



Use of Essential Oil of *Eucalyptus globulus* Leaves against *Sitophilus zeamais* Motsch

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Authors' contributions

This work was carried out in collaboration among all authors. Authors A. Kalonji-Mbuyi and MMM designed the study and coordinated all activities. Authors JNK, SMK and SYM collected samples of study. Authors AKM and GMB extracted essential oil. Authors ANN, NKK and BKK designed the protocol and dealt with analyses in the laboratory. Author ANN compiled the literature review. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess in laboratory conditions, the efficacy of essential oil of *Eucalyptus globulus* leaves against the infestation due to maize weevil *Sitophilus zeamais*, the main insect pest of stored maize grains.

Study Design: This study was conducted as two separate trials. Each trial was performed using a completely randomized design with 5 treatments repeated 3 times.

Place and Duration of the Study: The study was conducted in the laboratory Unit of Phytopathology and Crop protection, Faculty of Agronomy, University of Kinshasa, between February and April 2018.

Methodology: Two trials (in Petri dishes and in cotton bags) were conducted separately from each other, while treatments used were the same. Trials were conducted according to a completely randomized design with 5 treatments repeated 3 times. Treatments used corresponded to 5 different volumes (0, 15, 20, 25 and 30 μ l) of essential oil of *Eucalyptus globulus* leaves. In each trial, data collected were the rate of *S. zeamais* mortality and percentage of maize grains damaged.

Results: In general, statistical analysis showed significant differences ($P < 0.05$) among treatments used. There was variation over time of the percentage of *S. zeamais* mortality as a function of the volume of essential oil of *E. globulus*. The volume of 30 μ l of essential oil caused higher mortality compared to other treatments. The percentage of maize grains damaged increased on control, while it decreased on maize grains treated with essential oil of *E. globulus* leaves.

Conclusion: The results of the present study revealed that essential oil of *E. globulus* leaves can help to protect maize grains stored against infestation due to maize weevil, *Sitophilus zeamais*.

Keywords: Essential oil; *Eucalyptus globulus*; *Sitophilus zeamais*; stored maize grains.

1. INTRODUCTION

In developing countries such as the Democratic Republic of Congo (DRC), post-harvest losses and deterioration in the quality of stored seeds caused by pests are a major problem for agriculture. Seeds and stored food can be attacked by insects, fungi, bacteria and rodents. According to Ndomo et al. [1], on stored products, damages caused by insects are most significant compared to that due to other pests. In sub-Saharan Africa (SSA), the corn weevil, *Sitophilus zeamais* Motschulsky is the most important pest of stored grains [2]. *S. zeamais* is a reddish-brown to black beetle insect belonging to the family *Curculionidae*, who attacks not only maize grains, but also *Sorghum* [3], and other processed and unprocessed grains and products [4]. On cereals, attacks of *S. zeamais* can start in the field when the moisture content of the grains reaches 18 to 20% and continue during grain storage [3]. *S. zeamais* is also responsible for quantitative and qualitative damage on stored products and can cause weight losses varying from 20 to 90% on stored grains [5]. This demonstrates that *S. zeamais* can constitute a serious threat to the food security of many households living mainly from the consumption of cereals.

To control pests of stored products and foodstuffs, the use of synthetic pesticides has proven to be one of the effective control methods [6,7,8]. However, this control strategy has drawbacks which limit its use. These include the presence of pesticide residues in treated food, the development of resistant insects [8], environmental pollution, problems of intoxication and poisoning [9,10] of people and animals, and high prices and the scarcity of good quality products [1] in some countries.

The use of natural substances in protection of plants and stored products constitutes an alternative method to the use of synthetic pesticides which may pose risks to human and animal health [10]. To compensate for the use of synthetic pesticides, in certain regions of SSA, low-income agricultural producers often use plants with insecticidal or insect repellent properties to protect their foodstuffs and stored seeds. There is diversity of botanical plants known for their insecticidal or repellent properties. In most cases, it is often vegetable powders obtained from different parts of these plants that are used; and for some time, various studies have been carried out to isolate or identify secondary metabolites extracted from these plants having activity against insects [1]. Various other studies show that essential oils

extracted from these plants contain biocidal properties against various crop pests, as well as bacteria and fungi [11]. Among the many plants used for this purpose, *Eucalyptus globulus* occupies an important place. Indeed, its essential oil contains fifty-three chemical compounds. These include 1,8-cineole, limonene, α -pinene, aristolene, p-cymene, trans-verbenol, isosativene, α -myrcene, and terpinen-4-ol as major components [12]. These chemical compounds have antifungal, antibacterial, antiviral [13], insecticide and/or insect repellent properties.

