EFFICACY OF OZONE MIXED WITH CARBON DIOXIDE ON THE MORTALITY OF *RHYZOPERTHA DOMINICA* (F.) INSIDE FOOD PACKAGING

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ABSTRACT: The lesser grain borer, *Rhyzopertha dominica* (F.), is a primary beetle pest of stored grain in many regions of the world. Fumigation as a pest control method plays a key role in control and management of infestation stored commodities worldwide. This study was conducted to control the beetles in food packaging under modified atmosphere storage. Experiments were designed on three factors, including foodstuff (3 treatments), wrapper (4 treatments) and the concentration of ozone mixed with 40% carbon dioxide (3 levels). The results showed that the behavior of wheat is different from wheat flour and rolled oats foodstuffs. Accordingly, while the most mortality beetles in wheat (alive) was observed in the BOPP 40μm film (biaxially oriented polypropylene) with low permeability, the most mortality in wheat flour and rolled oats (non-alive) occurred in Non-woven PP (polypropylene) wrapper with high permeability. Mortality of beetles located in all of foodstuffs showed a significant decrease in 40% CO_2+150 ppm O_3, 40% CO_2+100 ppm O_3 and 40% CO_2+50 ppm O_3 in the level of 0.05 respectively. Arrangement of morality mean in the foodstuffs was significant as wheat four < rolled oats< wheat in 0.05 level.

KEY WORDS: Stored grain insect, Modified atmosphere, Spunbond, Perforated woven polypropylene

Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests (Rajendran and Sriranjini, 2008). The lesser grain borer, *Rhyzopertha dominica* (F.), is a primary beetle pest of stored grain in many regions of the world. This insect is injurious to cereals; breeds in corn, rice, wheat, and in other substrates containing starch (Edde, 2012). Fumigation as a pest control method plays a key role in control and management of infestation stored commodities worldwide. Therefore, numerous investigators have studied the application and effectiveness of fumigants to control stored-product insects. In addition, exposure of insects to toxic concentrations of atmospheric gases has been practiced for centuries and has been promoted in recent years as a bio-rational substitute for chemical fumigations (Sadeghi et al., 2011). O_3 gas, a powerful oxidant, has numerous beneficial applications and is very familiar to the food processing industry. This gas has regulatory acceptance by the Food and Drug Administration (USA) (FDA, 2001), and the Environmental Protection Agency's (USA) MSDS defines it as "pure air" (Mason et al., 2006). On the other hand, CO_2 is efficient only when concentrations higher than 40% are maintained for long periods. Exposure periods longer than 14 d are required to kill the insects when the concentration of CO_2 in the air is below 40% (Sadeghi et al., 2011).

Food packaging as one of the most important parts of food industry is related with food security. Food packaging provides not only a method for transporting food safely, but extends product's self-life via preventing from harmful bacteria, contamination and degradation (Chin, 2010). Furthermore packaging can be security for food product, insect can enter goods during transportation, storage in the warehouse, or in retail stores, and also it is possible that the initial contaminants develop and destroy foodstuffs (Allahvaisi et al., 2010). Accordingly, the use of type of packaging to eliminate probable contamination of the food and to prevent re-contamination is one of the underlying subjects in packaging industry. When an infected packaging with an insect's life stage, enters into the warehouse, it can spread the contamination to other packages and in addition reducing food quantity, the quality of food is annihilated.

