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Use of Ozone Gas as a Green Control Alternative to Beetles *Alphitobius diaperinus* (Panzer)Infestation in Aviary Bed Utilized in the Poultry Industry

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The control of poultry farming beetles (*Alphitobius diaperinus* Panzer – Coleoptera, Tenebrionidae) infestation around the world is based exclusively on Pyrethroid Group (cypermethrin) insecticide application (during the 45 days of poultry breeding). Some studies report beetles population's resistance, reinforcing the need of alternative methods for beetles control. Ozone (O_3) gas is considered a GRAS (generally recognized as safe) gas. The aim of this study was to determine the efficacy of O_3 gas treatment to eliminate beetles *A. diaperinus* development both, at adult and larvae stages. The insects were treated with three O_3 concentrations (30/40/60ppm) and exposure times (48, 36 and 24 h). All treatments were effective against its larvae stage. However, the most efficient (100%) treatment for adult beetles elimination was at 40 ppm, during 36 h of exposure.There is a need for future research on O_3 application in order to reduce the pesticides application/exposure, especially from the Pyrethroid Group, wildly spread for pest control in the poultry environments (roofs, floor, screens and/or curtains.

1. Introduction

Insect infestation in aviary beds such as by *Alphitobius diaperinus* Panzer (Coleoptera, Tenebrionidae), commonly called darkling beetle (considered the main poultry farming pest worldwide), is responsible for large losses in poultry farming. They concentrate mainly close to the drinkers/feeders and inside the shed´s wooden materials structures (Skov et al., 2004) Their presence (larvae stage) is detected inclusive in the poultry houses/sheds compacted soil and can reach 80 cm depth (Chernaki-Leffer et al. 2001). Apart from insects, also fungi (*Aspergillus, Penicillium*) and bacteria (*Campylobacter*, *Salmonella*), among others living organisms, have been reported able to contaminate aviary beds and chicks/chicken (Hazeleger et al., 2008; Singh et al. 2010; Soares et al. 2017b). When there is shortage of food during chicken growth, they begin to eat those insects (other living organisms contaminated – fungi, bacteria) leading to diseases development. Apart from that habit, which alters chicken feed conversion, it causes diarrhea, stress and reduces body weight (Matias, 1992; Despins and Axtell 1995; Skov et al., 2004).
Other *Alphitobius* species such as *A. laevigatus* (Fabricius), *A. stephens* and *A. piceus* (Oliver) have also been isolated, however, in stored grains and flours (Hagstrum, 2017). Those living organisms, find in the poultry house facilities, the proper nutrients that serve as good substrates for their development (Soares et al., 2017a). The beetles presence means chicken health problems, performance alterations and serious financial losses (Chernaki-Leffer et al. 2013; Oviedo-Rondon, 2008). Their control depends mainly on pesticide applications of the Pyrethroid Group (Marangi et al, 2012).

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Therefore, the poultry production sectors, have adopted Insects' Control procedures by applying mainly Pyrethroids Group insecticides. That application includes the poultry sheds environment (roofs, floor, screens and/or curtains) and the aviary beds (each 45 days of whole chicken growth cycle, or intermittent) to control undesirable insects mainly the *A. diaperinus* (Hays and Laws, 1991; Benabdeljelil and Ayachi, 1996).
Ozone (O_3) is an oxidizing gas that is highly toxic to many organisms including fungi, bacteria and viruses (Savi et al., 2014; Dubois et al., 2006). Its stability depends on factors such as pH and temperature.Its O_3half-life under atmospheric conditions is about 30 min and has its effect reduced with higher temperatures and lower pressures (Kim, 2000 and Sharma et al. 2004). According to Okpala (2017) there is a need to encourage further research using O_3 since is considered a green method (generally recognized as safe - GRAS). Regarding insects, some works have reported O_3 application for em *Tribolium castaneum*, *Sitophilus zeamais* , *Rhyzopertha dominica* and *Necrobia rufipes* (Rozado et al 2008; Subramanyam et al 2017; Hasan et al 2016).
The application of pesticides from the Pyrethroids Group for the *A. diaperinus* beetles control in poultry environment exposes the workers. It occurs directly (during handling - atomizer, spray or haul) at occupational activities by incorrect use (overdosis) and indirectly (by contact - dermal, eyes, ingestion and/or through - inhalation) or foods contamination (residues) (Spinosa, 2010).
As pest control in poultry farming is currently dependant on the pesticides utilization, there is a lack of information on green methods application to reduce contamination. Therefore the present study aimed to investigate the efficacy of O_3 gas treatments to eliminate *A. diaperinus* (Panzer) beetles (at adult and larvae stages).

