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Ozonation of Cowpea Grains: Alternative for the Control of *Callosobruchus maculatus* and Maintenance of Grain Quality

Gustavo Yves Rodrigues Ramos [1], Gutierres Nelson Silva [2], Ynayanna Nariza Medeiros Silva [3], Yago de Medeiros Silva [1], Izaias Santos Marques [1], Giovana Lopes da Silva [1], Marcela Silva Carvalho [4], Leda Rita D'antonino Faroni [5], Simone Kelly Rodrigues Lima [6], Daniel Dias Rufino Arcanjo [6], Massimo Lucarini [7], Alessandra Durazzo [7] and Douglas Rafael e Silva Barbosa [1,*]

1 Instituto Federal de Educação, Ciência e Tecnologia do Maranhão, Campus Codó 1, São Luís 65075-441, MA, Brazil
2 Instituto Federal de Educação, Ciência e Tecnologia do Mato Grosso do Sul, Campus Nova Andradina, Nova Andradina 79750-000, MS, Brazil
3 Agricultural Sciences Center, Universidade Federal do Piauí, Teresina 64049-550, PI, Brazil
4 Instituto Federal de Educação, Ciência e Tecnologia Baiano, Campus Teixeira de Freitas, Teixeira de Freitas 45985-970, BA, Brazil
5 Department of Agricultural Engineering, Universidade Federal de Viçosa, Rio Paranaíba 36570-900, MG, Brazil; lfaroni@ufv.br
6 Department of Biophysics and Physiology, Universidade Federal do Piauí, Teresina 64049-550, PI, Brazil
7 CREA-Research Centre for Food and Nutrition, 00178 Rome, Italy
* Correspondence: douglas.barbosa@ifma.edu.br

Abstract: Nowadays, the modified atmosphere with ozone is one of the main alternatives for controlling insect pests in stored grains, as it allows grain quality to be maintained while causing no environmental damage. In light of this, the purpose of this study was to assess the toxicity of ozone to *C. maculatus* in cowpea grains as well as how it affects the physiological and physical properties of the grains. The toxicity was determined by estimating lethal doses (mg/g) at the top and bottom of the fumigation chamber for 50% and 95% of adult insects (LD_{50} and LD_{95}). Cowpea grain samples were removed after being exposed to ozone, and the effect of ozonation on the physical and physiological quality of the grains was assessed. The electrical conductivity, water content, and germination were all examined in this manner. The lethal doses LD_{50} were 65.97 and 45.52 mg/g when the insects were distributed at the top and bottom of the fumigation chamber, respectively. As a result, ozone toxicity was higher at the bottom than at the top. The increase in ozone doses resulted in a decrease in water content. The germination rate exceeded 90% in both locations where ozone was applied. The electrical conductivity remained constant, ranging between 152.01 and 239.59 S cm^{-1} g^{-1}. The findings indicate that ozone is toxic to *C. maculatus* but has no effect on grain quality. Ozone may therefore be used to manage *C. maculatus* in cowpea that has been stored.

Keywords: cowpea; ozonation; alternative control; modified atmosphere; grain quality; cowpea weevil; *Callosobruchus maculatus*; toxicity

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1. Introduction

Cowpea, or *Vigna unguiculata* (L.) Walp, is grown in many places worldwide, but mostly in tropical and subtropical areas [1]. In 2021, with an annual production of 8.9 million tons, cowpeas were harvested on more than 14.4 million hectares globally [2].

Despite its high productivity, several obstacles remain, including the intense attack of insect pests, with *Callosobruchus maculatus* (F). (*Coleoptera*: *Chysomelidae*: *Bruchinae*) being one of the most destructive ones [3–6]. This insect can attack grains in both the field and during storage [7], causing major losses, such as reduced grain weight, market value, and germination capacity [8].

Chemical control has been one of the most widely used methods for the management of this insect, because of its high effectiveness, low economic value, and indiscriminate selling since the 1980s [9]. The main insecticides used are organophosphates and pyrethroids, which have prolonged effects [10,11], as well as phosphines, which are also widely used in the storage environment [10,11]. With its high toxicity, effectiveness, and lack of detrimental effects on grain viability, phosphine fumigation is particularly popular for controlling pests of stored goods, even though its indiscriminate and ongoing use has led to the emergence of resistant populations [12].

Therefore, research on alternative methods with high efficiency rates for pest control in stored products is required. In this regard, ozonation is an acceptable and economically viable method for treating grains during storage, leaving no residue, and hence environmentally friendly [13]. Accordingly, the use of ozone gas in grain storage may be a feasible strategy because it is a powerful oxidizing agent that is toxic to bacteria, viruses, fungi, protozoa, and insects [14], and does not affect grain quality [15].

