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Insecticidal Effect of Ozone against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Jacquelin Du Val (Coleoptera: Tenebrionidae): Influence of Commodity

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Abstract: Laboratory experiments were carried out in order to assess the insecticidal efficacy of ozone, against three major stored-grain beetle species, the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the confused flour beetle *Tribolium confusum* Jacquelin Du Val (Coleoptera: Tenebrionidae). The insects were exposed for 2, 4, 6 and 8 h, in vials treated with ozone at two dose rates, 115 and 55 ppm. At the highest dose rate, mortality of *R. dominica* and *S. oryzae* adults was close to 60% after 2 h of exposure, while 2 h later, all adults were dead. For *T. confusum* adults, mortality was negligible after 2 h of exposure, but reached 100% at the 6 h exposure interval. For *T. confusum* larvae, approx. 10% of the exposed individuals were dead after 2 h of exposure, while at the 4 h interval reached 70%. In vials treated with the low concentration, mortality was low, especially for *T. confusum* adults, where almost all the exposed individuals survived. The presence of wheat or maize in the vials reduced mortality, for all species tested, but there were no differences in mortality levels between these two commodities. Moreover, for all species, mortality levels varied at different distances from the ozone input point.

Key words: ozone, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium confusum*

Introduction

The range of insecticides that are currently used on stored grain and related commodities is becoming narrower, due to newer regulatory and legislation issues, aiming to reduce risk from pesticide residues on food, occupational exposure risks to workers and adverse effects on the environment. Among these pesticides, methyl bromide, a widely used fumigant for stored-product insect control, has been identified as an ozone depleter by the Montreal Protocol, resulting in its phase out. Phosphine (aluminium phosphide), the main alternative to methyl bromide, it is extremely toxic to mammals, and cannot be used in all types of facilities, since it is highly corrosive to certain mineral materials. On the other hand, other alternative insecticides, such as organophosphorus compounds, are slow acting in comparison with methyl bromide, and many of these substances are toxic to mammals and leave residues on food. Moreover, many stored product insect species are now resistant to phosphine and other substances[3], while several species are now considered as allergens and mycotoxin carriers, which seriously endanger human health[1,5,8]. Loss of fumigants, resistance to remaining fumigants and the consumers' demand for residue and contaminant-free food require the use of new, reduced – risk substances.

Ozone can be generated by electrical discharges in air and is currently used in the medical industry as a disinfectant against microorganisms, as a means of reducing odor, and for removing taste, colour, and environmental pollutants in industrial applications[7,9]. McKenzie et al.[11] noted that ozone treatment reduced the toxic effect of aflatoxin-contaminated maize fed to turkey pullets. Also, at 5 ppm, ozone inhibited surface growth, sporulation, and mycotoxin production by cultures of *Aspergillus flavus* and *Fusarium moniliforme*[10]. One other attractive characteristic of ozone is that it decomposes rapidly (half-life of 20 50 min) to molecular oxygen without leaving a residue. The use of ozone generators eliminates the need for handling, storage, and disposal problems of conventionally used fumigants and other pesticides. These characteristics make ozone an attractive candidate for controlling insects and fungi in stored grain. Erdman[4] observed mortality of

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larvae of the red flour beetle, *Tribolium castaneum* (*Herbst*) (*Coleoptera*: *Tenebrionidae*), *and* the confused flour beetle, *Tribolium confusum* Jacquelin Du Val (Coleoptera: Tenebrionidae), after exposure to 45 ppm of ozone. In the laboratory, 5 ppm of ozone resulted in 100% mortality of adult saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and *T. confusum*[9,10]. In the present study, we tested ozone against three major stored – grain beetle species, in empty containers, and in containers that contained grain.

Materials and Methods

Insects Commodities and Ozone Generator

The species tested were the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *T. confusum*. The first two species were reared in the laboratory on whole wheat kernels, while the third was reared on wheat flour plus brewer's yeast (5% by weight). All species were kept at 25℃ and 65℃. Adults were used in the tests with *S. oryzae* and *R. dominica*, while for *T. confusum*, both adults and larvae were tested. Untreated wheat (var. Mexa) and maize (var. Dias) were used in the tests. The generator type used was Ambiozon, Ser. 2000F (Ambiozon SL, Madrid, Spain). Two dose rates 115 and 55 ppm of ozone were used.

