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BIOFUMIGANT AND REPELLENT ACTIVITY OF CITRONELLA ESSENTIAL OIL ON COWBEE BEANS

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Abstract: The study aimed to evaluate the biofumigant and repellent action of citronella oil (*Cymbopogon nardus*) in a test with and without choice on cowpea weevil (*Callosobruchus maculatus* (Fabr.). In the no-choice experiment, concentrations 10 were used; 8.0; 6.0; 4.0; 2.0 and 0.0 µl/cm ^{3} and 4.0; 3.0; 2.0; 1.0 and 0.0 µl/cm ^{3} with a choice. In the first one, the concentrations were pipetted on the filter paper fixed on the inside of the lid of the polyethylene flasks, with 10g of cowpea cv BRS Guaribas added. Afterwards, ten non-sexed insects with 0 to 48h of age were added and taken to BOD for 96h. In the second, a circular arena formed by six flasks was used, the central container being symmetrically connected to the others by plastic tubes of 1cm in diameter and 10cm in length. 10g of cowpea grains cv BRS Guaribas were added to the peripheral flasks, the concentrations were added and mixed with the grains. Afterwards, 50 non-sexed *C. maculatus insects* aged between 0.0 and 48h were released into the central flasks of each arena, sealed with lids and taken to BOD for 24h. Then, the insects were removed from the treatments, counted to obtain the repellency index. The highest percentages of *C. maculatus mortality* were obtained with concentrations of 10µl; 8.0µl and 6.0µl and the efficiency of these same doses was respectively of 95.1%, 87.8% and 63.4%, while the total of eggs in any of the treatments with the oil varied from 11.2 to 23.0 and in the witness 112.2. The concentration of 10µl, in addition to causing the highest mortality of insects, provided 100% of the inviability of eggs. The essential oil of citronella (*C. nardus*) exerts efficient biofumigant activity on *C. maculatus* in stored cowpea grains, as well as all concentrations evaluated in the free-choice test have repellent action on *C. maculatus*.
Keywords: *Cymbopogon nardus, Vigna unguiculata, Callosobruchus maculatus,* essential oils, botanical insecticides.

INTRODUCTION

Cowpea (*Vigna unguiculata (L.) Walp.)* is considered an important food source for the human population, since it is rich in amino acids, thiamine, niacin, dietary fiber, as well as a protein content ranging, on average, from 23 to 25%, considered a food of great importance to compose the diet of the population in both rural and urban areas (VALE et al., 2017). Furthermore, it generates employment and income, which makes it important for tropical regions not only in Africa, but also in Central and South America (MOREIRA ARAUJO et al., 2018).

Although cowpea has economic and social importance, there are abiotic and biotic factors capable of affecting not only production, but also storage. Thus, the weevil, *Callosobruchus maculatus* ((Fabr.) is considered the most important pest of stored grains and seeds of cowpea, for causing indirect and direct damages such as the opening of galleries, consumption of the internal mass by the larvae, loss of of weight, reduction of the germinative power of the seeds, residues of increments, occurrence of secondary pests, as well as of microorganisms such as fungi, unpleasant odor, culminating with the impossibility for consumption, planting and commercialization (FILGUEIRAS et al., 2009; WANDERLEY et al., 2019).

Chemical control is done with the use of phosphine, for the disinfestation of the grains, but due to the non-observance of the recommendations for use, they have led to the emergence of insects resistant to the active ingredient of the synthetic product (ALMEIDA et al., 2006), contamination of the environment environment, food, which are consumed by people and trigger problems to human health. In this context, due to the clamor of society, research has sought healthier foods, conservation of the environment, new pest control techniques through the use of botanical insecticides based on essential oils for presenting insecticidal action, feeding inhibitors, deregulators growth rate of insects (SILVA et al., 2008), which has contributed to the conservation of the environment, maintenance of natural enemies, food free from chemical residues, as well as quality of life.

