mass spectra with those collected in the NIST-Wiley-MS library.

Bioactivity

Brine Shrimp lethality assay

The brine shrimp lethality test (BSLT) was used to predict the toxicity of the oil. The modified method of (Mac Laughlin& Rogers, 1998) [12] was employed in this study. Different concentrations (1000, 100, 10, 1 ppm) of the leaf essential oil of *E. robusta* were prepared using diméthylsulfoxide (DMSO 1 %). After 48 h, a drop of DMSO and 4 ml of sea water were added to each of the sample bottles containing the oil sample. Ten brine shrimp larvae of *Artemia salina* were carefully counted into each of the sample bottles and the volume of the sea water was made up to 5 ml.Tests for each concentration were carried out four times. A control experiment containing 5 ml of sea water, a drop of DMSO and ten brine shrimp

larvae was set along side. The experiment was maintained at room temperature for 24hrs, the number of surviving larvae were counted and recorded, and the data obtained were subjected to

Finney's probit analysis to determine the LC50 of the oil.

Anti-Varroa activity

Bees (*Apis mellifera*) colonies placed in Langstroth type hives in an experimental apiary located at Azzaba in Skikda town (36°45'41.1"N 7°03'50.3"E) were used. The apiary had some colonies parasitized with *V. destructor* previously identified. A number of 9 hives were selected for the study and divided randomly into three equal batches (three hives per batch). Essential oil treated batch(1ml/hive/week) is compared to positive batch infected and treated with thymol (1ml/hive/week) and control batch (infected without treatment).The method followed was the biological method "covers background" [13,14], diapers were greased with Vaseline, then the trays were placed in the frames of hives, diapers were then removed and carefully examined with a hand lens to detect the dead *Varroa.* The tests for each treatment were carried out three times, the experiment lasted 21 days during which the counting was done every two days, after each count diapers were thoroughly cleaned and then put back. The essential oil was deposited on a cardboard tab of 1 mm thickness to a width of 4cm and a length of 20 cm, the deposited volume was 1ml [13]. The tab was inserted through the main entrance of the hive; the treatment was repeated at 7th day and 14th day. The results were expressed in means of mortality ± standard deviation. The temperature during the experiment varied between 20°C and 22°C.

RESULTS:

Table 1: Yield of essential oil of *Eucalyptus robusta* from different geographic regions

Species	Yield (%)	Country	References
E. robusta	0.83	Algeria	Our result
	0.34	Brazil	[3]
	0.20	Brazil	[15]
	0.13	Democratic Republic of Congo	[5]

Table 2: Chemical composition of the leaf oils of *Eucalyptus robusta* from Algeria

Compound	Air (%)	RT (min)	RI
(+) spathulenol	1.12	33.523	1 578
1,8 - cineole	65.79	10.166	1 030
1-phellandrene	1.52	9.589	1 003
4-terpineol	1.03	14.270	1 177
Apigenin-4',7-dimethyl	2.84	52.245	2 964
alpha-pinene	1.89	8.003	939
alpha-terpineol	0.6	14.796	1 189
camphene	1.25	25.347	954
o-cymene	4.57	10.078	1 026
p-cymene	7.83	10.019	1 025
Pentacosane	2.36	53.142	2 500
Tetracosane	4.02	56.520	382.55
Tricosane	3.4	49.372	2 300
Heptacosane	1.10.	53.222	429.45
12-methoxy-3-methylcholanthrene	0.45	52.654	2815

RT: Retention time obtained by chromatography RI: Retentions indices, as determined on a HP-5MS column.

Table 3: Brine shrimp lethality assay of leaf extract of *E. robusta*

Plant Extract	Dose (µg/ml)	Nbre of tested shrimps	Nbre of survivors	% Mortality	LC50 (µg /ml)
E. robusta	1	40	5	35	9.42
	10	40	14	47.5	
	100	40	21	65	
	1000	40	26	87.5	

Table 4: Averages and standard deviations in the three batches

Batches	Averages± standard deviations
Batch 1 (*E.robusta*)	37,57 ± 1.95
Batch 2 (thymol)	41,8 ± 8.55
Batch 3 (Naturalfall)	8,19 ± 0.42

Table 5: Fisher (LSD) test result / Analysis of differences between modalities, 95% Confidence Interval for Mean

Modalities	Estimated means
T1(*E. robusta*)	5.5640 ^{A}
T2 (Thymol)	5.5337^{A}
T3 (Natural fall)	4.0401^{B}