Ozone gas has some advantages in its use for grain storage; for example, it can be generated in the place of use, thus eliminating the need for manipulation, storage, and disposal of chemical containers. Furthermore, its degradation results in the production of oxygen [14,16]. These traits have led to studies demonstrating the effectiveness of ozone gas in controlling insect pests in stored grains, including *C. maculatus*, *Zabrotes subfasciatus*, *Rhyzopertha dominica*, *Ephestia cautella*, *Plodia interpunctella*, *Trogoderma granarium*, and *Tribolium castaneum* [17–22].

Despite the potential of ozone as a fumigant insecticide for controlling insect pests in stored products, further research on the effects of ozone on *C. maculatus* and the quality of cowpea grains is needed. Accordingly, the objective of this study was to assess the toxicity of ozone gas on *C. maculatus* and its effect on the physical and physiological characteristics of cowpea grains.

2. Materials and Methods

2.1. Samples and Experimental Design

The cowpea (*Vigna unguiculata*) grains were purchased in the city of Timon (S 05°07′54.7″; W 42°49′13.9″), at the property São Judas Tadeu. The grains were maintained in a freezer at 10 °C for seven days to kill any insects that might have come from the field. They were then maintained at room temperature for 48 h. Prior to experimental implantation with ozone gas, the grains were characterized based on the following parameters: percentage of infestation, electrical conductivity, water content, and percentage of germination (Figure 1).

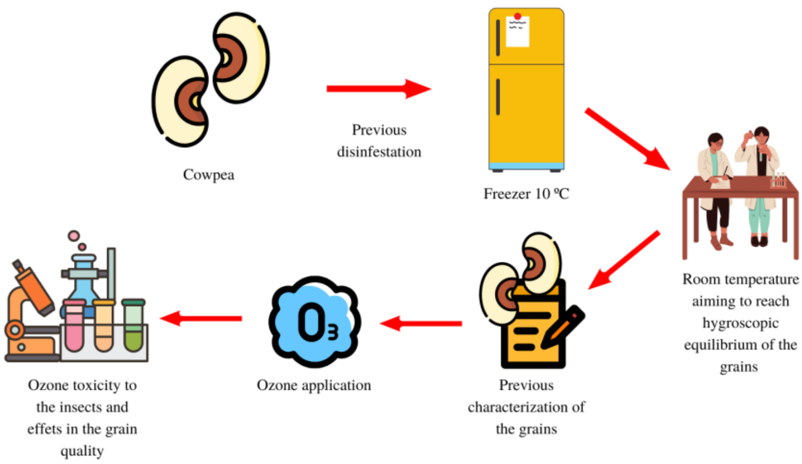

Figure 1. Scheme of the experimental plan.

The present work was carried out in the soil laboratory of the Instituto Federal de Educação, Ciência e Tecnologia do Maranhão—IFMA/Campus Codó.

The percentage of infestation was analyzed according to the methodology adapted from the Rules for Seed Analysis [23], based on the percentage of damaged grains in the batch used in the experiment, as well as egg, larvae, pupae, adult insects, and insect exit holes. The test was performed thrice with randomly selected 100 grains each time. It was performed by individually examining each grain and observing insect exit holes. Damaged and/or perforated grains were recorded as infested and were subsequently discarded. The remaining portions of each replicate that appeared unharmed by insects were submerged in water for 24 h. Each replicate was then cut apart, and the presence of eggs, larvae, pupae, or adults was noted. Finally, they were added to the total number of damaged and perforated grains for each repetition. The results were expressed in percentages by taking the average number of grains damaged by insects across three replicates.

2.2. Rearing of *Callosobruchus maculatus*

Adult *C. maculatus* that had infested cowpea seeds were used in the bioassays and rearing. The insects were raised in the laboratory and multiplied over several generations. They were placed in 1.5 L glass containers, covered with a perforated plastic lid and an inner fabric lining, and maintained under the following conditions: 27 ± 2 °C, 70 ± 5% relative humidity, and 12 h scotophase [24].

The insects oviposited in the confinement, and after a week, they were removed with a sieve and placed in a container with detergent and acetone to be discarded. The containers with the egg-infested grains were maintained at room temperature until the F1 generation emerged. This procedure was repeated for several generations to ensure the quality of adults required for the experiments.

2.3. Generation of Ozone Gas and Application System

An ozone generator, model O&L3.ORM (Ozone & Life, So José dos Campos, So Paulo, Brazil), operating in a dielectric barrier discharge system was used to generate ozone gas (DBD). Ozone was used at a volumetric flow rate of 2 L min^{-1}. An EverFlo oxygen concentrator (Philips Respironics, Murrysville, PA 15668, USA) generated moisture-free oxygen with a purity of 93%, which was used as an input for ozone production. The doses of ozone were monitored, applying the iodometric method [25]. Ozone was collected with flow control. The gas was directed to an Erlenmeyer containing a potassium iodide solution (KI 2%) and subsequently quantified by the iodometric method.

