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# Contribution to the Development of Burundi's Plant Heritage in the Management of Cereal Stocks: Case of *Eucalyptus Maidenii* Essential Oil and *Citrus limon* leaf powder against Insect Pests of Maize Grains

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**Abstract**

With the aim of contributing to the development of Burundi's plant heritage in the control of stored food pests, a study on the extraction and physico-chemical characterisation of the essential oil of *Eucalyptus maidenii*, the incorporation of this plant essence into *Citrus limon* leaf powder and the evaluation of the effectiveness of this mixture in the conservation of maize grains was carried out in the laboratory of Burundi University, from October 2021 to February 2022. The essential oil was extracted using locally manufactured equipment, and the physico-chemical characterisation involved determining the relative density, refractive index, acid index, peroxide index, carbonyl index and ethanol miscibility. Two types of mixtures of essential oil and *Citrus limon* leaf powder were then formulated with proportions of 1/20 (w/w) for the first mixture (M1) and 1/10 (w/w) for the second mixture (M2). Cleaned maize grains were distributed in 63 jars of 500 ml at a rate of 200 g per jar, then infested with 1 g of powder containing insect eggs. Each mixture was applied in 5 doses (0.05%; 0.5%; 1%; 1.5%; 2%) with 3 repetitions for each dose. The extraction process yielded 3.6% essential oil. After 4 months of storage, the average attack rate on maize grains was 1.58% in the control batches compared with 0.37% in the treated batches. On average, 7 insects appeared in the control batches compared with only 2 in the treated batches, and the insect mortality rate was 100% in almost all the treated batches.

**Keywords:** Essential Oil, *Citrus Limon*, Conservation, Cereal stocks, Maize-Migwa

## Introduction

Cereals are a staple food in the world in general and in Africa in particular. In Africa, out of 252 million ha of arable land, cereals occupy an area of 98.6 million ha (i.e., 39% of arable land) and produce 162 million tonnes [10]. Unfortunately, of the huge quantities grown and collected, a significant proportion is lost or spoiled each year during storage by insects, rodents and fungi. Insect pests, mainly Coleoptera, can cause losses of up to 30% in the tropics, or even the total loss of a stock [15, 4].

Synthetic pesticides have been considered the most effective and accessible means of controlling these insect pests. However, there is worldwide concern about the negative impact of these synthetic insecticides on the ozone, environmental pollution, toxicity to non-target organisms, adverse effect on human and animal health and often prove ineffective against insects over time [16, 9]. For this reason, a great deal of research is currently focusing on the use of plant-based insecticides, which are considered to be less toxic [5]. For example, phyto-pesticides formulated from the essential oils of aromatic plants are a serious possibility because essential oils and their constituents have been considered natural agents against insect pests because they are renewable, non-persistent in the environment and relatively safe for non-target organisms and humans [16, 17].

Given its tropical climate, with very abundant rainfall throughout most of the year, it is clear that Burundi has a very rich plant heritage but unfortunately little exploited. The main aim of the present work is to extract the essential oil of *Eucalyptus maidenii* using a device designed from local materials, and to mix it with *Citrus limon* leaf powder to test the effectiveness of this mixture on insect pests of maize grains in storage «*Sitophilus sp.*».

## Materials and Method

### Vegetal Material

The *Eucalyptus maidenii* leaves were harvested in September 2021 in the Migwa region in the Republic of Burundi. The *Citrus limon* leaves were harvested in Bujumbura city at the beginning of October 2021, then dried at room temperature (25 and

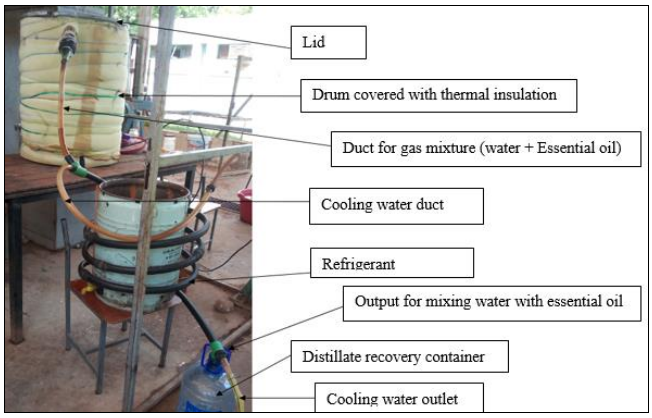
30°C) for 14 days for the *Eucalyptus maidenii* leaves and 21 days for the *Citrus limon* leaves in the hangar of the Faculty of Agronomy where the operations of designing the extractor, extraction and preparation of the insecticide mixtures took place. The maize grains of the local variety used in this work were bought at the popular market of Kinama. The choice of this variety was justified by its availability, its taste and the substantial income it provides for producers and sellers.