2. Experiment

2.1 Material

(a) Sample: insects (n=280) of *A. diaperinus* species (adults and larvae stages: 0.7 and 10.0 mm of mean length, respectively) extracted from aviary bed at the 45th day of chicken breeding (no insecticide application).
(b) Equipment: tweezers, Prolab (Sao Paulo, SP, Brazil); sieve system, 9-16 mesh (2.00- 1.00 mm/µm apert., 10-18 USM/ASTM),Beffer (Caieiras,SP, Brazil);vaccum pump, TE-58, Tecnal (São Paulo,SP,Brazil); O_3 gas generator, OP-35-5L, Interozone (Jundiaí, SP, Brazil), thermohigrometer, J-prolab (São José dos Pinhais, PR,Brazil). The O_3 chambers (50 and 70 mm for length and diameter, respectively) were made of glass with two apertures: one for the O_3 gas input (10 mm tube/0.3-inch tube) and other for the O_2 exit (a small output - 5 mm) (Figure 1).

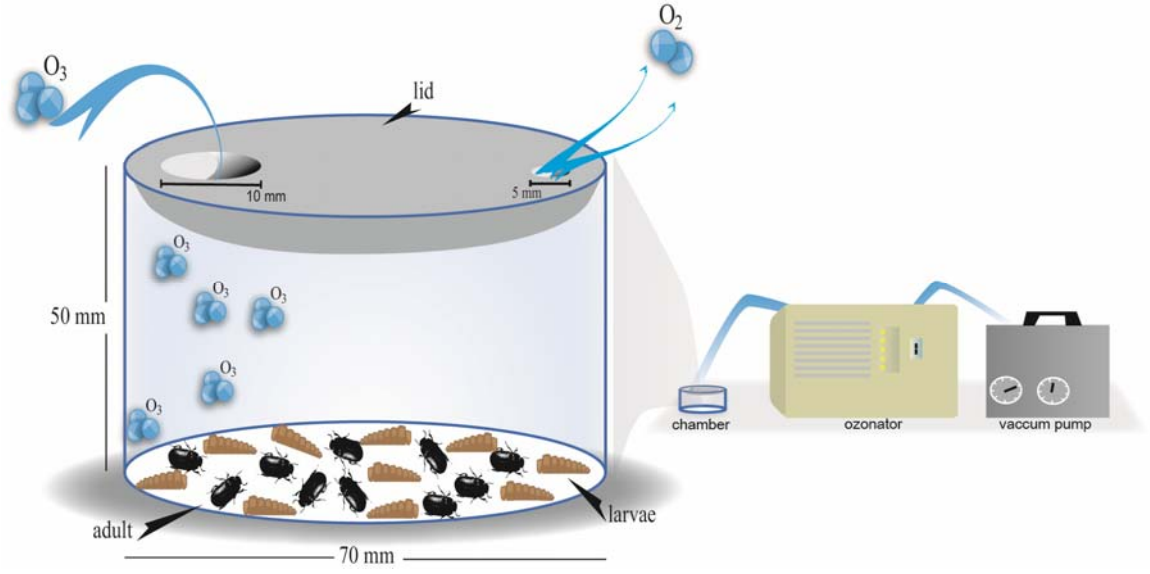


Figure 1: Pilot chamber scheme built for the in vitro ozone gas experiment for A. diaperinus (Panzer) inactivation at two development stages (larvae and adult).

2.2 Methods

1. (a) Sample collection and preparation:(a.1) collection –beetles samples (larvae and adult stages) were collected in the aviary bed, located at latitude -27º90'15" South and longitude -48º92'76" West, representatively from the floor of the shed (layer 10 cm in depth) after the birds transfer (45 days). Collection points: 4 points (n = 20 / each point) of the total area of the shed - the insect were maintained in aviary bed and chicken feed residues and sent to the laboratory at room temperature (25ºC); (a.2) preparation – groups of insects (n=10) were loaded in the glass chambers under constant conditions (23±2ºC and 77± 5% relative humidity -RH) and kept for the O_3 gas application.
(b) Ozone application: after the beetles (both stages) were loaded into the O_3 chambers, the generated O_3 gas was pumped (through the inlet entrance) into the vessels by a compressor (equipped with a filter to prevent the entry of moisture), at continuous flow rate (3 L min^{-1}). Three Groups for the different gas concentration treatments were prepared (30, 40 and 60 ppm: TI, TII, TIII) in duplicate (n=2). A group of beetles sample (both stages) was lodged in identical chamber to estimate their natural mortality (Control Group). O_3 gas insects mortality confirmation **-** insects death confirmation per O_3 gas concentration and exposure were registered. In order confirm their death, insects were transferred to petri dishes containing aviary bed for 5 hours.