Bioassays and Analysis

The tests were carried out in cylindrical columns, which could be separated in 12 equal segments. These columns were 70 cm high and 8 cm in diameter. Between two adjacent segments, there was a hole of 2.5 cm in diameter, covered with fine mesh, in order to prevent insects from moving among the segments. The ozonation was conducted from a small hole at the bottom of the column, from the first segment (segment 1). In each segment, 30 insects were placed separately for each species or life stage. These individuals were exposed for 2, 4, 6, and 8 h, and after the termination of each interval, mortality was counted. There were three types of bioassays. In the first bioassay, the columns were without grain and contained only insects, while in the other two bioassays the columns contained maize or wheat, respectively. The entire procedure was repeated three times., For the data analysis, the GLM-proc or SAS[13] was used separately for each species/stage. Insect mortality was the response variable and segment, exposure, dose and column containment were the treatment variables.. Means were separated by the Tukey-Kramer HSD test.

Results

Mortality of *R. dominica* Adults

From the main effects, only dose and exposure were significant, while interactions were not significant (Table 1). In the empty columns, at the high dose, after 2 d of exposure, mortality was similar in all segments, and ranged between 60 and 79% (Table 2). After 4 h of exposure, mortality was high (>93%), while all adults were dead at the 6 – h exposure interval. At the same dose, in the columns that contained maize or wheat, mortality was notably reduced, especially in the last segments. Hence, after 2 h of exposure, more than two thirds or half of the exposed individuals were still alive in segment 1, for maize and wheat, respectively, while mortality was negligible in the last segment (segment 12). At longer exposure intervals, mortality was increased, and reached 100%, but reduced mortality levels were also noted in the last segments. At the low dose, mortality was notably lower in comparison with the respective figures for the high dose, and did not exceed 65%. Nevertheless, mortality was also reduced when the column contained the two types of grains.

Mortality of *S. oryzae* Adults

For *S. oryzae* adults, only dose was significant (Table 1). The mortality levels noted were similar to those recorded for *R. dominica* adults (Table 3). At the high ozone concentration, after 2 h of exposure in the empty columns, mortality ranged between 63 to 74%, and was similar in all segments. All adults were dead after 6 d of exposure. At the same dose, in columns that contained grains, mortality was reduced after short exposures (2 – 4 h), especially in segments 6 – 12. After 8 h of exposure, survival occurred in segments 10 – 12 and 9 – 12, for maize and wheat, respectively. At the low ozone concentration, mortality was generally lower than the respective figures of *R. dominica*, especially in the case of the two types of grains, where all adults survived in most of the segments examined.

Mortality of *T. confusum* Larvae

All main effects, regardless of the segment, were significant (Table 1). Moreover, dose x exposure and treatment x dose were also significant. In the empty columns, at the high dose and after 2 d of exposure, mortality was lower in comparison with the previous species. Mortality was similar in all segments (Table 4). All larvae were dead after 6 d of exposure. On the other hand, at the 2 h exposure interval, mortality was negligible in the columns that contained grains, and did not exceed 65% at the 6 h interval. At the 8 h exposure, with the exception of segments 1 – 3 with maize, survival occurred in all segments examined. At the low ozone concentration, for all three treatments, all adults survived after 4 h of exposure. At the 8 h exposure, mortality did not exceed 33, 10 and 14%, in empty columns, columns with maize and columns with wheat, respectively.

Mortality of *T. confusum* Adults

As in the case of larvae, treatment, dose, dose x exposure and treatment x dose were significant (Table 1). Mortality was generally lower in comparison with *T. confusum* larvae (Table 5). At the high concentration, after 2 h in empty columns, mortality was extremely low, but 2 h later ranged between 60% – 78%. All adults were dead at the 8 h exposure. In columns that contained grains, at the 8h exposure, with the exception of segments 1 – 3 in maize, survival occurred in all segments examined, while it was only 30 and 21% in segment 12, for maize and wheat, respectively. At the low concentration, practically, no mortality was recorded.