In the DRC, there are few studies documented on the use of various essential oils against seed pests. It is with this in mind that this study is situated, which explores the possibilities of using the essential oil of *E. globulus* in the conservation of maize grains attacked by *S. zeamais*. Before starting this research, two main questions were asked. First, from what quantity of essential oil of *E. globulus* can we observe *S. zeamais* mortality? Second, by what mode of action does essential oil of *E. globulus* act effectively on *S. zeamais*? The objective of this study is to assess under laboratory conditions the insecticidal efficacy (by fumigation and by contact) of essential oil of *E. globulus* against *S. zeamais*, the main pest of stored maize grains.

2. MATERIALS AND METHODS

2.1 Presentation of the Study Environment

In the present study, two separate trials were conducted in the laboratory of Phytopathology and Crop Protection Unit, Faculty of Agronomy, University of Kinshasa, DR-Congo. The average daily temperature and humidity recorded in the laboratory indicated $26\pm 1^{\circ}\text{C}$ and $85\pm 3\%$, respectively.

2.2 Biological Material Used

The biological materials used consisted of maize seeds of the Samaru variety, a population of *S. zeamais* and fresh leaves of *Eucalyptus globulus*. The maize seeds were obtained at the laboratory of "Service National de Semences" (SENASAEM). The population of *S. zeamais* was collected in a maize warehouse located in the MBANZA-LEMBA market, in the municipality of Lemba, Kinshasa. The fresh leaves of *E. globulus* used for the extraction of essential oil

were harvested in the Experimental Garden of the Faculty of Sciences, University of Kinshasa. The geographic coordinates of Experimental garden (where fresh leaves of *E. globulus* were collected) recorded with the GPS (extrex Summit Garmin) indicated $4^{\circ}19'S$ latitude, $15^{\circ}8'E$ longitude, and 330 m of altitude. This site falls within the Aw4 climate type according to Köppen classification characterization with 4 months of dry season (from second mid-May to first mid-September) coupled with 8 months of rainy season (from second mid-September to first mid-May). Daily temperature averages 24.5°C and accuses small variations, and the annual rainfall is close to 1500 mm. The relative humidity is highest in April and May, and is minimum in September and October [14]. The soil of Experimental garden where fresh leaves of *E. globulus* were taken was sandy, and the characteristic vegetation was dominated by *Imperata cylindrica*.

2.3 Extraction of Essential oil of *E. globulus* Leaves

The extraction of essential oil from fresh leaves of *E. globulus* was made using the hydrodistillation technique. This technique consisted in introducing the *E. globulus* leaves in a water bath, then bring the whole to a boil. The water vapor which escapes from this boiling pass through a serpentine pipe where it is cooled by tap water, then collected in a graduated cylinder. The essential oil was thus obtained after having separated it from the hydrosol.

2.4 Trials Conduct

The 2 trials were conducted separately from each other, while the treatments used were the same. Each trial was conducted according to a completely randomized design with 5 treatments repeated 3 times. The 5 treatments corresponded to 5 different volumes of essential oil of *E. globulus*. The volumes used were 0, 15, 20, 25 and 30 μl . The first trial was carried out in Petri dishes measuring 9cm in diameter. In each Petri dish, a disc of Whatman filter paper (N^o2 measuring 9 cm in diameter) was placed. According to each treatment, a volume of essential oil of *E. globulus* leaves was collected using a tips pipette (Finnpipette, Thermo Fisher scientific, Ireland) and placed in a corner of the filter paper, except for the control. Forty grains and 40 corn weevils were then placed on filter

paper in each Petri dish. All of Petri dishes were then covered with a white cloth held in place with an elastic wrapper to prevent the exit of the corn weevils.

The second trial was carried out using small cotton bags measuring 15 cm x 15 cm. Except for the control, the maize grains were placed in a Petri dish, then a volume of essential oil of *E. globulus* leaves corresponding to the treatment was added. The Petri dish was then covered with a lid, and the whole was stirred for 1 to 2 minutes. The maize grains thus coated with essential oil of *E. globulus* leaves were then placed in the cotton bag, and 40 corn weevils were added.