3. Results and Discussion

The effect of O_3 gas as a clean inactivation strategy for beetle proliferation (genus *Alphitobius*) presented variations among the conditions applied (concentration x exposure time) and so for the beetle development stages. Table 1 shows data obtained for larvae and adult behaviour.

Table 1: Percentage of Alphitobius diaperinus (Panzer) *insect mortality (larvae e adult stages) after ozone gas application at different concentrations and exposure times*

Beetle development[a]	Ozone gas treatments		Insect death
	Concentration (ppm)	Exposure time (h)	Number[b] (mean[c] %)
LARVAE 	30	24	20 (100)
	40	36	20 (100)
	60	48	20 (100)
	30	48	20 (100)
	40	24	20 (100)
	60	36	20 (100)
ADULTS 	30	48	20 (100)
	40	36	20 (100)
	60	24	16 (80)
	30	36	4 (20)
	40	24	8 (40)
	60	48	20 (100)
	30	24	0 (0)
	40	48	20 (100)
	60	36	20 (100)

[a] *Alphitobius diaperinus* (Panzer) [b] total insects:10 [c] *n*=2

3.1 Effect of O_3 gas on treatments with *A. diaperinus* (larvae and adults stages)

Larva estage - the larvae were more susceptible to O_3 than the adult beetles, with their death registered since the lowest concentration and time applied (30 ppm/24 h). During all treatment, the larvae began ecdise for continuity of development, and not for pupa formation. Thus, all O_3 treatments in which the larvae were submitted proved to be effective on eliminating them (100%) under the conditions of temperature and RH applied in the current study (23±2ºC and 77± 5%). Using high concentrations over short periods (1800 ppm / 30 min) McDonough et al. (2011) were able to eliminate an average of 74.8% of *larvae* from *Tribolium castaneum* (Coleoptera: Tenebrionidae) species. By exposing the larvae longer (60 and 90 min), authors reported results of 92 and 100%, respectively, corroborating with the current work (showing the *larvae* high

sensitivity when to exposed O_3). Holmstrup et al. (2011) reported laboratory tests exposing adults and larvae of *Tribolium castaneum* with 40 ppm for 6 h was enough to eliminate 25%, and exposure was required for 24 h to reach 100% mortality.

Adult stage - with 60 ppm of O_3 for 24 h of gas exposure, the adult beetles remained totally motionless with their paws adhering to the body. They were removed from the chambers and left at room temperature (23°C) and RH (82%) for confirmation of death. At the time of removal, the movement of some individuals was observed, which means that the treatment was not effective, eliminating only 80% of the population. The efficacy of the test was demonstrated with exposure of 40 ppm for 36 h, where 100% mortality of larvae and adult beetles was obtained. Table 1 shows the results obtained by the effect of O_3 gas on larvae and adult beetles.

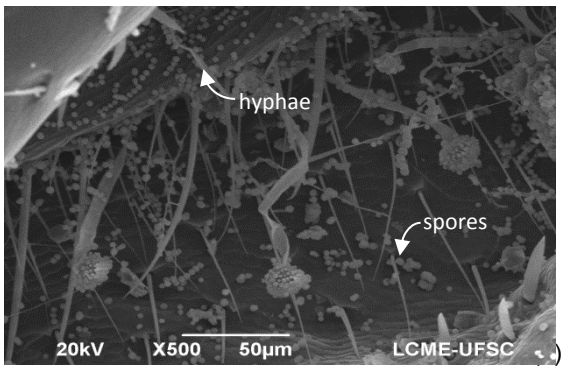
Laboratory tests results obtained by Hasan et al. (2016) registered also the efficacy of that gas, however, showed that larvae of the *N. rufipes* species are more resistant to ozone when compared to adults exposed to the same concentrations and time (66 ppm / 36h). Using two phases (sterilization or passivation) and (treatment stage) with O_3 applications Campabadal et al. (2013) obtained 100% results in *Tribolium castaneum* mortality in grains by exposing to the gas concentrations of 3.600 ppm/h and (25 or 50 ppm) for 3 to 6 days. Other species of grain beetles stored as *Tibolium confusum* were also exposed to 5,400 ppm-h concentration, resulting in 100% mortality (Leesch, 2002). On the other hand, at adult corn weevils (*Sitophilus zeamais*), by applying high concentrations and short time (1.188 ppm/h) Rozado et al. (2008) obtained 95% mortality.