2.4. Fumigation Chamber

Samples of cowpea grains were ozonized inside PVC chambers (20 cm in diameter × 100 cm in height) with a volume of 31.4 L and connections at the bottom and top for the ozone inlet and outlet, respectively [22,26]. Each sample contained 10 kg cowpea. 50 unsexed adult insects (0–48 h of age) were placed in plastic bottles (7.5 cm in diameter and 10.0 cm in height) containing 300 g cowpea. The cages were distributed within the grain mass (10 kg) at two different points: in the plenum and on the surface. The upper and lower parts of the cages were made of organza fabric to allow the passage of ozone and oxygen gas (control). The toxicity of the ozone gas was determined at these two different points. This procedure was repeated four times, with each cage corresponding to one repetition for each exposure time for ozone and oxygen gas (control). Oxygen, free of moisture and with a purity of 90 ± 3% was used as control.

2.5. Ozone Toxicity for *C. maculatus*

Dose–response curves were developed using bioassays in which insects were exposed to increasing doses of ozone gas in order to determine the lethal doses (LD_{50} and LD_{95}). Consequently, the time between doses was also determined. Initially, preliminary tests were performed to estimate the highest doses at which no insect death occurred (lower end) and the highest dose at which maximum mortality occurred (upper end). Based on the data obtained, dose intervals for definitive bioassays were established for the gas injection point. Five doses each were used at the top (29.2, 47.1, 57.8, 117.0, and 186.9 mg/g) and the bottom (29.2, 47.1, 57.8, 87.4, and 117.0 mg/g). Ozone was used with a volumetric flow rate of 2 L min^{-1}, at a concentration of 1.61 mg L^{-1} at the inlet, a temperature of 25 °C, and 70% humidity. (Figure 2). The Van Leeuwen method [27] was used to calculate ozone doses. For the control treatment, cowpea grains and insects were exposed to oxygen gas under conditions similar to those used in the ozone treatment. After every dose, mortality was evaluated.

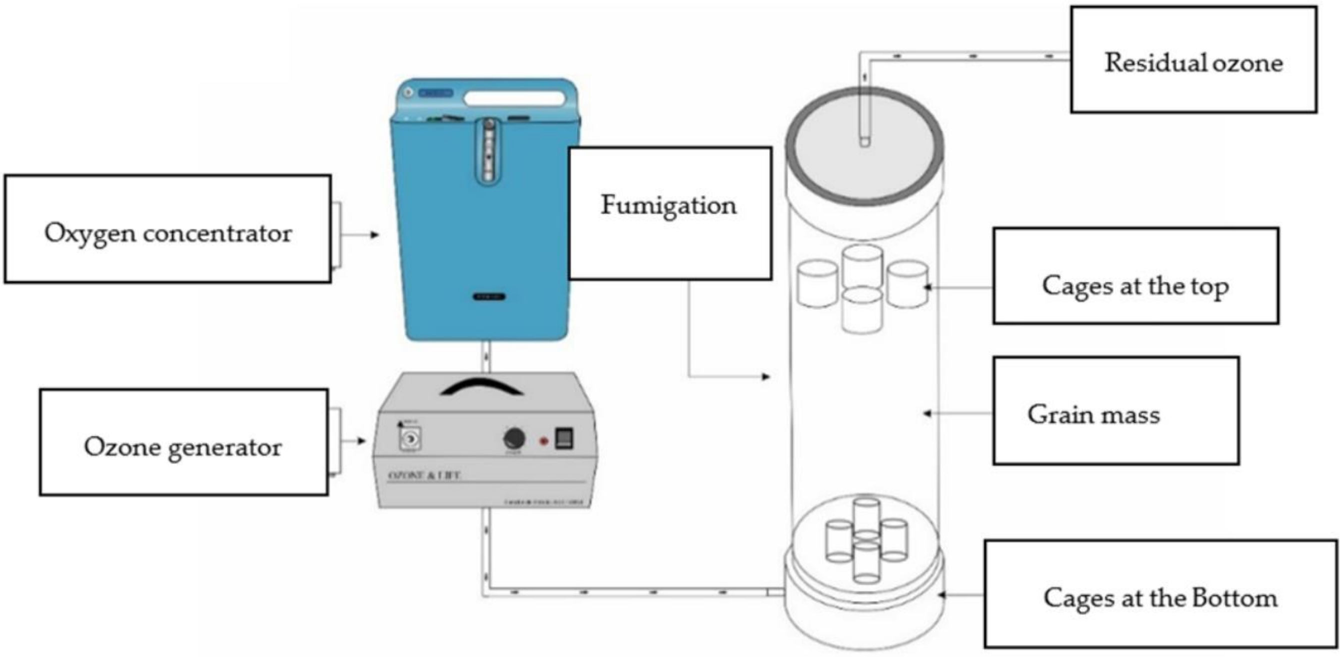

Figure 2. Schematic diagram of the experimental setup.