2.5 Data Collected and Statistical Analysis

After maize seeds treatment, data were collected every 48 hours. Parameters recorded were based to percentage of dead weevils, and the number of damaged maize grains. The percentage of dead weevils in maize seeds treated were corrected using Abott's formula (1925) cited by Thiaw [15], taking into account the natural mortalities observed in the untreated maize grains, using the following formula: $N_c = [(N_o - N_t)/(100 - N_t)] \times 100$, where N_o : number of dead corn weevils in the maize grains treated; N_t : number of dead corn weevils in untreated maize grains; and N_c : corrected percentage of dead corn weevils. Data recorded were subject to analysis of variance at the 5% probability level. The means comparison was separated using the least significant difference (LSD) test. Statistical analysis was made using Statistix 8.0 software (free version), and the graphical results were done using Microsoft Excel 2003 software.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Trial 1: Insecticidal efficacy of essential oil of *E. globulus* leaves in Petri dishes

3.1.1.1 Percentage of *S. zeamais* mortality

The percentage recorded on *S. zeamais* mortality in Petri dishes are illustrated in Fig. 1. Significant differences ($P < .05$) were observed between treatments throughout the experimental

period. It was observed that there is a variation over time of the percentage of *S. zeamais* mortality as a function of the volume of essential oil of *E. globulus* used. The volume of 30 μ l of essential oil caused higher mortality compared to other treatments. Forty-eight hours after maize grains treatment, the rate of mortality recorded in the control was 3.3% and reached 8.7% at 10 th day of observation (doo). In maize grains treated, the mortality rate recorded at the 2nd and 10 th doo was respectively 6.9 and 30.5% with 15 μ l, 10.3 and 53% with 20 μ l, 15.5 and 56% with 25 μ l, and 22.4 and 91.1% with 30 μ l of essential oil of *E. globulus* leaves.

3.1.1.2 Percentage of maize grains damaged by *S. zeamais*

Results related to the percentage of maize grains damaged by *S. zeamais* are illustrated in Fig. 2. In general, according to the doo, the percentage of maize grains damaged increased on control, while it decreased on maize grains treated with essential oil of *E. globulus* leaves. Significant differences ($P < .05$) were observed between treatments used. On the control, at the 2nd doo, the maize grains damaged represented 11.7% and increased to 33.3% at the 10 th doo. On the maize grains treated with essential oil of *E. globulus* leaves, the percentage of maize grains damaged recorded at the 2nd and 10 th doo was, respectively, 8.3 and 6% with 15 μ l, 6.7 and 4.3% with 20 μ l, 5.8 and 3.3% with 25 μ l, and 4.2 and 0.7% with 30 μ l.

3.1.2 Trial 2: Insecticidal efficacy of essential oil of *E. globulus* leaves in cotton bags

3.1.2.1 Percentage of *S. zeamais* mortality

The percentage of *S. zeamais* dead recorded in the cotton bags is illustrated in Fig. 3. For each experimental period, analysis of variance revealed significant differences ($P < .05$) between treatments. In general, it noted that mortality rate of *S. zeamais* increases with increasing volume of essential oil of *E. globulus* leaves. In the control, the mortality rate of *S. zeamais* recorded on the 2nd doo was 5.8%, then decreased to 2.7% at the end of the observations. For each volume of essential oil used, the mortality rate recorded on the 2nd and 8th doo was respectively 10.6 and 51.6% with 15 μ l, 14.2 and 65.3% with 20 μ l, 21.2 and 69.8% with 25 μ l, and 35.4 and 84.5% with 30 μ l.