**Mass Rearing of Insects «*Sitophilus sp*.»**

In accordance with the method used by [12] and [3], but also with a view to obtaining a large population of adult insects capable of producing sufficient eggs for the various trials, mass rearing was carried out using adult insects collected from maize cobs stored in the hangar of the Faculty of Agronomy at the Burundi University. These insects were introduced into a bag containing 2 kg of local variety maize, then stored at room temperature (23 to 30°C) with relative humidity varying between 75 and 90% in the same laboratory. After two months of storage, the fully colonised maize grains were sieved to recover the powder used to infest the bioassays.

**Extractor Design**

Obtaining an essential oil of *Eucalyptus maidenii* is essential for our study. For this reason, we set out to build an extraction device using a iron barrel of 250 litres, two electrics heating elements of 1500 watts and two pipes of different cross-sections of 6 meters long.



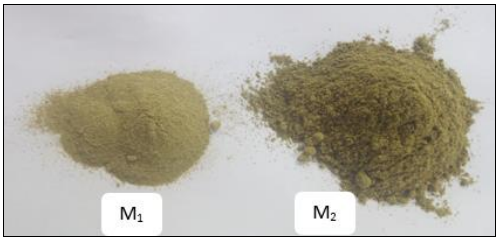

**Extraction and Physico-Chemical Characterisation of the Essential Oil**

The essential oil was extracted from 17 kg of dry *Eucalyptus maidenii* leaves by steam distillation for 6 hours using the apparatus described above, then dehydrated with anhydrous sodium sulphate (Na2SO4). It was kept refrigerated at 4°C until use.

The physico-chemical parameters were determined in the chemistry laboratory of Sciences Faculty, such as the relative density by the pycnometric method, the refractive index by direct reading on the classic NOVEX refractometer at 20°C, the miscibility with ethanol as well as the acid, carbonyl and peroxide number all by the titrimetric method.

**Incorporating the Essential Oil into *Citrus Limon* Powder**

Two mixtures were made: for the first mixture (M1), 100 grams of powder with 5 grams of essential oil were transferred in a flask of 250 ml. The flask was placed on a hot plate at 200°C and stirred regularly until the essential oil was completely incorporated into the powder. For the second mixture (M2), 100 grams of powder with 10 grams of essential oil were treated in the same way.




**Conduct of the Trial**

Samples of 200 grams of maize grains were divided into 63 jars. These samples were infested with 1 gram of powder containing eggs of the insect «*Sitophilus sp*.». After infestation, the 30 jars were immediately treated with the insecticide mixtures (powder incorporated with essential oil), while the other 30 jars were treated three weeks after infestation. Each mixture was applied in 5 doses (0.05%; 0.5%; 1%; 1.5%; 2%) with three repetitions for each dose. The remaining three jars were used as controls. The jars treated in this way were placed in the Faculty of Agronomy laboratory at room temperature for four months.

**Statistical Analysis**

Statistical analyses were carried out using STATA 15 software. This enabled us to determine the correlation, find the regression lines and carry out the analysis of variance (ANOVA test) at the 0.05 threshold.

## Results and Discussion

### Extraction Yield

Extraction of the essential oil using local equipment gave a yield of 3.6%. This yield is close to that of [13] who achieved a yield of 4% by extracting the essential oil of *Eucalyptus maidenii* from Gisozi using the Clevenger apparatus, but much lower than that of [11] who achieved yields of 5% for *E. globulus maidenii* 17746 and 5.38% for E. globulus maidenii 2130, two Iranian varieties similar to *E. maidenii*, using the Clevenger apparatus.

The low yield can be explained by the fact that the apparatus we used had leak holes on the closure. This resulted in losses of the gas mixture (steam + essential oil), so improvements are needed to achieve a higher yield.