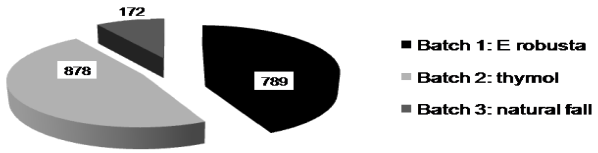


Fig.1: Total number of dead *Varroa*

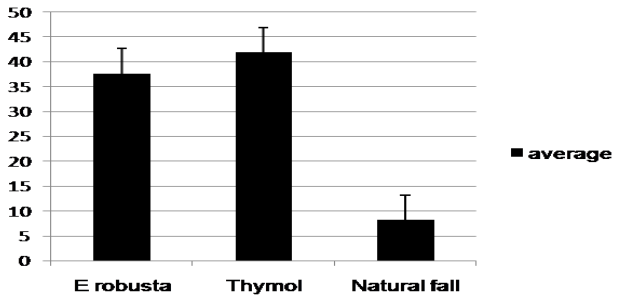


Fig.2: Averages of dead *Varroa* and standard deviations

Yield, compositions, contents and identification of the leaf essential oil

In this study, the yield of the oil obtained from the hydro-distillation of the leaves of *Eucalyptus robusta*(0.83%)was compared with those of the same species from different geographic regions (Table1, here).Results obtained for the compositions contents and identification of the leaf essential oil of *Eucalyptus robusta* oil are depicted in Table 2, where the major component was 1,8-cineole (65.87%). We compared our results to those published for the same species in other geographical areas, including China, Brazil and the Democratic Republic of Congo. We also compared them with other species of Eucalyptus (globulus, sideroxylon.) from Algeria and several other parts of the world, the composition was similar or different depending on the species and region. (Table2 here)

Toxicity and acaricidal activity

The current research showed that this oil was toxic towardsthe brine shrimp larvae *Artemia salina*, the result of the brine shrimp lethality assay of *E. robusta* is in Table 3 (here).The acaricidal activity against *Varroa destructor* is illustrated by Figure 1(here). The average of dead*Varroa* during treatment for the three batches and the standard deviation is shown in Table 4 and Figure 2.

The statistical analysis of variance (ANOVA) using the Statistica 10 software showed that there was a difference between essential oil of *E.robusta*, thymol and natural fall of *Varroa* at the threshold of $\alpha = 5\%$ which was significant **($p^* \leq 0.0065$),** the multiple comparison of the means was performed with the post hoc Fisher Test (LSD) indicated that the oil of *E. robusta* (T1) and the thymol (T2) belonged to the same group A, so had the same acaricidal activity against *Varroa destructor*, which is different of mites natural fall (T3) (Table 5).

DISCUSSION:

The yields of the oils obtained from the hydro-distillation of the leaves of *Eucalyptus robusta* was 0.83 %, it is higher than the same species from Brazil [15,16], and from Democratic Republic of Congo [17] (Table1). It's relatively lower than other plants as a source of essential oil: *Eucalyptus microtheca* (2.3%), *Eucalyptus tereticornis* (3.4%) and *Eucalyptus grandis* (4.7%) [18].

The essential oil composition of *Eucalyptus robusta* obtained of this study where 15 compounds were identified, which made up 99.95 % of the total essential oil gave 1.8-cineole (65.97%)as the major constituent, other components present in appreciable contents were:p-cymene (7.83 %), o-cymene (4.75 %), α-pinene (1.89%), 1-phellandrene (1.52%), camphene (1.25%) and (+) spathulenol (1.12%) (Table2). These results showed a relative difference to those published for other geographical regions: alpha-pinene (30,18%), 1,8-cineole (26,08%), and globulol (4,44%), were reported as the major component in the essential oil of *Eucalyptus robusta* from China [19], α-phellandrene (36.6%), α-pinene (16.6%), *p*-cymene (14.8%), andβ-pinene (11.8%) from Brazil [20], p-cymene (27.3%), myrtenal (12.8%), β-pinene (6.3%), and α-terpineol (6.3%) from Democratic Republic of Congo (Cimanga et al.,2002). Also it is similar to the chemical composition of leaves essential oil of *Eucalyptus globulus* were the major constituent was 1,8-cineolefrom Algeria [21-25], from Argentine [26], from Australia [27], from Egypt [28], from India [29],from Pakistan [30], and from Ethiopia (Mekonnen et al.,2016).It is similar too to the composition of leaves essential oil of *Eucalyptus sideroxylon* from Algeria [31], from Tunisia [32] and from Australia [33], these results are similar to those found in *Eucalyptus viridis* and *Eucalyptus oleosa* from Iran[34]. Previous studies of the leaf oil compositions of *Eucalyptus* species used commercially as a natural source of 1.8-cineole have been reported [35,36].