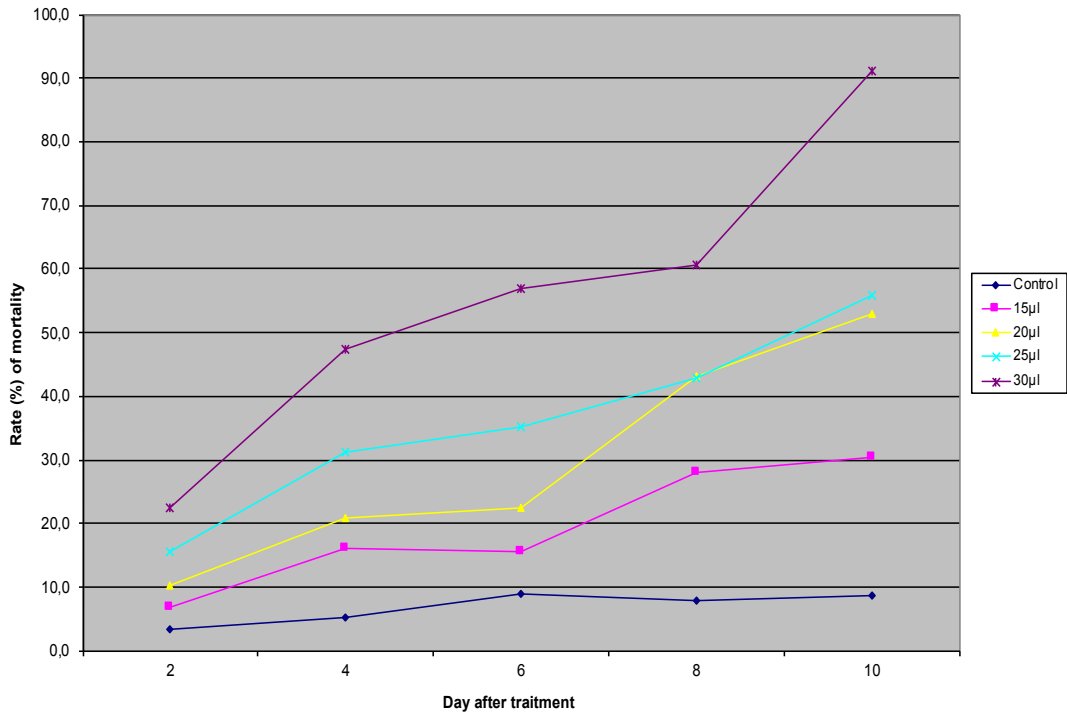


Fig. 1. Rate (%) of *Sitophilus zeamais* mortality in Petri dishes

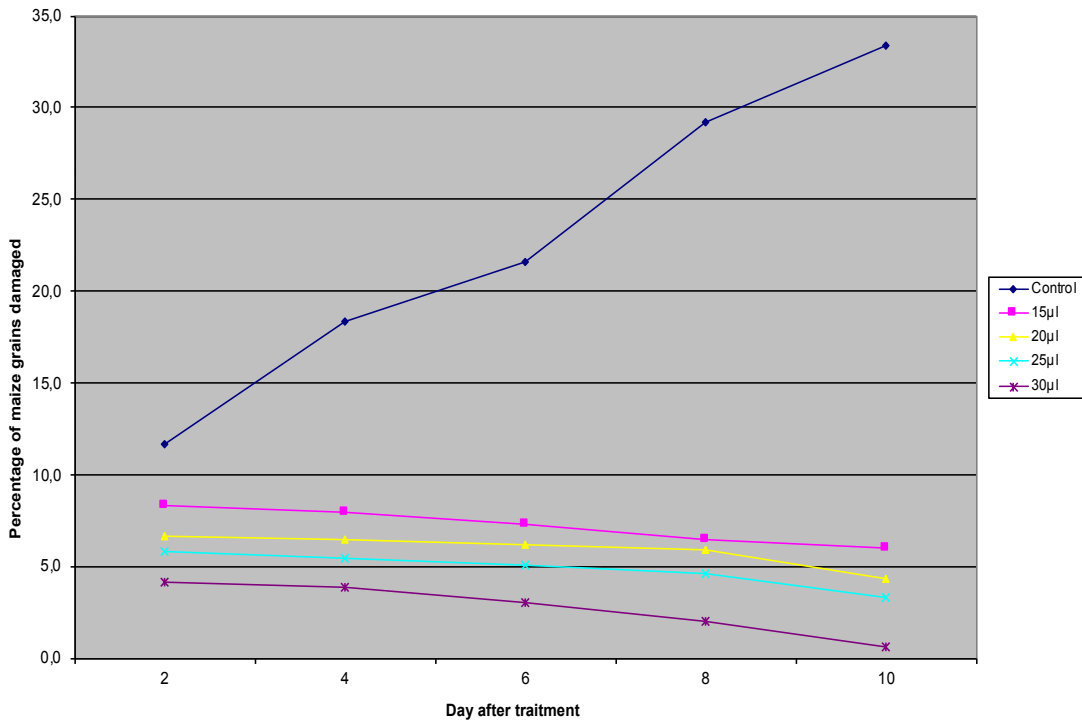


Fig. 2. Percentage of maize grains damaged by *Sitophilus zeamais* in Petri dishes