2.6. Quality Evaluation of Cowpea Grains

Following the characterization of cowpea grains prior to ozonation, samples from the toxicity test conducted at the bottom and top of the fumigation chamber were used to assess grain quality following ozonation.

2.7. Characterization of Cowpea before Treatment with Ozone and Oxygen

The initial characterization showed that the grains had the following characteristics: water content of 8.61%, electrical conductivity of 113.94 µS cm^{-1} g^{-1}, germination of 87.5%, and a low degree of infestation by insects (1.34%).

2.8. Water Content and Germination

2.8.1. Water Content

The water content of cowpea grains was measured at 103 ± 1 °C for 72 h [28]. Grain samples (30 g) were used for each treatment. The results are expressed as a percentage of the wet basis.

2.8.2. Germination

Cowpea seed germination was evaluated according to the Norms for Seed Analysis, using four replicates [23].

2.9. Electrical Conductivity

The electrical conductivity of the solution with the cowpea grains was determined by the "mass conductivity" method [29].

2.10. Experimental Design and Statistical Analysis

Toxicity data were subjected to probit analysis, using the PROBIT procedure of SAS software, version 8.02 [30], to generate concentration–mortality curves. The experiment was carried out in a completely randomized design, in a split-plot scheme: plots (atmospheric air and ozone) and subplots (lethal doses in the top (29.2, 47.1, 57.8, 117.0 and 186.9 mg/g) and bottom (29.2, 47.1, 57.8, 87.4 and 117.0 mg/g)), with four repetitions. Means were compared using Tukey's test at 5% probability. The models were chosen based on the significance of the regression coefficients, using the "*t*" test at 5% probability and the coefficient of determination (R^2). Descriptive statistics were used with the mean and standard error of the parameters for which regression adjustments were not possible. For the ANOVA, SAS Software, version 8.02 [30] was used while SigmaPlot Software, version 7.0 [31] was used for the regression analysis.

3. Results

The results were as follows: (i) toxicity of ozone to *C. maculatus* and (ii) maintenance of cowpea grain quality after ozone treatment.

3.1. Ozone Gas Toxicity

When the insects were distributed at the top and bottom of the fumigation chamber, the LD_{50} of the insects were 45.52 and 65.97 mg/g, respectively, and the LD_{95} of the insects were 236.95 and 254.78 mg/g (Table 1). The dose–mortality curves for the top and bottom, where grains and insects were exposed to ozone, revealed equations with low chi-square (χ^2) values (4.00) and high *p*-values (>0.05). This indicated that the data were suitable for the PROBIT model for the estimation of lethal doses because the observed data were close to the estimated data. A lower LD_{50} value at the bottom, as indicated by the non-overlapping confidence intervals, indicates greater toxicity. Ozone gas was in fact 1.44 times more toxic at the bottom than at the top. However, because the confidence interval values overlapped, there was no discernible difference between the top and bottom groups for the lethal dose LD_{95}. High values of the slope of the dose–mortality curves for both ozone application positions showed that small dose variations caused large mortality variations.

Table 1. Ozone toxicity (LD_{50} and LD_{95} mg/g) for *Callosobruchus maculatus* in cowpea grains, at the dosage of 1.61 mg L^{-1} and a volumetric flow rate of 2 L min^{-1}.

Position	n	DF	Slope ± SE	LD_{50} (95% CI)	TR_{50}	LD_{95} (95% CI)	TR_{95}	χ^2	*p*
Bottom	1000	3	2.29 ± 0.20	45.52 (41.10–49.64)	1.44	236.95 (189.49–324.08)	1.07	3.55	0.31
Top	1000	3	2.80 ± 0.16	65.97 (61.38–70.87)	-	254.78 (217.68–309.83)	-	0.76	0.85

n = number of insects used in the tests; DF = degree freedom; SE = standard error; CI = confidence interval; TR = toxicity ratio, χ^2 = chi-square, *p* = probability.

3.2. Quality Evaluation of Cowpea Grains

3.2.1. Water Content

In terms of the water content of cowpea grains, a significant difference was observed between treatments with ozone gas vs. control ($F_{1;6}$ = 87.14; $p < 0.05$) when the grains were exposed to the bottom of the fumigation chamber. No significant difference was observed in the water content between the treatments ($F_{1;6}$ = 0.74; $p > 0.05$), in the case of exposure at the top of the fumigation chamber.