**Table 1:** Physico-chemical parameters of *Eucalyptus maidenii* essential oil

| Parameters | Values | | | |
|---|---|---|---|---|
| | Essay 1 | Essay 2 | Essay 3 | Average |
| Relative density $D_{25}$ | 0,9306 | 0,9147 | 0,9250 | 0,9234 |
| Refractive index | 1,4650 | 1,4640 | 1,4644 | 1,4645 |
| Miscibility with ethanol | 1V d'HE/9V d'EtOH 70% | 1V d'HE/9V d'EtOH 70% | 1V d'HE/9V d'EtOH 70% | 1V d'HE/9V d'EtOH 70% |
| Acid index (mg de KOH/g of EO) | 2,244 | 2,201 | 2,222 | 2,222 |
| Carbonyl index (mg de KOH/g of EO) | - | - | - | - |
| Peroxide Index (méq.$O_2$/Kg of EO) | 8,2 | 8,5 | 8,4 | 8,3 |

The results obtained are close to those found by other authors for the other varieties of eucalyptus: [1] found 1.45952 as the refractive index for the essential oil of *E. globulus* and 1.45215 for the essential oil of *E. citriodora*. [6] found 0.925 as relative density and 1.451 as refractive index for E. globulus essential oil. When determining the carbon index, we did not observe the appearance of the blue colouration, indicating the total absence of carbonyl compounds in our plant essence. The same case was observed by [14] on the essential oil of *Ammoides verticillata* from the Adrar region. The acid number highlights the behaviour and quantity of free acids present in our essential oil. It can also tell us about the essential oil's susceptibility to changes, particularly oxidation. In our case, the essential oil of *Eucalyptus maidenii* has a value of 2.222 mg KOH/1g EO: this value is slightly higher than that of [2] where they found 1.268 mg KOH/1g *Eucalyptus globulus* EO. From the point of view of the peroxide value, the essential oil showed a value of 8.3 meq.O2/Kg of EO. This value is slightly higher than that of [14] who found 7.25 meq.O2/Kg of *Ammoides verticillata* EO from the Adrar region.

### Organoleptic Quality and Relative Humidity of Grains Before and After Storage

The organoleptic test carried out with ten people after winnowing the grains, revealed that all the batches treated had not undergone any change in colour, odour or taste after four months of storage. The same observation was made by [15], where maize grains treated with *Citrus limon* powder at a dose of 10% did not change in colour, odour or taste after six months' storage. Another finding was that no trace of the characteristic taste and odour of *Eucalyptus maidenii* essential oil was detected in any of the treated batches. This phenomenon can be explained by the high volatility of the essential oil.

Before conservation, the sample of maize taken had a moisture content of 10.95%, but after four months of conservation it had a moisture content of 12.85%, i.e. 1.9% of the rate of increase. This means that moisture had accumulated in the grains through the aeration holes in the storage jars, bearing in mind that the study was carried out during the rainy period when the atmosphere is saturated with moisture. The same finding was made by [15], who observed a 16.18% increase in moisture in maize grains treated with various plant powders after six months of storage.

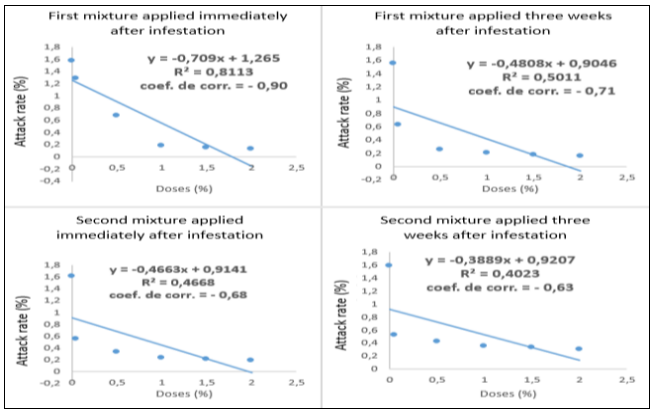
**Table 2:** Average rate of increase in grain weight (%)

| Doses applied (%) | Treatments | | | |
|---|---|---|---|---|
| | $M_1$ AIAI | $M_1$ ATWAI | $M_2$ AIAI | $M_2$ ATWAI |
| 0 (Aw) | 0,46 | 0,46 | 0,46 | 0,46 |
| 0,05 | 0,55 | 0,67 | 0,60 | 0,50 |
| 0,5 | 0,62 | 0,78 | 0,64 | 0,51 |
| 1 | 0,64 | 0,65 | 0,67 | 0,54 |
| 1,5 | 0,56 | 0,67 | 0,56 | 0,44 |
| 2 | 0,54 | 0,52 | 0,55 | 0,44 |
| *Corr. coef.* | *0,24* | *-0,09* | *0,10* | *-0,44* |