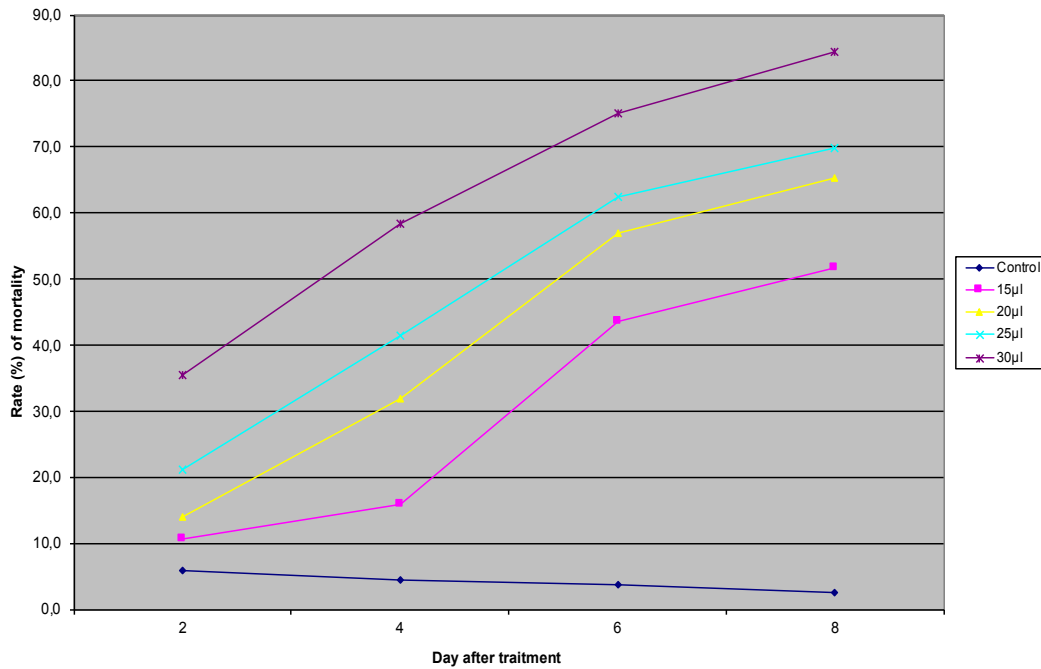


Fig. 3. Rate (%) of *Sitophilus zeamais* mortality in cotton bags

3.1.2.2 Percentage of maize grains damaged by *S. zeamais*

The results relating to the percentage of maize grains damaged by *S. zeamais* in cloth bags are illustrated in Fig. 4.

The analysis in Fig. 4 indicates that the trend curve for the percentage of damaged maize grains increases over time in the control, while it decreases in the other treatments. Analysis of the variance relative to the percentage of damaged maize grains revealed significant differences ($P < .05$) between treatments at threshold of 5% probability. In the control, the percentage of damaged maize grains recorded on the 2nd and 8th day was 10.8 and 48.3%, respectively. In the treated maize grains, the percentage of damaged recorded on the 2nd and 8th day, was respectively, 6.9 and 4.7% with 15 µl, 4.3 and 3.0% with 20 µl, 4.2 and 2.7% with 25 µl, and 4.0 and 1.0% with 30 µl of essential oil of *E. globulus* leaves.

3.2 Discussion

The use of essential oils to protect stored seeds against insects has been the subject of several studies [13,15,16]. These different studies revealed that toxic and/or repellent properties of

essential oils against insects of stored foodstuffs depended on their chemical compositions [13,17], and on the level of sensitivity of insects [13].

Results of the present study revealed that essential oil of *E. globulus* leaves is toxic to *Sitophilus zeamais*, both on filter paper and on cotton bags. This toxicity is linked to its eucalyptol (1,8-cineole) content. The insecticidal properties of eucalyptol have already been demonstrated against several insects such as *Tribolium confusum*, *T. castaneum*, *Prostephanus truncatus*, *Callosobruchus maculatus* and *Rhyzopertha dominica* [18,19,20]. It was also recognized that eucalyptol has insect repellent properties. Indeed, Obeng-Ofori et al. [18] have demonstrated the repulsive effect of 1,8-cineole against the weevils (*S. zeamais* and *S. granarius*) of stored foodstuffs. The repellent activity of *E. globulus* essential oil was also evaluated against various insects. For example, Chandel et al. [21] revealed that *E. globulus* essential oil has significant repellency against *Rhyzopertha dominica*. Similar finding was also observed by Auysawasdi et al. [22] with the same essential oil against *Anopheles dirus*. Ndomo et al. [1] also observed that essential oil of *Callistemon viminalis*, whose eucalyptol content was 58.49%, was able to repel adults of