Figure 3A shows the regression curves describing the water content of cowpea grains exposed to ozone and atmospheric air at different grain doses positioned at the bottom of the fumigation chamber.

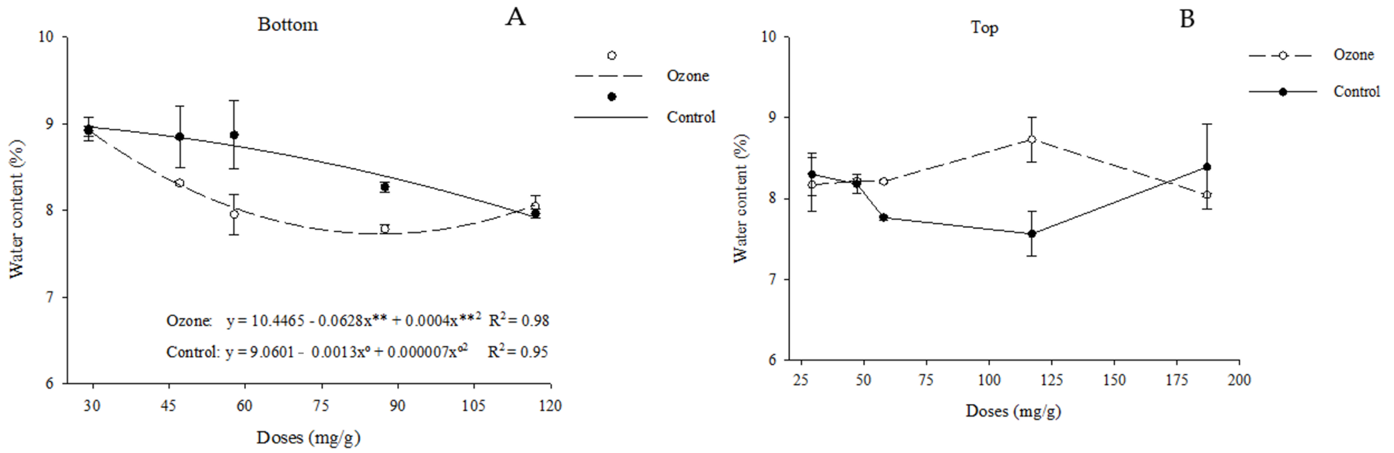

Figure 3. Water content (mean ± SE) of cowpea exposed to ozone and oxygen (control), in different doses. (**A**) Bottom. (**B**) Top. ^{0} Significant at 10%, * Significant at 5%, ** significant at 1% and 'ns' not significant by *t*-test.

When the cowpea grains were exposed at the top, it was not possible to adjust the regression; therefore, descriptive statistics were used (Figure 3B). The values of the water content of the grains exposed to the ozone and control treatments ranged from 7.54% to 8.73% b.u, regardless of the dose, in case of exposure at the top of the fumigation chamber.

3.2.2. Germination

Regarding germination, no difference was observed between treatments with ozone gas and the control ($_{F1;6}$ = 2.46; $p > 0.05$) when cowpea grains were exposed to the bottom of the fumigation chamber, as well as at the top ($F_{1;6}$ = 0.15; $p > 0.05$).

Figure 4A shows regression curves describing the germination of cowpea grains at the bottom of the fumigation chamber, exposed to different doses of ozone gas and the control treatment. When the cowpea grains were exposed at the top of the fumigation chamber, it was not possible to adjust the regression; therefore, we used descriptive statistics (Figure 4B).