Discussion

The present study indicates that the efficacy of ozone is greatly affected by the target species, life stage, exposure interval, dose rate and the presence of commodity. Furthermore, we examined relatively shorter exposure intervals in comparison with the majority of the previous available studies. For instance, Kells et al. [6] reported 92% – 100% mortality at 50 ppm after 3 d of exposure, for adults of the maize weevil, *Sitophilus zeamais* (Motsch.), adults of *T. castaneum* and larvae of the Indian meal moth, *Plodia interpunctella* (H bner) (Lepidoptera: Pyralidae). In that study, the authors tested ozone in maize, and noted that ozone fumigation had two phases: rapid degradation and slow movement in the grain mass, and reduced degradation with free movement. The latter phase is considered as a direct result of saturation[6]. In our study, regardless of the species tested, the presence of grain caused a significant reduction of mortality, suggesting that the rate of degradation was high, despite the fact that ozone flow was continuous. On the other hand, in the empty column, mortality was high, especially in the case of *R. dominica* and *S. oryzae*. Also, the presence of fine material may also negatively affect ozone efficacy[6,12]. Based on the present results, at least in some of the combinations tested, 115 pm of ozone can be effective even after 8 h, and in some cases, even after 6 h of exposure. In contrast, the reduction of concentration to 55 ppm was not effective, indicating that longer exposures are needed at this ozone level.

Apart from mortality in the entire column, ozone efficacy was notably varied among different column parts. Thus, the efficacy of ozone was reduced with the increase of distance from the ozone introduction point. This trend was expressed more vigorously in the case of maize and wheat, while there was little or no effect in the empty column. Generator performance is affected by several factors, and, at least in the present study, any fluctuations were not ’corrected’’ by the continuous ozone flow. Ozone concentration is decreased with depth[6]; therefore the introduction of ozone from the lowest part of a grain mass may cause delayed effectiveness of ozone. However, most insect populations exist at the top layer of bulked grains[2], and for this reason, the introduction of ozone from the top may be advantageous. Nevertheless, populations can often be established in lowest layers[14]. From a practical standpoint, the use of fans or other air circulation techniques can assist ozone penetration[12]. Generally, ozone mortality was similar for the two types of grains, suggesting that the mechanisms that reduce ozone efficacy are similar for both maize and wheat. This is in accordance with previous reports by Kells et al. [6,] and Mendez et al. [12], where penetration was similar for both maize and wheat, despite the fact that the porosity of these grains is different.

From the species tested, adults of *R. dominica* and *S. oryzae* was much more susceptible than adults and larvae of *T. confusum*. Also, the rate of reduction in mortality level of *T. confusum* larvae was increased from segment 1 to segment 12 in comparison with *R. dominica* and *S. oryzae*. The latter two species are characterized as primary pests, which mean that they are able to damage sound grain kernels, and allow the secondary pests, such as *T. confusum* to continue the infestation. For this reason, the increased susceptibility of *R. dominica* and *S. oryzae* to ozone can be considered as an important characteristic, since fast mortality reduces the concomitant damage by the secondary colonizers. However, the immature stages of these species develop inside the grain kernel, and, given that ozone is not as penetrating as other gases, such as phosphine, it is expected that these life stages will remain unaffected. Maier et al.[9] noted that a carefully designed ozonation system could be compatible with other fumigation techniques, such as phosphine, at the same exposure interval. This system should provide proper penetration, and continuous standardized flow to adjust variations in gas concentration. The results of the present work show that ozone, at increased concentrations, is effective at shorter exposures, especially in empty areas, but also into the grain mass. This characteristic, in combination with the other advantages of ozone, such its fungicidal effect and the fact that ozonation does not affect the final product[9], makes ozone a good candidate for further evaluation. For its wider use, additional experimental work is required to establish the feasibility of ozonation, in conjunction with cost considerations.

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Table 1. ANOVA parameters for main effects and interactions for the species tested (total df = 863)

		R. dominica		S. oryzae		T. confusum (larvae)		T. confusum (adults)	
Source	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	2	0.43	0.6464	0.22	0.8025	11.58	<0.0001	7.15	0.0009
Dose	1	118.47	<0.0001	130.56	<0.0001	84.30	<0.0001	77.84	<0.0001
Treatment x dose	2	0.62	0.5372	0.17	0.8393	11.27	<0.0001	5.85	0.0030
Segment	11	0.22	0.9960	0.18	0.9983	0.39	0.9581	0.40	0.9553
Treatment x segment	22	0.03	0.9999	0.03	0.9999	0.07	0.9999	0.05	0.9999
Dose x segment	11	0.,05	0.9999	0.09	0.9999	0.16	0.9991	0.24	0.9937
Exposure	3	3.86	0.0093	1.69	0.1667	8.04	<0.0001	12.69	<0.0001
Treatment x exposure	6	0.19	0.9773	0.01	0.9999	0.19	0.9769	0.95	0.4567
Dose x exposure	3	2.53	0.0562	1.27	0.2812	5.98	0.0005	11.42	<0.0001
Segment x exposure	33	0.01	0.9999	0.01	0.9999	0.08	0.9999	0.06	0.9999