The genus Cymbopogon (Family Poaceae) has approximately 120 species, its center of origin is in tropical and subtropical regions, including Brazil. The plant species of this family hold a wide range of chemical constituents, many of which are used not only in the food industry, but also in the cosmetics industry and extraction of essential oils for use in pest control (BARBOSA, 2011).

In this context, the objective of this work was to evaluate the biofumigant and repellent activity of citronella essential oil *(*Cymbopogon *nardus* (L.) on *C. maculatus* in stored cowpea grains.

MATERIAL AND METHODS

The work was carried out at the Laboratory of Entomology of Embrapa Meio-Norte under controlled conditions in BOD with temperature of 27±1°c, humidity 60±10% and photophase of 12:00h. The insects used in the bioassays came from farms kept in the laboratory, the leaves of citronella (*C. nardus*) were harvested from plants grown in the experimental field of Embrapa Meio-Norte and the essential oil was extracted by distillation in a Clevenger apparatus, the which was placed in a hermetically sealed opaque glass container and placed in a freezer until the bioassays were assembled.

For infestation of treatments, ten unsexed adults of *C. maculatus* aged 0 to 48 h were used in 10 g of cowpea beans cv BRS Guariba packed in polyethylene bottles with screw caps and a capacity of 45 cm $^{3.}$

The treatments consisted of concentrations: 0.0 (control); 2.0 µl; 4.0 µl; 6.0 µl; 8.0µl and 10 µl of the oil that were applied by means of an automatic pipette on a filter paper fixed on the inside of the lid of the flasks, soon after, closed and packed in BOD Mortality was observed 96 h after application of the oil and at 15 days, counting the total number of eggs and unviable eggs. Concentration efficiency was calculated using the Abbott formula (1925) and the percentage of $\%E= T- I/T \times 100$ nonviable eggs using the simple rule of three. Analysis of variance (ANOVA) using the F test and the means compared by the Tukey test at 5% probability in both experiments. For the construction of the regression graph, the Excel program was used.

The repellency test was performed using circular arenas composed of five flasks with the same capacity as those used in the no-choice test, symmetrically connected to the central flask by means of plastic tubes 10 cm long and 3 mm in diameter. 10g of beans, cv BRS Guariba, were added to each flask and, afterwards, the treatments were randomly selected, which consisted of the following concentrations: 0.0 (control); 1.0 µl; 2.0 µl; 3.0 µl and 4.0 µl, which were added to the grains present in the peripheral flasks and mixed with a glass rod. Then, in the central flask of each arena, 50 non-sexed insects with 0 to 48 h of hatching were released. All flasks were sealed and the arenas taken to the BOD for 24h. The replications consisted of five arenas and the experimental tests were in a completely randomized design.

The number of attracted insects and the repellency index (RI) were evaluated in the repellency $IR= 2G/(G+P)$ test, where, where RI= repellence index, G=% insects attracted by treatment and P= % of insects attracted to the control. The RI values range from 0.0 and 2.0.

RESULTS AND DISCUSSION

The highest mortality percentages of *Callosobruchus maculatus* were obtained using concentrations of 10µl; 8.0µl and 6.0µl, which did not differ statistically from each other. The efficiency of these same concentrations was respectively 95.1%, 87.8% and 63.4%, while the total number of eggs in any of the treatments with the oil ranged from 11.2 to 23.0, not differing statistically between si, but it was different from the witness 112.2. The concentration of 10µl, in addition to causing the highest insect mortality, resulted in 100% of the eggs being unviable (Table 1). The evaluation of the insecticidal activity of essential oils is necessary to be carried out at different stages of insect development to ensure the reduction of pest populations under storage conditions (NYAMADOR et al., 2010).