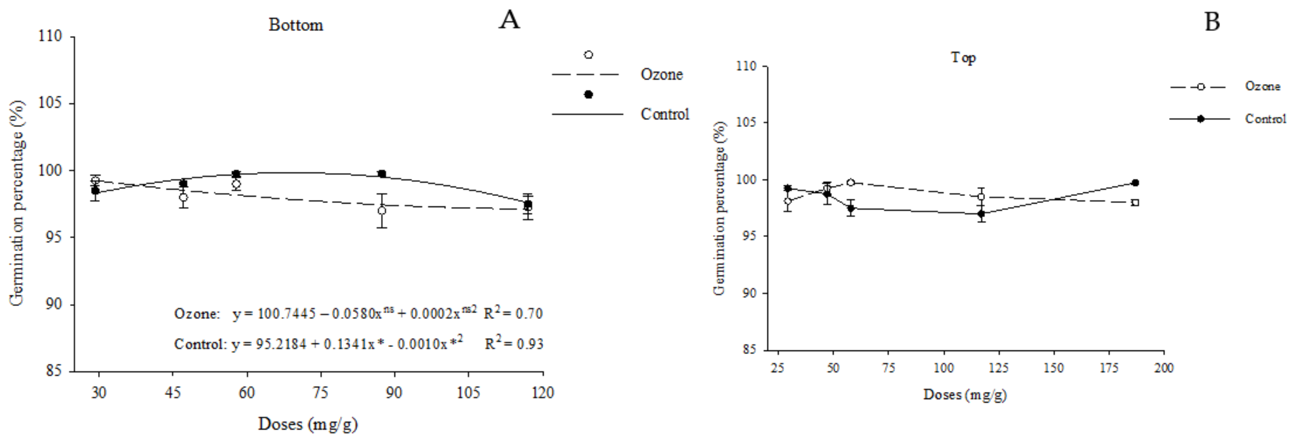

Figure 4. Germination percentage (mean ± SE) of cowpea exposed to ozone and oxygen (control), in different doses. (**A**) Bottom. (**B**) Top. ^{0} Significant at 10%, * Significant at 5%, ** significant at 1% and 'ns' not significant by *t*-test.

3.2.3. Electrical Conductivity

A significant difference was observed in the electrical conductivity of cowpea grains at the bottom when treated with ozone gas vs. the control ($F_{1;6}$ = 87.74; $p < 0.01$). Furthermore, a significant difference was also observed in the electrical conductivity of cowpea grains between the treatments ($F_{1;6}$ = 12.87; $p < 0.05$), when the grains were at the top of the fumigation chamber.

Figure 5A shows the regression curves describing the electrical conductivity of the grains at different doses of ozone and atmospheric air (control), at different positions in the fumigation chamber (Figure 5A,B). Regression adjustment was not possible for cowpea grains positioned at the top. The electrical conductivity ranged between 152.01 and 239.59 µS cm^{-1} g^{-1} regardless of the atmospheric air or ozone gas (Figure 5B).

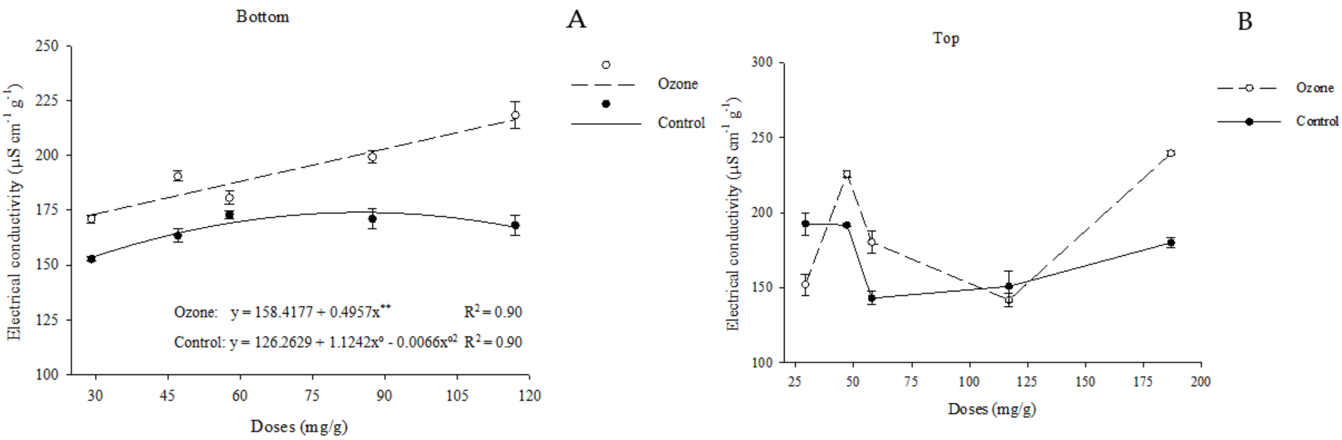

Figure 5. Electrical conductivity µS cm^{-1} g^{-1} (mean ± SE) of cowpea exposed to ozone and oxygen (control), in different doses. (**A**) Bottom. (**B**) Top. ^{0} Significant at 10%, * Significant at 5%, ** significant at 1% and 'ns' not significant by *t*-test.

4. Discussion

The results of the present study revealed that the dose and exposure time necessary to cause mortality in *C. maculatus* adults increased as the cages containing insects moved away from the gas at the point of injection. When the insects were located close to the injection point in the bottom part of the fumigation chamber, the lethal times, LT_{50} and LT_{95}, were 12.62 h and 23.76 h, respectively, for *S. zeamais*, with an LT_{50} of 43.52 h and $LT9_5$ of 64.19 h for *T. castaneum* when the insects were distributed on the plenum (bottom) [15]. In the present study, the lethal doses LD_{50} and LD_{95} corresponded to lethal times LT_{50} and LT_{95} of 7.80 and 40.58 h. These results showed a difference in the action of ozone gas depending on the family, genus, or species of stored cowpea grain insects evaluated; the lethal doses at the bottom for *Z. subfasciatus* (LD_{50} and LD_{95}) were 29.78 and 87.79 mg/g respectively [22]. Thus, the lethal dose LD_{50} which is used as a measure of toxicity was higher (45.52 and 236.95 mg/g for LD_{50} and LD_{95}) for *C. maculatus*, although the insects belonged to the same family (*Chrysomelidae*).