Table 2. Mean mortality (% ± SE) of *R. dominica* adults after exposure to the ozone – treated column (within each row, for each column containment and dose, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05)

Segment (1 input, 12 output)	Empty column (115 ppm)				Maize (115 ppm)				Wheat (115 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	63.2 ±7.3a	100 ±0.0b	100 ±0.0b	100 ±0.0b	66.6 ±7.3a	100 ±0.0b	100 ±0.0b	100 ±0.0b	50.4 ±8.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b
2	73.4 ±3.4a	100 ±0.0b	100 ±0.0b	100 ±0.0b	62.2 ±4.3a	100 ±0.0b	100 ±0.0b	100 ±0.0b	50.9 ±7.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b
3	72.3 ±2.6	96.4 ±2.1b	100 ±0.0b	100 ±0.0b	60.0 ±5.4a	97.4 ±1.2b	100 ±0.0b	100 ±0.0b	42.7 ±10.4a	98.4 ±1.1b	100 ±0.0b	100 ±0.0b
4	79.0 ±4.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b	47.9 ±7.8a	94.3 ±3.9b	100 ±0.0b	100 ±0.0b	30.2 ±5.4a	90.3 ±3.9b	100 ±0.0c	100 ±0.0c
5	62.5 ±7.4a	96.5 ±2.3b	100 ±0.0b	100 ±0.0b	53.4 ±6.5a	90.4 ±8.1b	100 ±0.0b	100 ±0.0b	26.3 ±7.8a	90.2 ±7.1b	100 ±0.0c	100 ±0.0c
6	71.3 ±5.3a	97.4 ±2.4b	100 ±0.0b	100 ±0.0b	50.4 ±4.8a	95.6 ±3.2b	100 ±0.0b	100 ±0.0b	21.5 ±9.5a	87.4 ±5.9b	100 ±0.0c	100 ±0.0c