***Aw**: Absolute witness, **$M_1$ AIAI**: First mixture applied immediately after infestation, **$M_1$ ATWAI**: First mixture applied three weeks after infestation, **$M_2$ AIAI**: Second mixture applied immediately after infestation, **$M_2$ ATWAI**: Second mixture applied three weeks after infestation, **Corr. coef.**: Correlation coefficient*

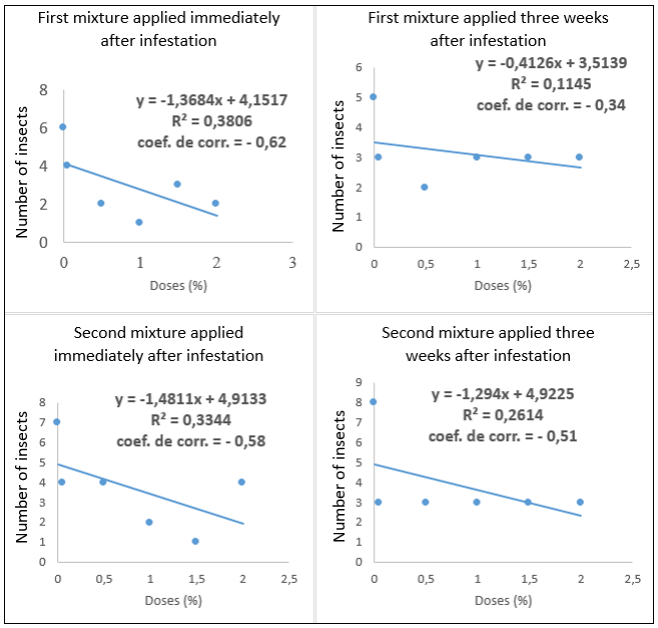
The correlation test shows that there is no statistical relationship between the increase in grain weight and the variation in doses of the insecticide mixtures (essential oil + *Citrus limon* powder) except for the second mixture applied after three weeks, which shows a weak negative correlation. This means that the increase in grain weight was not caused by the treatments we carried out. This increase would be due to the accumulation of moisture by the grains, as the determination of their relative humidity showed that this increased to a rate of 1.9%. In fact, the study of [7] showed that the use of plant powders in the control of insect pests greatly reduces the weight losses of bean seeds as the doses increase.

### Grain Attack Rate



The correlation test shows that there is a strong negative relationship between dose variation and grain attack rate. From the point of view of the time of application, the correlation coefficients given by the statistical test show that immediate treatment of the batches significantly reduces the grain attack rate compared with late treatment. The analysis of variance showed that there was no significant difference between the use of the 5% essential oil blend and the 10% essential oil blend (P = 0.6780 and 0.7631 > 0.05). In other words, regardless of the type of blend used, the same efficacy is obtained. It is therefore better to use the first mixture with 5% essential oil to avoid wasting this essential oil.