It should also be considered that differences in the order of insects can also be decisive in determining which position of ozone gas application is more efficient. For instance, for *Ephestia kuehniella*, a moth, belonging to the order Lepidoptera, 5 h of exposure resulted in almost complete mortality of all life stages of this insect, when it was positioned on top of 2 kg of wheat [32], which differs from the results observed in the present study for *C. maculatus*, belonging to the order Coleoptera, in which there was greater mortality when the gas was injected in the bottom region of the fumigation chamber.

Similarly, for *R. dominica*, the lowest mortality time was observed when the insects were distributed at the bottom of the fumigation chamber, with an LT_{50} value of 8.69 h and LT_{95} value of 11.28 h [26]. An explanation for this difference in toxicity according to the position of the ozone gas injection in the fumigation chamber could be the resistance of the grains to the passage of the gas, reducing its concentration along the grain mass and thereby indicating that the closer to the gas injection point, the greater the toxicity [26].

The process of introducing ozone gas into the grains is divided into two stages. In the first stage, the gas degrades quickly and slowly as it moves through the grain mass.

In the second phase, ozone flows freely through the grain, with little degradation due to the saturation of the degradation sites [33]. However, the same authors stated that the saturation rate was affected by the rate or flow of ozone injected into the medium, which degrades to oxygen with a short half-life (20–40 min). This could explain why the use of long periods of time, and, consequently, higher doses of ozone were required to cause mortality at the top of the fumigation chamber in the current study. The dose of ozone gas varies along the column because of the reaction between the gas and the grain. Consequently, ozone decomposes, causing a delay in the increase in its dose in the intergranular space in relation to the airflow [22].

Studies conducted to test the toxicity of ozone gas at different life stages *of C. maculatus* under laboratory conditions showed that adult insect mortality was observed in cases of treatments with 4 and 5 h of direct exposure at a concentration of 1.2 mg L^{-1} [18]. The insecticidal effect of ozone on *C. maculatus* infestation was optimized by increasing the concentration and dosage (exposure time) [20]. As a result, in addition to exposure time, ozone concentration influences the mortality of *C. maculatus*. In the present study, the ozone concentration, while initially fixed, increased insect mortality with increased exposure to the gas. Our study, unlike the one carried out with different life stages of the insect [18], aimed to evaluate the effect of ozone on the mortality of *C. maculatus* adults, simulating a more realistic condition with the presence of grain mass, and insects distributed at different points. Consequently, our results of the exposure period, as well as the dose, were different, since these parameters affect the effectiveness of ozone, and consequently, the mortality of adult insects. It is worth mentioning that our study also evaluated the quality of the grains after the use of ozone gas, which was not observed in the study with different stages of life [18].

To facilitate the use of ozone gas for grain treatment while avoiding as much degradation as possible, it is recommended to inject ozone at several points, in structures capable of inverting the ozone direction after a certain period, thereby treating both the bottom and top with high concentrations throughout the fumigation chamber [34]. This application strategy could represent a solution for the differences in mortality found between ozone injections at the top and bottom of the grain mass, as reported in the present study. Furthermore, the movement of ozone through the grain mass can be hampered by a phenomenon known as medium ozone demand [35]. Consequently, the highly reactive nature of ozone restricts its movement [36]. Thus, the surfaces of many materials act as catalysts for ozone degradation, and the rate of degradation is generally determined by the surface properties of the materials with which the gas comes into contact and the temperature [37]. Although there is information about the losses related to the position of the gas injection and its degradation, the results of the toxicity to *C. maculatus* obtained in the present study provide important information regarding the mortality of this insect in its adult phase.

In general, increasing the ozone dose resulted in a decrease in the water content. This property was also observed in a wheat experiment, where after 60 days of storage, increasing the exposure period (dose) of ozone gas reduced the water content of the grains [26]. The reduction in water content for brown and golden flaxseed packages after ozonation ranged from 7.74% to 7.54% for golden flaxseed and 8.39 to 7.98% for brown flaxseed [38]. For lima beans (*Phaseolus Lunatus* L.) exposed to ozone at different doses, the water content was reduced from 11.2% b.u to 10.38% b.u with increasing doses [22]. This drying phenomenon may be related to an ozone-containing gas mixture that, owing to the flow of gases passing through the grains, reduces humidity and favors product dehydration [39].