3.2 Insects O_3 gas behaviour / dormancy

Important to emphasize that some insects, including beetles, take advantage of their physiological processes of dormancy when exposed to adverse conditions (i.e., lack of O_2, including the O_3 at the current experiments) and re-establish quickly when those adversities are of short duration (Begon et al., 2007). During the tests applied in the current study it was taken into account that physiological behaviour and it was observed that: after only 12 h of O_3 application directly on the dorsal region of the *adult* beetles and *larvae* (at the highest concentration - 60 ppm) and flow 3 L / min, the *adults* highly reduced their moving activity (semi-static), however came back to movements/life after the confirmation time set 5h). According to Sousa et al (2012) lower respiration rates may potentially lead to reduced O_3 absorption. Despite that, the *larvae* showed motionless activity. The live and dead insects (*larvae* and *adult*) were removed from the O_3 chambers and counted 5 h after the bioassays. Sousa et al. (2008) observed that O_3reduces the *S. zeamais* walking activity by reducing the chances of insects escaping from fumigation exposure in the early stages.

A.diaperinus acts as a vector of toxigenic fungi in poultry farms. Using scanning electron microscopy it is possible to observe the presence of reproductive structures of toxigenic fungi (*Aspergillus*) adhered to the exoskeleton of beetles (Figure 2). The use of ozone gas corroborates both control of beetles and control of fungi (Christ et al., 2016).

(a)



(b)

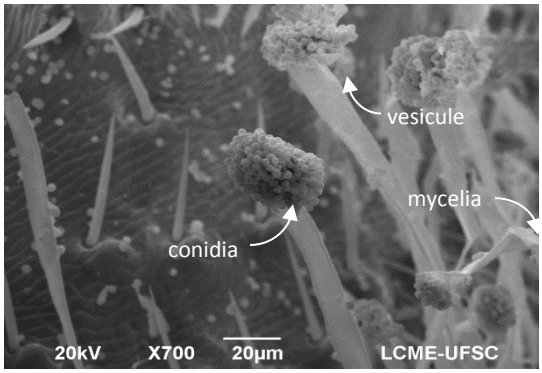


Figure 2: Scanning electron micrographs of Alphitobius diaperinus (VENTRAL (LEGS) isolated from aviary bed FUNGI CONTAMINATIO/INFECTED (a,b) showing fungi reproductive structures do (Aspergillus) [500 to 700X].

4. Conclusion

The O_3 gas effect on the *A.diaperinus* (Panzer) beetles proliferation at the stages (larvae & adult) studied, showed to be effective and should be further studied and applied as a clean inactivation strategy instead of pesiticide application in aviary beds.

Some techniques of poultry bed treatments recommended by the industries can reduce beetles infestation due to temperature elevation by fermentation, however reported not to be totally efficient. In that context, the O_3 gas application *in situ* has shown be better efficiency.

There is a need of research developments especially on O_3 application directly on aviary bed as well as equipment development. Thus reducing pesticides use for control of the *A. diaperinus*, mainly cypermethrin.

This monitoring strategy produces food without the risk of residues and contributes to the well-being of poultry and the rural workers.

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Reference

Begon M., Townsend C.R., Harper J.L., Eds., 2009, Ecology: from individuals to ecosystems. Artmed, Porto Alegre, Brazil

Campabadal C.A., Maier D.E., Mason L.J.,2013,Efficacy of fixed bed ozonation treatment to control insects in stored bulk grain, Applie. Eng. Agric. 29, 693-704.

Chernaki-Leffer A.M., Biesdorf S.M., Almeida, L.M., 2001, Thermal requirements, period of development and survival of immature *Alphitobius diaperinus*(Panzer) (Coleoptera: Tenebrionidae), Neotrop. Entom. 30, 365-368.

Chernaki-Leffer A.M., Bueno R., Raspantini L.E., Gorniak S.L., 2013, Effects of exposure of higher doses of cypermethrin in layers hens, Int. J. Poult. Sci. 12, 362 -366.

Christ, D., Savi, G. D., & Scussel, V. M. (2016). Effectiveness of ozone gas in raw and processed food for fungi and mycotoxin decontamination–a review. *Journal of Chemical, Biological and Physical Sciences*, *6*, 326-348.