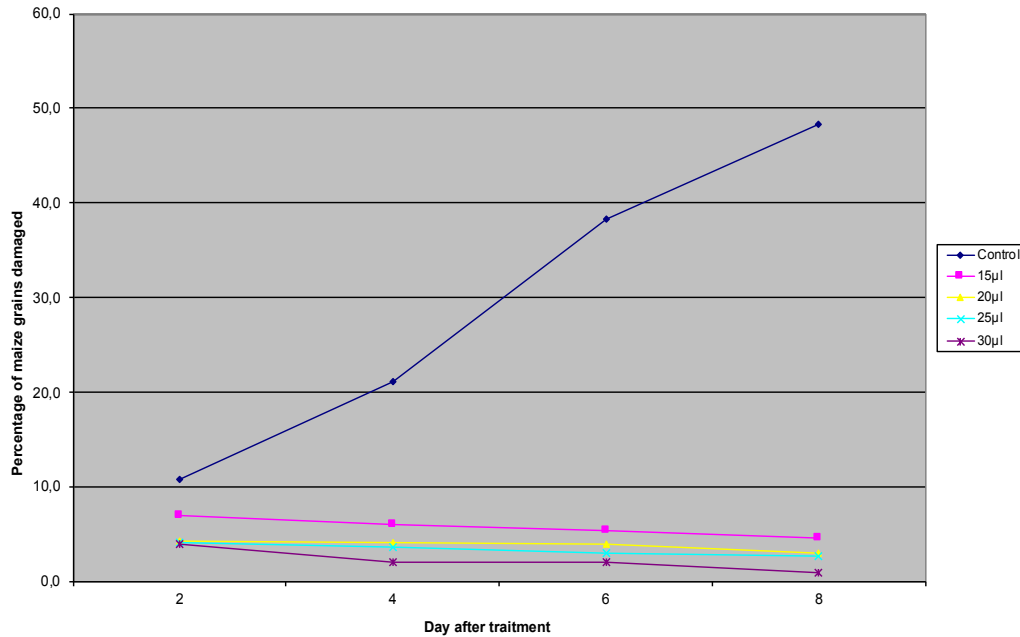


Fig. 4. Percentage of maize grains damaged by *Sitophilus zeamais* in cotton bags

Acanthoscelides obtectus. Although eucalyptol is the principal compound (72.71% content) of essential oil of *E. globulus* leaves, the insecticidal activity of this essential oil could also be due to certain minority constituents or to a synergistic effect several chemical constituents of *E. globulus*.

It was observed in the two trials that mortality rate of *S. zeamais* increased with increasing of the volume of essential oil of *E. globulus* (Figs. 1 and 3) and the exposure time of insects. This finding corroborates observations of Ndomo et al. [1] who noted the high percentage of mortality of adult *A. obtectus* as a function of time and dose of essential oil of *C. viminalis*. Although cases of mortality were observed in the control, their percentage remained below 10%. These mortalities could be naturally due to advanced age of insects used to conduct this study. In the control (Figs. 2 and 4), the percentage of damaged maize grains increased over time, while in the other treatments, it decreased as the volume of essential oil of *E. globulus* was increasing. The increase in the percentage of damaged maize grains in the control is consistent with observations of Ngamo & Hance [23], who point out that in the absence of any treatment of stored cereal grains, the damage due to *S. zeamais* can reach 90% during 5 months of storage.

4. CONCLUSION

The objective of this study was to evaluate in the laboratory conditions, the efficacy of the essential oil of *Eucalyptus globulus* against the corn weevil, *Sitophilus zeamais*. Results obtained revealed that the essential oil of *E. globulus* leaves had insecticidal properties regardless of the volume used. It was observed in both trials that increasing the volume of the essential oil conducted to increase the mortality rate of *S. zeamais*. In the control, the mortality rate of *S. zeamais* was lowest, while the percentage of damaged maize grains was higher compared to other treatments. Overall, the results of these trials suggest that the essential oil of *E. globulus* can act by fumigation and by contact. Comparing the two modes of action, it noted that essential oil used acts effectively by contact. The essential oil of *E. globulus* leaves represents an important solution for protection of seeds and foodstuffs against infestations due to insect pests of stored.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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