### Cumulative Number of Insects «*Sitophilus sp*.»

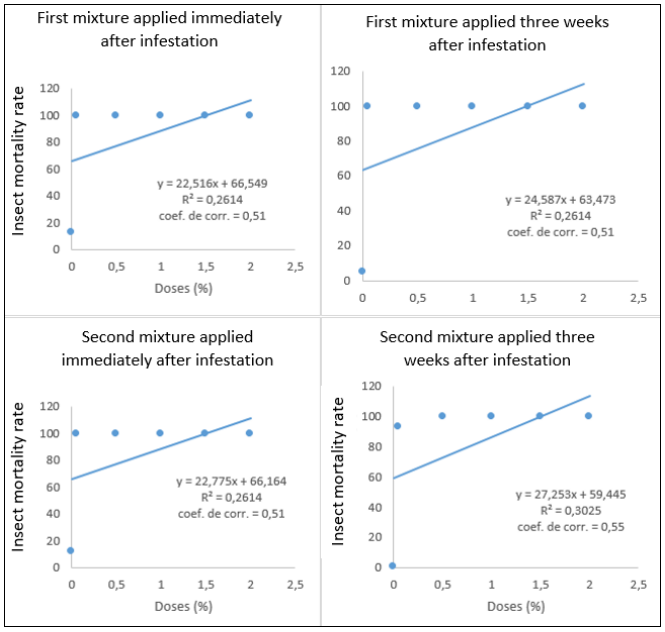



The number of insects that appeared in the untreated batches (control batches) was greater than the number of insects that appeared in the treated batches, but the statistical test showed that there was a weak negative correlation between the variation in treatment doses and the number of insects that appeared in the batches, except for the treatment carried out with the first mixture, which showed an average negative correlation of -0.62. This can be explained by the fact that all the insects identified had developed inside the grains, so they had a good chance of reaching adulthood before coming into contact with the active ingredients. This can be explained by the fact that all the insects counted had developed inside the grains, so they had a good chance of reaching adulthood before coming into contact with the active molecules of the essential oil and *Citrus limon*.

In fact, as in the case of grain attack rate, immediate treatment of batches is always more effective than late treatment in preventing insect accumulation, given the degree of linearity «$R^2$». In other words, when batches are treated late, insects find time to accumulate.

Analysis of variance showed that there was no significant difference between the two types of insecticide mixtures used (P = 0.5634 and 0.4876 > 0.05).

### Insect Mortality Rate




After four months of storage, the control batches showed lower mortality rates than the treated batches. The mortality rate was only 8% for the control batches and 100% for the treated batches. These values exceed those of [15], where *Citrus limon* powder applied to maize grains at a dose of 10% killed 44.2% of insects after six months of storage. They also exceed those of [8], who achieved 66.66% mortality of *T. castaneum* adults treated by inhalation with *Eucalyptus gomphocephala* essential oil for four hours. On the other hand, the results of our study coincide with those of [12] who tested the toxicity of the essential oil of *Callistemon viminalis* leaves on filter paper against *Acanthoscelides obtectus* (Say) adults where the dose of 0.251 µl/cm$^2$ caused total mortality (100%) of the bruchids after four days of exposure.

There was no significant difference between the 5% essential oil blend and the 10% essential oil blend (P=0.9937 and 0.9390 > 0.05).

### Conclusion and Outlook

On the basis of the results obtained and the observations made, we can conclude that:

Burundi's plant heritage can be developed by producing essential oil for export and for various uses in different fields. And this can be done in the traditional way, using locally-made equipment. Blends of *Citrus limon* essential oil and powder can be produced on a large scale for use as an alternative or complementary solution to the increased use of synthetic pesticides in the fight against food pests with a view to sustainable development.

But to make this possible, we plan to improve and expand the study: by performing a chromatographic analysis of the essential oil obtained from the local production equipment; improving extraction equipment to achieve high yields; by improving and completing the study using appropriate equipment where temperature and humidity conditions are well controlled; improving the technique for mixing

essential oils and powders to obtain a stable, homogeneous mixture; extending the test to essential oils and powders from other plant species; extending the test to other types of cereals and pulses; by testing essential oils for other uses, such as myciculture, preserving food products to minimise the overuse of chemical additives, treating diseases, and in agriculture: against weeds, for example.