Dubois M., Coste C., Despres AG., Efstathiou T., Nio C., Dumont E., Parent-Massin D., 2006, Safety of Oxygreen®, an ozone treatment on wheat grains. Part 2. Is there a substantial equivalence between Oxygreen-treated wheat grains and untreated wheat grains?, Food. addit . contam. 23, 1-15.

Hagstrum D., Tomaz k., Bhadriraju S., Nawrot J., Eds., 2017, Atlas of stored-product insects and mites. Elsevier, Minesota, the United States

Hasan M.M., Aikins M.J., Schilling W., Phillips T.W., 2016, Efficacy of controlled atmosphere treatments to manage arthropod pests of dry-cured hams, Insec. *7*, 44.

Hazeleger W.C., Bolder N.M., Beumer R.R., Jacobs-Reitsma W.F., 2008, Darkling beetles (*Alphitobius diaperinus*) and their larvae as potential vectors for the transfer of *Campylobacter jejuni* and *Salmonella enterica*s erovarparatyphi B variant java between successive broiler flocks, App.Environ. Microbiol.74, 6887-6891.

Holmstrup M., Sorensen J.G., Heckmann L.H., Slotsbo S., Hansen P., HansenL S., 2011, Effects of ozone on gene expression and lipid peroxidation in adults and larvae of the red flour beetle (Tribolium castaneum), J.Stored.Prod. Resea. 47, 378-384.

Kim J.G., Yousef A.E., 2000, Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone, J.Food. Scie. 65, 521-528.

Marangi M., Pati S., Camarda A., Cafiero M.A., Giangspero A., 2012, Acaricide Residues in Laying Hens Naturally Infested by Red Mite *Dermanyssus gallinae*, PLoS. ONE. 7,e31795, doi:10.1371/journal.pone.0031795

Matias R.S., 1992,Control of *Alphitobius diaperinus* on a bed and poultry bed, Braz. Agric.Resea.27, 205-207.

Oviedo-Rondón E.O., 2008,Technologies to mitigate the environmental impact of broiler production, J. Braz. Zootec. 37, 239-252.

Savi G.D., Scussel, V.M., 2014, Effects of ozone gas exposure on toxigenic fungi species from Fusarium, *Aspergillus*, and *Penicillium* genera, Ozone: Scie. Eng. 36,144-152.

Sharma R.R., Demirci A., Puri V.M., Beuchat L.R., Fett W.F., 2004. Modeling the inactivation of Escherichia coli O157: H7 on inoculated alfalfa seeds during exposure to ozonated or electrolyzed oxidizing water, Transac. ASAE. 47, 173.

Singh S., Yadav A.S., Singh S.M., Bharti P., 2010, Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance, Food. Res. Intern. 43, 2027-2030.

Skov M.N., Spencer A.G., Hald B., 2004, The role of litter beetles as potential reservoir for *Salmonella enterica* and thermophilic *Campylobacter* spp. between broiler flocks, Avi.Dise.48, 9-18.

Soares C.E., Ferreira B.C.F., Dutra, M.O., Maria, G.S., Martins, C.S., Scussel, V.M., 2017a, Vectors of Toxygenic Fungi in chicken farms, VI Sacta. Florianópolis, Brazil.

Soares, C.E., Weber, A., Scussel, V.M., 2017b,Stereo and Scanning Electron Microscopy Characteristics of Poultry Breeding Beetle (*Alphitobius diaperinus)* – a filamentous toxigenic fungi carrier, Emi. J. F. Agric. 7.

Sousa A.D., Faroni L.D.A., Guedes R.N.C., Tótola M.R., Urruchi W.I., 2008, Ozone as a management alternative against phosphine-resistant insect pests of stored products, J. Stored. Prod. Res. 44, 379-385.

Sousa A.H., Faroni L.R.A., Silva G.N., Guedes R.N.C., 2012,Ozone toxicity and walking response of populations of Sitophilus zeamais (Coleoptera: Curculionidae), J.Econ. Entomol. 105, 2187-2195.

Spinosa H.S., Górniak S.L., Palermo-neto J., 2010, Eds., Toxicology Applied to Veterinary Medicine. Manole, São Paulo, Brazil

Subramanyam B., Li, B., 2017, Efficacy of Ozone against Phosphine Susceptible and Resistant Strains of Four Stored-Product Insect Species, Insec. 8, 42.