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
7	60.4 ±12.6a	95.4 ±3.0b	100 ±0.0b	100 ±0.0b	46.2 ±8.3a	80.0 ±5.9b	100 ±0.0c	100 ±0.0c	24.5 ±7.5a	70.4 ±5.6b	100 ±0.0c	100 ±0.0c
8	65.4 ±3.2a	96.5 ±2.1b	100 ±0.0b	100 ±0.0b	36.4 ±5.4a	77.6 ±7.9b	100 ±0.0c	100 ±0.0c	20.3 ±5.8a	73.7 ±7.9b	100 ±0.0c	100 ±0.0c
9	63.5 ±7.9a	96.5 ±2.0b	100 ±0.0b	100 ±0.0b	36.5 ±4.5a	80.9 ±10.4b	100 ±0.0c	100 ±0.0c	13.4 ±4.4a	63.9 ±5.9b	96.7 ±2.2c	100 ±0.0c
10	70.3 ±4.0a	100 ±0.0b	100 ±0.0b	100 ±0.0b	20.3 ±6.5a	72.5 ±8.9b	97.4 ±2.1c	100 ±0.0c	6.8 ±3.0a	56.9 ±9.9b	93.0 ±4.3c	100 ±0.0c
11	63.6 ±4.4a	93.5 ±3.9b	100 ±0.0b	100 ±0.0b	10.9 ±4.3a	47.9 ±8.5b	84.3 ±10.9c	90.4 ±5.5c	3.5 ±2.1a	41.4 ±8.9b	90.2 ±5.1c	92.6 ±5.2c
12	62.8 ±6.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b	3.3 ±2.3a	36.0 ±8.0b	68.4 ±8.3c	83.3 ±6.4c	0.0 ±0.0a	40.3 ±9.0b	89.3 ±9.0c	100 ±0.0c
Segment (1 input, 12 output)	Empty column(55 ppm)				Maize(55 ppm)				Wheat (55 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	0.0 ±0.0a	16.9 ±5.8b	30.5 ±5.4b	53.4 ±7.8c	0.0 ±0.0a	6.5 ±4.3b	15.7 ±6.7bc	30.4 ±8.9c	0.0 ±0.0a	3.5 ±2.1ab	13.5 ±5.4bc	23.4 ±7.5c
2	0.0 ±0.0a	13.9 ±4.3b	40.5 ±5.5c	60.0 ±8.5d	0.0 ±0.0a	3.4 ±2.3ab	12.4 ±4.4bc	25.5 ±5.9c	0.0 ±0.0a	3.6 ±2.5ab	13.4 ±6.5bc	23.3 ±6.5c
3	0.0 ±0.0a	23.3 ±5.4b	40.0 ±6.4c	65.0 ±8.4d	0.0 ±0.0a	3.5 ±1.4ab	10.6 ±5.6bc	22.3 ±6.5c	0.0 ±0.0a	0.0 ±0.0a	6.5 ±2.3b	14.6 ±7.0b
4	0.0 ±0.0a	10.3 ±5.4b	36.9 ±8.4c	60.4 ±10.5d	0.0 ±0.0a	3.5 ±1.3ab	11.5 ±7.6bc	20.3 ±4.5c	0.0 ±0.0a	0.0 ±0.0a	6.5 ±2.5b	13.4 ±5.6b
5	0.0 ±0.0a	10.3 ±4.6b	35.4 ±7.4c	63.0 ±4.5d	0.0 ±0.0a	3.0 ±2.1ab	6.7 ±2.6b	16.9 ±4.4c	0.0 ±0.0a	0.0 ±0.0a	3.7 ±1.4ab	10.3 ±5.1b
6	0.0 ±0.0a	12.1 ±4.7b	36.6 ±8.4c	62.4 ±8.4d	0.0 ±0.0a	0.0 ±0.0a	3.0 ±2.0ab	10.3 ±5.1b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	5.5 ±3.2
7	0.0 ±0.0a	16.4 ±6.5b	37.5 ±6.5c	60.3 ±5.5d	0.0 ±0.0a	0.0 ±0.0a	3.3 ±1.7a	13.5 ±4.5b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	2.5 ±2.0
8	0.0 ±0.0a	12.3 ±6.5b	23.0 ±7.0b	40.5 ±7.4c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
9	0.0 ±0.0a	13.9 ±7.5b	30.3 ±8.9b	56.5 ±9.0c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0a	16.5 ±7.4b	40.3 ±6.8c	65.1 ±7.8d	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
11	0.0 ±0.0a	20.2 ±6.4b	43.4 ±4.3c	70.3 ±9.6d	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
12	0.0 ±0.0a	16.4 ±5.4b	36.5 ±7.0c	60.4 ±9.0d	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Table 3. Mean mortality (% ± SE) of S. *oryzae* adults after exposure to the ozone – treated column(within each row, for each column containment and dose, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05)