This oil was toxic towards the brine shrimp larvae *Artemia salina*,Finney's probit analysis of the result revealed an LC50 value of 9.42 µg/ml (Table 3) indicating cytotoxic potentials [37]. The results ascertained documented use of the plant extracts as repellant and insecticidal agents [35]. It is worth noting that the toxicity of our oil is very close to the LC50 of *E. globulus*from Nigeria (9.59 µg /ml) [39], andhigher compared to *Eucalyptus baileyana* and *Eucalyptus major* from Australia with an LC50 (µg/ml) values of 216 and 762 respectively [36]. This difference is due to the chemical composition of each species, in the specie of Nigeria, Tepinen-4-ol (23.46%), γ-terpinene (17.01), apple oil (5.55%), α-pinene (4.16%), and α-phellandrene (2.20%) were reported as the major components. However our specie showed: 1.8-cineole (65.97%), p-cymene (7.83 %), o-cymene (4.75 %), α-pinene (1.89%), and 1-phellandrene (1.52%) as major constituents.

Eucalyptus robusta essential oil was found to be active against *Varroa destructor* of bees a significant effect was observed ($p^* \leq 0.0065$) (Table 5),it showed a total number of dead *Varroa* of (789) very close to that given by thymol (878), and very significant compared to natural fall of varroa (172) (Figure 1). According to the results obtained on the mortality of *Varroa* by the different treatments, we

note that the standard deviations are very high regardless of the colony studied or the hive lot processed. This indicates an heterogeneity which manifests itself in the different colonies. Each colony is thus, infested to its level according to the factors of tolerance and resistance of its own (Table 4 and Figure 2).

Natural essential oils are excellent candidates to fight *Varroa* since they are safe for bee colonies and humans.Several works have been carried out by researchers all over the world, particularly in Algeria, to study the effectiveness of essential oils against *Varroadestructor*, essential oils of: *Eucalyptus globulus*, rosemary, thyme from Algeria [33], olive, clove, garlic from Pakistan [34], bitter orange ,grapefruit, Citronella volatile oils from Egypt [32] and *Eucalyptus camaldulensis* from Iran [37].

CONCLUSION:

This study revealed that *Eucalyptus robusta* essential oil is rich in 1, 8-cineole, it is toxic and has an acaricidal effect on *Varroa destructor*. The purpose of the test of essential oil was to demonstrate that *Eucalyptus* could be used as a natural and alternative means of combating *Varroa* without danger to the environment, Bee and honey. Finally, this study is initiated on the basis that thymol is a product whose efficacy in the control of mites is established and that this effect could be potentiated by combining it with local *Eucalyptus* essential oils. The ultimate goal is to develop new products which are more efficient, but above all overcoming the problem of increasing pest resistance to treatments.

ACKNOWLEDGEMENTS:

We are very grateful to Mr. Mokadem Hamadi, who allowed us to work in his apiary and helped us a lot.

Conflict of Interest

No conflict of interest associated with this work.

REFERENCES:

1. Akin M, A., Aktumsek, A., Nostro. (2010).Antibacterial activity and composition of the essential oils of *Eucalyptus camaldulensis* Dehn.and*Myrtus communis* L. growing in Northern Cyprus. *African Journal of Biotechnology*,9 (4): 531-35.
2. Edris, A.E.(2007).Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents.*Phytotherapy Research*,21 (4): 308-23.
3. Filomeno, C.,Filomeno, C.A.,Barbosa, C.A.,Pereir, J.L., Pinheiro, A.L., Fidenco,P.Het al.(2016).The chemical diversity of *Eucalyptus* spp. essential oils from plants grown in Brazilia.*Chemical Biodiversity*, Doi:10.1002/cbdv.201600097.
4. Xin Chao, L.,Qi Zhi, L.,Wang Peng, S.,Zhi Long, S.(2014). Evaluation of activity of the essential oil of *Eucalyptus robusta* Smith leaves and its constituents compound against over wintering Cocopsylla chinensis (Yang et Li) (Hemipta: psyllidae). *Journal of Enthomology and zoology Studies*, 2(4): 27-31.
5. Cimanga,K.,Kambu, K.,Tona,L., Apers,S., De Bruyine,T., Hermans,N, et al 2002 Correlation between chemical composition and antibacterial activity of EOs of some aromatic medicinal plants growing in the Democratic Republic of Congo. *Journal.Ethnopharmacology* 79: 213–20.
6. Kluser S.,Neumann,P., Chauzat, M.P., Pettis, J.S. (2010). Global Honey Bee Colony Disorder and Other Threats to Insect Pollinators.UNEP Emerging Issues 1.
7. Mahmoud, R., Asad,S., Raja,S., Ul Moshina,A., Wagchoure,E.S., Sarwar, G, et al.(2014). Control of *Varroa destructor* (Acari: varroidae) in *Apis mellifera* (Hymnoptera: apidae) by using plant oils extract. *Pakistan Journal of Zoology*, 46(3): 609-15.
8. Habbi-Cherif Aisssa.(2014).Etude de la dynamique de la population du parasite*Varroa destructor* de l'abeille domestique (*Apis mellifera*) etévaluation de l'efficacité de quelques huiles essentiellesdans la lutte contre ce parasite.Mémoire de Magister. Université Mouloud Mameri, Tizi Ouzou, Algeria.
9. Shengping, P.J.Y.Q.Q., Lihua, L.C.W.(2011).Study of the niche transition of lesser Asian mite (Tropilaelaps clareae) in honey bee colony just after infection desynchronisation of larger varroa mite (*Varroa destructor*). *Apiculture of China*.
10. Bogdanov, S., Charriere,J.D.,Imdorf, A.,Kilchenmann, V.(2002).Determination of residues in honey after treatment with formic and oxalic acid under field conditions.*Apidolology*, 33: 399.
11. Umpiérrez, M.L., Santos,E., González,A., Rossini, C. (2011). Essential oils from Asteraceae as potential biocontrol tools for.tomato pests and diseases. Phytochemical Review,11(4):339-50.
12. Mac Laughlin, J.L., Rogers, L.L. (1998). The use of biological assays to evaluate botanicals.*Drug Information Journal*, 32:513-24.
13. Labeste, L.(2013). Protocole de traitement aux huiles essentielles – activité acaricide sur le