This work is in line with that carried out by Alves et al., (2015), who, when studying the insecticidal action of essential oils in the control of *C. maculatus,* concluded that the concentration of 4.0 µl /cm ^{3} inhibited laying and emergence. of 99% and 100%, respectively, showing the insecticidal potential of the oil on the biology of the insect, since essential oils can act in different ways, whether in biology, behavior, as an attractant or repellent, have action on digestive enzymes and neurological affecting the insect and preventing it from feeding, causing immobilization and culminating in its death (ISMAN, 2006).

In this sense, Wanderley et al. (2019) evaluated the activity of fixed moringa oil, citronella and fennel essential oils, and commercial neem. They observed that citronella oil at doses of 1.5x10 ^{5} and 2.0 x10 ^{5} L resulted in higher insect mortality. France et al. (2012) found the insecticidal action of *C. nardus oil* on *Zabrotes subfasciatus* when using a dose of 0.5ml/kg in *Phaseolus vulgares* L. concluded that the essential oil of *C. citratus* showed a 90.6% reduction in oviposition, while, *C. nardus,* the reduction was 93.3%.

C. maculatus responses to *C. nardus* essential oil concentrations were dependent, since the higher the concentration, the higher the insect mortality. The oil was toxic to

Concentration	Mortality (%)	Efficiency (%)	Total of eggs (DP)	Variable eggs (%)
10 µl	96 a	95,1	13,0 ± 2,2 b	100,00 a
8,0 µl	90 ab	87,8	11,2 ± 3,1 b	81,46 b
6,0 µl	70 abc	63,4	16,2 ± 1,9 b	60,24 cd
4,0 µl	42 bcd	29,3	18,8 ± 3,6 b	72,8 0 bc
2,0 µl	32 cd	17,1	23,0 ± 4,4 b	46,06 d
Witness	18 d	-	112,2 ± 28,2 a	16,26 e
CV (%)	23,24	-	29,21	22,5

Means followed by the same letter in the column do not differ statistically from each other by the Tukey test at 5% probability. SD= standard deviation. Data transformed into √x.

Table-1. Effect of *Cymbopogon nardus essential oil* on mortality, efficiency and oviposition of *Callosobruchus maculatus* in cowpea seeds.

adults of *C. maculatus* from a concentration of 6 µl with an increasing linear response ($y= 8,4571x + 15,714$; $R^2 = 0,9733$; $P<0,01\%$) (figure 1).

As for repellency, it can be observed that all concentrations evaluated were classified as repellents and the control behaved as neutral to *C. maculatus.* Thus, it is noteworthy that the lowest concentration of 1.0 µl, classified as repellent, attracted more insects than the others, but not enough to be classified as attractive (Table 2). This work is in line with that carried out by Wanderley et al, (2019), as they observed that in the control of *C. maculatus,* the essential oil of *C. nardus* had a lower attractive effect than the other oils evaluated regardless of the doses studied.

Similar results to this research were found by Girão et al. (2014) who carried out work with *C. nardus* in powder form on *Zabrotes subsfasciatus* found a repellent effect. Results found by Santos, Silva and Brito (2018), using *C. nardus powder for C. maculatus* repellency, showed that the concentration of 1.0g showed repellent activity to *C. maculatus* in cowpea cv. BRS Guaribas.

However, França et al. (2012) obtained contrary results when evaluating the repellent effect *of C. nardus oil* 0.5ml/kg on *Zabrotes subfasciatus,* as it did not repel and was classified as neutral. The collection of the part of the plant to be used for oil extraction, as well as weather conditions, harvest time, handling and storage can interfere with the amount of chemical products of the plant species (SOARES & TAVARES DIAS, 2013).

Lima- Mendonça et al. (2013) when evaluating the repellent effect of *Cymbopogon nardus* on *Zabrotes subfasciatus,* found a repellent effect. This shows that both the oil and the powder of that species have potential to control not only *C. maculatus,* but also *zabrotes subsfasciatus,* showing the spectrum of plant control action on pest species. Plants are capable of producing secondary metabolites, which have toxic activity to insects, contributing to population reduction, preventing the emergence of resistant insects, since plant-based products, such as essential oils, exert control pressure on insects., but with the combined action of several substances present in them.