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	71.3 ±9.0a	100 ±0.0b	100 ±0.0b	100 ±0.0b	73.6 ±5.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	76.5 ±9.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b
2	64.3 ±5.4a	95.6 ±3.5b	100 ±0.0b	100 ±0.0b	69.6 ±8.7a	100 ±0.0b	100 ±0.0b	100 ±0.0b	73.5 ±8.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b
3	73.4 ±8.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b	70.5 ±7.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	62.4 ±5.4a	100 ±0.0b	100 ±0.0b	100 ±0.0b
4	63.4 ±7.4a	96.4 ±3.2b	100 ±0.0b	100 ±0.0b	63.2 ±8.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	63.2 ±5.4a	96.8 ±2.1b	100 ±0.0b	100 ±0.0b
5	63.3 ±7.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b	69.6 ±7.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	63.9 ±6.5a	95.4 ±2.3b	100 ±0.0b	100 ±0.0b
6	56.4 ±4.5a	95.5 ±2.3b	100 ±0.0b	100 ±0.0b	53.4 ±10.0a	100 ±0.0b	100 ±0.0b	100 ±0.0b	60.4 ±7.9a	90.4 ±5.4b	100 ±0.0c	100 ±0.0c
7	65.4 ±7.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b	49.5 ±5.4a	93.4 ±5.5b	97.6 ±1.2b	100 ±0.0b	60.9 ±10.5a	86.5 ±6.8b	100 ±0.0c	100 ±0.0c
8	74.6 ±11.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	44.4 ±6.5a	90.3 ±5.4bc	97.6 ±1.2cd	100 ±0.0d	56.5 ±8.9a	83.4 ±7.0b	92.4 ±6.3bc	100 ±0.0c
9	74.3 ±8.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b	38.7 ±7.5a	90.0 ±5.7bc	93.4 ±4.0cd	100 ±0.0d	55.5 ±7.5a	80.5 ±10.5b	91.5 ±3.4b	94.6 ±2.9b
10	63.5 ±8.5a	96.5 ±3.0b	100 ±0.0b	100 ±0.0b	25.4 ±8.5a	60.5 ±8.9b	73.5 ±5.4b	88.7 ±6.5c	53.4 ±10.4a	70.5 ±6.5ab	88.4 ±7.4bc	90.4 ±4.6c
11	63.4 ±8.9a	96.6 ±2.2b	100 ±0.0b	100 ±0.0b	30.4 ±7.6a	60.9 ±5.6b	69.8 ±9.3b	75.4 ±7.5b	50.4 ±8.9a	69.8 ±12.5ab	75.4 ±6.8b	82.2 ±5.7b
12	73.2 ±11.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b	20.3 ±5.9a	50.0 ±6.5b	65.6 ±5.2c	73.4 ±6.5c	49.5 ±9.5a	64.5 ±5.4ab	73.4 ±6.7b	77.7 ±8.0b
Segment (1 input, 12 output)	Empty column(55 ppm)				Maize(55 ppm)				Wheat (55 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	0.0 ±0.0a	3.4 ±2.1ab	3.4 ±2.3ab	8.7 ±4.3b	2.1 ±1.3a	5.4 ±3.2ab	13.4 ±5.4bc	23.4 ±7.9c	0.0 ±0.0a	5.4 ±3.5ab	12.4 ±3.5bc	22.7 ±7.0c
2	0.0 ±0.0a	6.5 ±3.1b	13.4 ±4.5bc	20.0 ±5.3c	0.0 ±0.0a	3.2 ±2.0a	5.4 ±3.7ab	9.5 ±4.3b	0.0 ±0.0a	7.5 ±3.5b	8.5 ±4.3b	15.4 ±5.6b

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
3	0.0 ±0.0a	3.5 ±2.1a	16.5 ±5.5b	32.2 ±5.9c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.5 ±2.4	0.0 ±0.0a	0.0 ±0.0a	2.5 ±1.5a	12.0 ±5.1b
4	0.0 ±0.0a	10.5 ±5.1b	13.0 ±5.9b	22.5 ±4.8b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.4 ±1.4
5	0.0 ±0.0a	6.9 ±3.4b	17.4 ±5.4bc	30.0 ±8.5c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
6	0.0 ±0.0a	6.0 ±3.1b	10.5 ±4.3bc	25.8 ±8.5c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
7	0.0 ±0.0a	6.7. ±2.8b	12.3 ±5.1bc	22.1 ±5.9c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
8	0.0 ±0.0a	4.8 ±3.1b	17.9 ±7.8c	25.4 ±6.5c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
9	0.0 ±0.0a	9.5 ±5.4b	19.7 ±4.8bc	32.6 ±12.4c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0a	5.4 ±2.5b	13.4 ±5.4bc	37.7 ±5.8c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
11	0.0 ±0.0a	6.1 ±3.2b	18.5 ±5.9c	29.8 ±7.5c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
12	0.0 ±0.0a	3.0 ±1.4a	15.4 ±5.7b	24.6 ±6.8b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Table 4. Mean mortality(% ± SE) of *T. confusum* larvae after exposure to the ozone – treated column(within each row, for each column containment and dose, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05)

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	42.5 ±5.9a	70.9 ±5.4b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	30.4 ±5.7b	60.4 ±12.5c	100 ±0.0d	0.0 ±0.0a	25.4 ±6.5b	57.4 ±8.5c	98.9 ±0.8d
2	52.9 ±8.0a	77.4 ±8.5b	100 ±0.0c	100 ±0.0c	5.4 ±3.2a	32.5 ±7.0b	64.6 ±10.6c	100 ±0.0d	0.0 ±0.0a	27.8 ±6.5b	59.5 ±7.5c	90.4 ±7.4d
3	44.5 ±6.5a	70.3 ±5.4b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	25.7 ±7.9b	55.5 ±7.9c	100 ±0.0d	0.0 ±0.0a	22.5 ±9.0b	53.3 ±9.1c	85.4 ±5.9d
4	55.4 ±6.8a	78.9 ±5.4b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	25.8 ±8.6b	58.6 ±6.9c	92.1 ±5.4d	3.2 ±2.1a	22.4 ±6.5b	50.2 ±5.4c	83.2 ±6.0d
5	54.5 ±6.8a	88.0 ±5.7b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	20.5 ±6.0b	48.0 ±8.4c	84.5 ±5.4d	3.0 ±1.7a	23.5 ±7.4b	44.3 ±5.1c	78.5 ±7.0d