varroa. Apisevices-Galerie Virtuelles Apicoles, France.
14. Ghomari, F.N., Kaouache,B.,Arous,A.,Cherchali, S. (2014). Effet de traitement par fumigation du thym (Thymus vulgaris) sur le *Varroa destructor* agent de la varroase des abeilles. *Revue Nature et Technologie*,10 : 34-38.
15. Jaimand, K., Rezaee,M.B., Kandy, N.B.H.(2009).Volatile Oil Constituents of the *Eucalyptus viridis* R. T. Baker and *Eucalyptus oleosa*
16. T.E., Ebadah,M.A., Zidan, E.W.(2006).Evaluation of some volatile plant oils and maurik against *Varroa destructor* in honey bee colonies. *Journal of Applied Science Research*, 2(8): 514-21.
17. Boland, D.J.,BrophyJ.J.(1991). *Eucalyptus* leaf oils: use, chemistry, distillation and marketing. Melbourne, A.P.N. House, technical editor Inkata Press, p: 25–155. ISBN 0909605- 69-76.
18. Ghasemi, V., Moharramipour,S., Tahmasbi, G. (2011). Biological activity of some plant EOs against *Varroa destructor* (Acari: Varroidae),an ectoparasitic mite of *Apis mellifera* (Hymenoptera: Apidae). *Exp. Appl. Acarol*, 55: 147–54.
19. Islaka, A.,Ogunwande, N.O.,Olawore, K.A., Adeleke,K.A.,Konig, W.A. (2003). Chemical composition of the essential oils from the leaves of three *Eucalyptus* species growing in Nigeria.*Journal of essential oils Research*, 15(5): 297-301.
20. Ghaffar, A.,Yameen,M., Kiran,S., Kamal,S., Jalal,S., Munir,R.B, et al.(2015) Chemical Composition and *in-vitro* Evaluation of the Antimicrobial and Antioxidant Activities of Essential Oils Extracted from Seven *Eucalyptus* Species. *Molecules*, 20: 20487–98.
21. Dethier, M., Nduwimana,A., Coerdier,Y., Menut,C., Lamaty,G. (1999). Aromatic plants of tropical central Africa XVI Studies on essential oils of five *Eucalyptus* species grown in Burundi.*Journalof Essenial Oil Research*, 6: 469-73.
22. Olayinka, A.J, Olawumi, O.O., Olalekan,A.M., Abimbola,A.S., Idiat,I.D., Theophillus, O.A. (2012). Chemical composition, antioxidant and cytotoxic effects of *Eucalyptus globulus* grown in north -central Nigeria.*Journal of NaturalProductPlant Resource*, 2(1): 1-8.
23. Mekonnen, A., Yitayew,B., Tesema,A., Taddese, S.(2016).In Vitro Antimicrobial activity of essential oil of *Thymus schimperi*, *Matricaria chamomilla*, *Eucalyptus globulus*, and *Rosmarinus officinalis.International Journal of Microbiology*, 1–8.
24. Clarkson, C., Maharaj,V.J., Crouch,N.R., Grace,O.M., Pillay,P., Matsabisa, M.G,et al. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *Journal of Ethnopharmacology*, 92: 177-91.
25. Cock, Ian Edwin.(2008). Assessment of the toxicity of selected Australian native plant extracts using the *Artemia franciscana* nauplii bioassay.:The Internet Journal of Toxicology Pub 1-13.
26. Roh, H.S, Lee,B.H., Park, C.G.(2013).Acaricidal and repellent effects of myrtacean EOs and their major constituents against *Tetranychus urticae* (Tetranychidae).*Journal of Asia-Pacific Entomology*, 16: 245–49.
27. Nait Achour, K.(2012). Etude de la composition chimique des essences de quatre espèces d'*Eucalyptus* poussant dans la région de Tizi Ouzou. Mémoire de Magister, Algeria.
28. Daroui-Mokadem, H., Kabouche,A., Bouacha,M., Soumati,B., El Azzouny,A., Bruneau, C,et al 2010 GC/MS analysis and antimicrobial activity of the essential oil of fresh leaves of *Eucalyptus globulus* , and stems of *Smyrnium olusatrum* from Constantine (Algeria). *Natural Product Communication,* 5(10): 1669-72.
29. Elaissi, A., Rouis, Z., Salem,N.A.B., Mabrouk, S., Salem,Y.B., Salah, K.B.H, et al. (2012). Chemical composition of 8 *Eucalyptus* species' EOs and the evaluation of their antibacterial, antifungal and antiviral activities.*Complementaryand Alternative Med*icine12: 81.
30. Ebadollahi, A., Safalizadenh,M.H., Pourmirza,A.A., Nouri-Ganbalan, G.(2010). Comparison of fumifant toxicity of *Eucalyptus globulus* Labill and *Lavandula stoechas* Lagainst different stages of *Tribolium castanum* Herbst. *Indian Journal of Agricultural Research, 44 (1): 26-31.*
31. Harkat-Madouri, L., Boudria,A., Khodir,M., Bey-Ould,Z., Si Saida,K., Rigouc,P.D, et al. (2015). Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria. *Industrial Crops and Products*, 78: 148–53.
32. Yang, Y., Choi,H., Choi,W., Clark,J.M., Ahn, Y.(2004). Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). *Journal of Agricultural and Food Chemistry*,52 : 2507–11.

33. Pant M.S., Dubey, P.K., Natanjali,p.s., Naik,S.N., Sharma, S.(2014).Insecticidal activity of *Eucalyptus* oil nanoemulsion with karanja and jatropha aqueous filtrates.*International Biodeterioration Biodegradation*, 91: 119–27.
34. Sartorelli, P., Marquioreto,A.A., Baroli,M.E.L., Liwa, P.R.H., Moreno, J. (2007) Chemical composition and antimicrobial activity of the essential oils from species of *Eucalyptus* (*robusta – saligna*). *Phytotherapy*, 21(3): 231-33.
35. Taleb-Toudert, K. (2015). Extraction et caractérisation des huiles essentielles de dix plantes aromatiques provenant de la région de Kabylie (Nord Algérien). Evaluation de leurs effets sur la bruche de niébé *Callosobruchus maculatus* (Coleoptear : Bruchidae). Thèse de Doctorat. Université Mouloud Mameri, Tizi Ouzou, Algeria.
36. Yones, D.A., Baki,H.Y., Bayoumi, S.A.L.(2016).Chemical composition and efficacy of some selected plant oils against *Pediculus humanus capitis* in vitro.*Parasitology Research*115: 3209–18.
37. Russo, S., Cabrera,N., Chludil,H.,, Yaber-Grass,M., Leicah, S.(2015). Insecticidal activity of young and mature leaves essential oil from *Eucalyptus globulus* Labill. against*Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). Journal of Agricultural Research, 7: 375–79.