Experiments with two years of sampling revealed that ozone treatment had no significant effect on the moisture and water content of wheat grains; however, these parameters were significantly affected by sampling location, with differences between the top and bottom of the tank. All samples collected from the top of the tanks during the drying process had a higher water content [40]. In the present study, the greatest reduction in the water content occurred when ozone was applied to the bottom at the dose of 87.4 mg/g

(15 h of exposure), whereas for the top the dose was 117 mg/g (20 h of exposure). This confirmed that there was a trend of greater water loss when ozone was injected into the bottom of the storage containers.

In the present study, the germination percentage was greater than 90%, and no significant differences in the germination test were observed at either location of ozone application. Similar results were obtained when ozone was used as an alternative fumigant to control *C. maculatus* in cowpea, in which grain germination was not affected by the use of this gas [24].

Similar results were found in maize, in which ozone did not influence germination [15]. Cowpea seeds were protected from attacks and weight loss caused by *C. maculatus* and *C. chinensis* by ozone treatment at a concentration of 2.0 g/m^3, which did not significantly reduce seed germination compared to the control [18]. In a study using *P. lunatus* lima beans, increasing doses of ozone gas at a concentration of 1.61 mg resulted in an increase in the percentage of germination from 67.25% to 93.75% [22].

For the grains positioned at the bottom, there was a positive increase in conductivity as the dose of ozone gas increased. The electrical conductivity test measures the number of ions leached in a solution, in which the grains are directly related to their degree of deterioration [26]. Low conductivity indicated high-quality seeds and grains with high quality [41].

In a study aimed at evaluating the physiological and sanitary qualities of ozonized soybean seeds during drying, there was an increase in electrical conductivity ranging from 118.11 to 173.99 µS cm^{-1} g^{-1}, after storage [42]. Similar results were obtained in this study, in which the electrical conductivity ranged from 152.01 to 239.59 µS cm^{-1} g^{-1} for the bottom. Another study verified an increase in the electrical conductivity of corn grains exposed to ozone gas at the top, from 24 h onwards, while there was no significant difference at the bottom [15]. An increase in the electrical conductivity of ozonized wheat grains was also observed [22]. A similar increase in the electrical conductivity of corn subjected to ozone gas and atmospheric air at temperatures of 20, 30, 35, and 40 °C was observed [43]. This increase in electrical conductivity was observed in the present study, with 29.2 mg/g at the bottom (5 h of exposure), starting from 171.05 µS cm^{-1} g^{-1} with 29.2 mg/g and a value of 190.55 µS cm^{-1} g^{-1} with 47.1 mg/g (8 h); then, for the dose of 57.8 mg/g (10 h) there was a decrease in the electrical conductivity value, but there was an increase afterward with the dose of 87.4 mg/g (15 h), starting from 199.29 µS cm^{-1} g^{-1} and reaching a maximum peak of 218.34 µS cm^{-1} g^{-1} for the dose of 117 mg/g (20 h).

Ozone damage to the cell membrane of the grain tissue may promote a greater release of exudates by increasing the leaching of electrolytes into the solution [44]. The results of the present study corroborate those found by the authors but with a slight decrease after 10 h of exposure (57.8 mg/g). Thus, grain quality parameters such as electrical conductivity must be constantly monitored by considering their relationship with the deterioration of these grains when subjected to long periods of exposure to ozone gas, which can cause a reduction in quality.

The present study provides important information about the toxicity of ozone gas on *C. maculatus* present at different points in cowpea grains, thus simulating real conditions since species belonging to the order Coleoptera can survive at different depths in the grain mass, especially due to their morphology and physiology. Furthermore, ozone gas did not affect the grain quality under these study conditions. The results obtained in this study, together with the information available in the literature, confirm that the use of ozone gas at higher doses can reduce the lethal exposure time of insect pests and, consequently, have a greater control efficiency. It is important to point out that one of the prerequisites for maintaining susceptibility patterns of insect populations to ozone is the adoption of integrated management measures, including the use of the correct dose and application of ozone alternately or interspersed with other insecticides.

5. Conclusions

In the present study, ozone gas proved to be effective in controlling *C. maculatus* in cowpea grains, regardless of the insect's distribution in the mass of cowpea grains. The effectiveness in the control of *C. maculatus* by ozone was influenced by the dose (period of exposure) of the gas. In general, ozone treatment did not affect grain quality. This study is expected to have a significant scientific and practical impact, as it provides an alternative solution for chemical control, which is associated with insect resistance and several environmental and human health risks. Moreover, this study opens up new perspectives for further investigation of the mechanisms underlying the insecticidal activity of ozone and its potential applications in practice. Overall, this study highlights the importance of exploring ecological and sustainable alternatives to mitigate the damage caused by *C. maculatus* in stored cowpea grains.

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