Segment (1 input, 12 output)	Empty column (115 ppm)				Maize (115 ppm)				Wheat (115 ppm)			
6	50.6 ±8.9a	89.0 ±6.0b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	23.2 ±5.6b	44.4 ±7.6c	80.8 ±5.9d	0.0 ±0.0a	19.5 ±6.9b	40.9 ±5.4c	65.4 ±8.5d
7	49.7 ±7.7a	87.5 ±7.8b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	18.3 ±6.3b	33.8 ±7.5b	63.5 ±5.0c	0.0 ±0.0a	17.5 ±8.4b	33.4 ±5.1b	62.5 ±5.4c
8	44.5 ±6.8a	79.6 ±6.6b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	10.7 ±5.4b	25.4 ±5.4c	63.0 ±10.3d	0.0 ±0.0a	15.4 ±7.3b	30.9 ±5.4b	52.5 ±7.8c
9	42.6 ±7.6a	81.4 ±8.7b	100 ±0.0c	100 ±0.0c	2.4 ±1.5a	18.5 ±5.8b	30.4 ±7.4b	62.8 ±7.4c	0.0 ±0.0a	12.5 ±4.6b	27.5 ±5.4c	41.5 ±8.2c
10	44.6 ±7.6a	78.7 ±4.5b	100 ±0.0c	100 ±0.0c	2.1 ±1.2a	12.5 ±5.1b	25.4 ±6.5b	53.0 ±8.9c	0.0 ±0.0a	6.2 ±3.2b	19.5 ±5.4c	34.4 ±4.5d
11	52.5 ±8.0a	72.5 ±8.0b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	11.3 ±3.4b	15.4 ±5.7b	34.1 ±6.5c	0.0 ±0.0a	5.4 ±3.9b	16.1 ±6.7bc	30.4 ±7.5c
12	50.6 ±7.4a	74.6 ±5.7b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	6.5 ±2.9b	11.4 ±3.9b	31.1 ±9.4c	0.0 ±0.0a	2.3 ±1.4a	5.5 ±3.9a	20.0 ±5.9b
Segment (1 input, 12 output)	Empty column (55 ppm)				Maize (55 ppm)				Wheat (55 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	0.0 ±0.0a	0.0 ±0.0a	2.3 ±1.5a	32.4 ±6.5b	0.0 ±0.0a	0.0 ±0.0a	2.3 ±1.3ab	9.5 ±5.4b	0.0 ±0.0a	0.0 ±0.0a	12.1 ±3.4b	12.5 ±4.5b
2	0.0 ±0.0a	0.0 ±0.0a	5.0 ±2.3a	29.5 ±5.4b	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a	8.5 ±3.2b	0.0 ±0.0a	0.0 ±0.0a	8.9 ±3.4b	11.2 ±3.2b
3	0.0 ±0.0a	0.0 ±0.0a	7.5 ±4.7b	32.5 ±5.4c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	4.3 ±2.3	0.0 ±0.0a	0.0 ±0.0a	7.7 ±3.4b	13.3 ±3.5b
4	0.0 ±0.0a	0.0 ±0.0a	3.5 ±2.3a	29.4 ±4.3b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.2 ±2.3	0.0 ±0.0a	0.0 ±0.0a	5.4 ±2.7ab	13.3 ±3.5b
5	0.0 ±0.0a	0.0 ±0.0a	3.4 ±2.3a	32.4 ±5.4b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0a	0.0 ±0.0a	9.0 ±3.6b	9.8 ±4.4b
6	0.0 ±0.0a	0.0 ±0.0a	10.1 ±4.6b	20.4 ±5.4b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.1 ±2.0
7	0.0 ±0.0a	0.0 ±0.0a	9.0 ±5.4b	22.4 ±8.6b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
8	0.0 ±0.0a	0.0 ±0.0a	7.4 ±4.3b	21.0 ±9.0b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
9	0.0 ±0.0a	0.0 ±0.0a	9.0 ±4.5b	18.9 ±8.1b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0a	0.0 ±0.0a	5.4 ±2.9a	23.3 ±6.7b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
11	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a	20.2 ±5.8b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
12	0.0 ±0.0a	0.0 ±0.0a	1.5 ±0.9a	22.4 ±6.5b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Table 5. Mean mortality(% ± SE) of *T. confusum* adults after exposure to the ozone – treated column (within each row, for each column containment and dose, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05)