Cruz et al. (2012) using a concentration of 2% per 20g of cowpea grains proved the repellent activity of *C. nardus oil* on *C. maculatus.* Olivero-Verbel et al., (2009) found 96% repellency to *Tribolium castaneum* exposed to *C. nardus oil* when using a concentration of 0.2 µl/cm $^{2.}$ These results corroborate those found in this work.

CONCLUSIONS

The essential oil of citronella (*C. nardus*) has efficient biofumigant activity against *C. maculatus* in stored cowpea grains.

C. nardus shows repellent action to *C. maculatus* in stored cowpea grains.

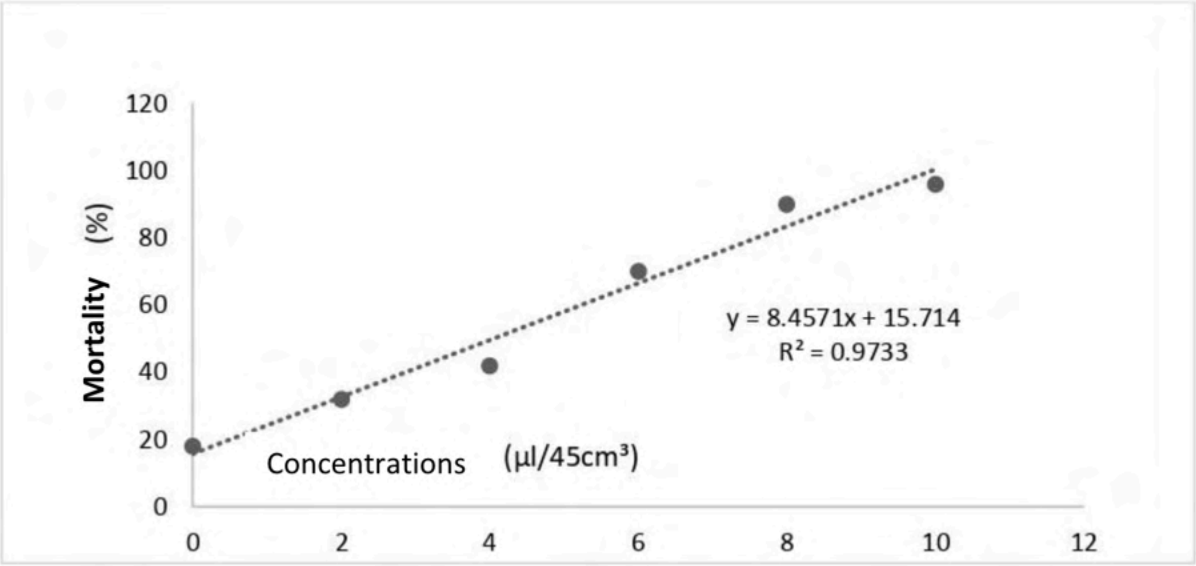

Figure 1. Graphic representation and regression equation between *C.* nardus essential oil concentrations after 96 h of application and mortality percentage of Callosobruchus maculatus in cowpea grains.

Concentrations	Insects attracted (DP)	repellency index	Rating
4,0 µl	1,4±0,5 c	0,1	Repellent
3,0 µl	1,6±0,5c	0,1	Repellent
2,0 µl	2,0±1,1c	0,1	Repellent
1,0 µl	6,4±3,2b	0,3	Repellent
0,0 µl	31,8±4,7a	1	Neutral
CV	33,90%		

Cymbopogon nardus oil repellency on *C. maculatus* in a free choice test.

Means followed by the same letter vertically do not differ statistically from each other by the Tukey test at 5% probability. SD= standard deviation. Data transformed into √x.

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