**References**


1. Abdo MB. Physico-Chemical Profile and Antioxidant Activities of Eucalyptus globulus Labill and Eucalyptus citriodora Essential Oils in Ethiopia. Med Aromat Plants. 2019; 8:p332. Doi: 10.35248/2167-0412.19.8.332
2. Alexander E, Richard E, Victor S, John S. Physicochemical Properties and Antimicrobial activities of Essential oil extracted from Eucalyptus globulus leaves. International Journal of Chemical Sciences. 2019; 3(5):1-4 (available on http://www.chemicaljournals.com)
3. Camara A. Lutte contre Sitophilus oryzae L. (Coleoptera: Curculionidae) et Tribolium castaneumherbst (Coleoptera: Tenebrionidae) dans les stocks de riz par la technique d'étuvage traditionnelle pratiquée en Basse-guinée et l'utilisation des huiles essentielles végétales. Thèse, Université du Québec à Montréal, 2009.
4. Guèye MT, Seck D, Wathelet JP, Lognay G. Lutte contre les ravageurs des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale: Synthèse bibliographique. Biotechnol. Agron. Soc. Environ. 2011; 15(1):183-194 (available on https://orbi.uliege.be/bitstream/2268/89355/1/Publi.1%20Momar.pdf)
5. Karahacane T. Activité insecticide des extraits de quelques plantes cultivées et spontanées sur les insectes du blé en post-récolte. Thèse de doctorat, Ecole Nationale Supérieure Agronomique, 2015.
6. Kassahun A, Feleke G. Chemical Composition and Physico-Chemical Analysis of Eucalyptus Globulus Leave and Oil. Science Journal of Chemistry. 2019; 7(2):36-38. Doi: 10.11648/j.sjc.20190702.11
7. Khelfane Karima G. Etude de l'activité insecticide des huiles essentielles et des poudres de quelques plantes à l'égard de bruche du haricot Acanthocelides obtectus Say (Coleoptera, Chrysomelidae, Bruchinae) et comportement de ce ravageur vis-à-vis des composés volatils de différentes variétés de la plante hôte (Phaseolus vulgaris L.). Thèse de doctorat, Université Mouloud Mammeri de Tizi-Ouzou, 2014. (available on https://www.ummto.dz/dspace/bitstream/handle/ummto/1576/th%c3%a9se%20de%20doctorat.pdf?sequence=1)
8. Khouloud BH, Nadjate M. Effet insecticide par inhalation de l'huile essentielle d'Eucalyptus gomphocephala sur les adultes et les larves de l'insecte ravageur du blé en post-récolte Tribolium castaneum (Herbst). Mémoire de Master, Université Djillali Bounaama de Khemis-Miliana, 2019
9. Lindquist DA. Les pesticides: La chimie au service de la survie. AIEA Bulletin. 2000; 23(3):37-39. (available on https://www.iaea.org/sites/default/files/23305483739_fr.pdf)
10. Macauley H. Les cultures céréalières: riz, maïs, millet, sorgho et blé. Dakar, Centre international de conférences Abdou Diouf, 2015. (available on https://www.afdb.org/fileadmin/uploads/afdb/Documents/Events/DakAgri2015/Les_cultures_c%c3%a9r%c3%a9ali%c3%a8res_riz__ma%c3%afs__millet__sorgho_et_bl%c3%a9.pdf)
11. Moghaddam EH, Karamian R, Shaaban M, Mohammadian A. Identification and Comparison of Some Essential Oils Components in Seven Eucalyptus Species Cultivated in Khoramabad. Journal of Medicinal Plants and By-products. 2020; (1):59-66 (available on https://jmpb.areeo.ac.ir/article_121751_c478ddfc63523290d0ff799c92f50420.pdf)
12. Ndomo AF, Tapondjou AL, Tendonkeng F, Tchouanguep FM. Evaluation des propriétés insecticides des feuilles de Callistemon viminalis (Myrtaceae) contre les adultes d'Acanthoscelides obtectus (Say) (Coleoptera; Bruchidae). Tropicultura. 2009; 27(3):137-143.
13. Nduwimana A. Contribution à l'étude des possibilités de valorisation par extraction des plantes du Burundi: Cas des Eucalyptus species et des Aframomum species. Mémoire de licence, Université du Burundi, 1994.
14. Seddik M. Analyse physico-chimique, chromatographique et spectroscopique de l'huile essentielle d'Ammoides verticillata de la région d'Adrar. Étude de son activité biologique et antioxydante. Mémoire de Master, Université d'ORAN ES-SENIA, 2010. (available on https://theses.univ-oran1.dz/document/TH3194.pdf)
15. Semacumu G, Kulimushi E, Bahige P. Étude de l'efficacité des poudres de quelques plantes locales dans la lutte post-récolte contre les insectes ravageurs des grains de maïs (Zea mays) en conservation à Goma. Cahiers Africains des Droits de l'Homme et de la Démocratie, 2011, 304-316.
16. Sendi JJ, Ebadollahi A. Biological Activities of Essential Oils on Insects. RPMP. 2013; 37:130-150 (available on https://www.researchgate.net/publication/270273498_Biological_Activities_of_Essential_Oils_on_Insects)
17. ST Ngamo L, Hance T. Diversité des ravageurs des denrées et méthodes alternatives de lutte en milieu tropical. Tropicultura. 2007; 25(4):215-220. (available on https://www.researchgate.net/publication/45266304)