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	2.5 ±1.4a	77.7 ±6.5b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	32.6 ±6.5b	64.5 ±7.5c	100 ±0.0d	0.0 ±0.0a	27.8 ±5.6b	56.4 ±5.6c	97.8 ±0.9d
2	3.2 ±1.5a	72.5 ±8.9b	100 ±0.0c	100 ±0.0c	5.5 ±2.3a	34.5 ±6.5b	61.6 ±4.9c	100 ±0.0d	0.0 ±0.0a	25.6 ±7.1b	54.5 ±4.3c	92.5 ±3.6d
3	0.0 ±0.0a	74.6 ±6.3b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	27.5 ±8.4b	52.3 ±5.8c	100 ±0.0d	0.0 ±0.0a	22.5 ±7.4b	52.1 ±6.5c	86.6 ±6.7d
4	0.0 ±0.0a	74.3 ±8.0b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	25.4 ±6.9b	54.4 ±8.4c	91.5 ±6.0d	3.2 ±2.0a	24.6 ±8.1b	49.0 ±6.5c	80.4 ±7.5d
5	3.2 ±1.5a	69.5 ±6.5b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	22.2 ±5.4b	44.4 ±7.0c	84.6 ±5.4d	3.3 ±2.3a	22.5 ±5.4b	47.7 ±7.6c	76.9 ±11.6d
6	0.0 ±0.0a	62.5 ±10.3b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	21.5 ±4.0b	42.5 ±6.5c	80.5 ±8.7d	0.0 ±0.0a	22.5 ±6.9b	42.5 ±6.3c	66.7 ±5.9d
7	0.0 ±0.0a	62.5 ±7.5b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	11.5 ±5.4b	32.0 ±6.5c	65.4 ±9.0d	0.0 ±0.0a	17.9 ±5.4b	36.6 ±6.3c	60.4 ±9.5d
8	0.0 ±0.0a	72.5 ±8.9b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	11.1 ±3.5b	22.5 ±6.5b	63.5 ±6.5c	0.0 ±0.0a	12.5 ±4.1b	30.3 ±7.3c	53.1 ±6.0d
9	0.0 ±0.0a	73.3 ±7.8b	100 ±0.0c	100 ±0.0c	4.4 ±1.3a	17.6 ±6.7b	32.5 ±6.9b	65.0 ±7.0c	0.0 ±0.0a	12.3 ±4.3b	25.4 ±7.1b	45.4 ±6.4c
10	0.0 ±0.0a	62.0 ±6.5b	93.3 ±4.3c	100 ±0.0c	0.0 ±0.0a	7.5 ±3.3b	19.6 ±7.8b	55.5 ±10.3c	0.0 ±0.0a	7.5 ±3.4b	15.4 ±6.5bc	32.3 ±8.5c
11	0.0 ±0.0a	63.5 ±6.7b	97.1 ±1.5c	100 ±0.0c	0.0 ±0.0a	8.0 ±3.1b	15.4 ±6.1b	32.3 ±4.0c	0.0 ±0.0a	4.6 ±2.3ab	13.4 ±4.3bc	30.5 ±7.8c
12	0.0 ±0.0a	59.9 ±10.2b	93.2 ±3.5c	100 ±0.0c	0.0 ±0.0a	5.4 ±2.7ab	13.3 ±5.4bc	30.2 ±10.3c	0.0 ±0.0a	3.5 ±1.5a	6.7 ±2.3a	20.9 ±4.7b
Segment (1 input, 12 output)	Empty column(55 ppm)				Maize(55 ppm)				Wheat (55 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	0.0 ±0.0	0.0 ±0.0	3.2 ±1.7	3.2 ±1.7	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.2 ±1.5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.7 ±2.1
2	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.9 ±2.1	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	2.4 ±1.2	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.1 ±1.4

Segment (1 input, 12 output)	Empty column (115 ppm)				Maize (115 ppm)				Wheat (115 ppm)			
3	0.0 ±0.0a	0.0 ±0.0a	3.5 ±2.4ab	6.7 ±3.3b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
4	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
6	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
7	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
8	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
9	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
11	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
12	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0