Highland & Wilson (1981) believed that polypropylene has a higher resistance than polyethylene to insect penetration (with equal thickness). Bowditch (1997) found that the polypropylene film tested was resistant to penetration by 1st-instar larvae of *Ephestia cautella* (Walker). In another study, from 4 kinds of used polymers such as polyethylene, polypropylene, polyvinylchloride and cellophane, polypropylene had the least permeability against the pest insect as most of the pests were unable to penetrate this polymer and if penetration occurred, it was less (Allahvaisi et al., 2010). On the other hand, in recent years, especially biaxially oriented polypropylene films (BOPP) have become one of the most popular high-growth films in the world market (Lazic et al., 2010). In another study on BOPP 80μm laser films was expressed that BOPP films without holes and with the maximum number of holes were the most suitable for controlling of *Tribolium confusum* Jacquelin du Val. in alive and non-alive foodstuffs respectively (Nateq Golestan et al., 2015). Sacks made from woven polypropylene are replacing jute sacks for commodity storage in developing countries. Woven polypropylene (WPP) sack manufacture was developed in Japan in the late 1960s and was quickly adopted in Europe, South Africa, Australia and North America. These sacks are lighter and relatively stronger than jute (Kennedy & Devereau, 1994). Spunbonded bags made of synthetic polymers were commercialized by the technology of Freudenberg (Germany) and Du Pont (USA) in the 1950s and 1960s. Many polymers, including polypropylene, polyester, polyethylene, polyamide, polyurethane, etc. are used in the spunbond process. Among various polymers, isotactic polypropylene (PP) is the most widely used polymer for spunbond non woven production. Non woven products made by using the spunbond process are used in different industries such as packaging (Lim, 2010). Food packaging with non woven wrappers is developing and now is used for packaging products such as rice. This wrapper has a high permeability to gases and vapor.

When a product is packaged, it may be contamination or initial contamination may be developed, and because percentage of insect's penetration and contamination development can depend on the type of packaging material, finding the best wrapper for packaging is inevitable. This study examine the simultaneous effects of mixture of ozone and carbon dioxide gas, current wrappings of food packaging and type of food on mortality of stored pest. Furthermore, it suggests the appropriate wrapper based on type of food.

MATERIAL AND METHODS

This study was carried out at Department of Plant Pest and Disease, Razavi Khorasan Research Center for Agriculture and Natural Resources during the years 2012-2013. Mixed concentrations of 50, 100 and 150 ppm O_3 along with 40% CO_2 gas was tested on packages made of 4 wrappers, including 2 BOPP films with 40 and 80μm width, woven polypropylene wrapper laminated/perforated (WPP-L/P) and non-woven polypropylene fabric (Spunbond) filled with wheat, rolled oats wheat flour foodstuffs.

Insect

The lesser grain borer, *Rhyzopertha dominica (F.)* (Coleoptera: Bostrichidae) was prepared from laboratory of Department of Plant Protection, College of Agriculture, Urmia University in Urmia (37°33'N 45°04'E) a city in Iran. Cultures were established and maintained on healthy uncontaminated food at 25±2°C and 65±10% r.h. in plastic bottles and were closed with pieces of muslin cloth fixed by rubber bands. Rearing medium used was composed of wheat. All insects were cultured under moderately crowded conditions to ensure proper development and equal size of the resultant adults.

Supply of gases

Ozone gas was generated by ozone Generator, Ozonica series, Oz 100 models (WWW.ozoneab.com), that generate 100 gram/hour ozone from purified oxygen with 4 reactors. Purified oxygen produce by oxygen generator, LFY-I-5F-W model, provided by Longfei Group Co. Ltd., and produce purified oxygen 93%±3% with flow rate 0-5 L/min. Specified O_3 concentration was measured based on the volume of the chamber and the default generator. A local factory supplied CO_2 gas needed inside cylinders of 40 kg with 99.9% purity.

Wrappers

Woven polypropylene wrapper laminated/perforated was taken from Kabir Industrial Group located in Tehran, Iran and made of 95% PP+2% PE+2% $CaCO_3$+1% Color material and perforated by needle rollers with a distance 5 mm from each other. Non-woven polypropylene fabric was taken from Baftineh Ltd. located in Tehran, Iran and made from 100% PP with 90 gram/m^2 and white color. BOPP film rolls with 40μm width was taken from *Poushineh* Industrial Group located in Tehran, Iran. We laminated 2 BOPP film rolls with 40μm width together and produced film 80μm.

At the first, the packages 20×30 cm were filled with 1 kilogram of wheat and rolled oats separately. Then a cage (10×10 cm) containing 40 insects and 3 gr. food was entered into each package and sealed with a plastic press machine. Subsequently, packages transferred into chamber 70×120×180 cm and placed horizontally at the bottom it and the chamber closed tightly. Afterwards, CO_2 gas (CO_2 cylinder with purity 99.9%) was injected into the upper left, and air exited from the bottom right until concentration of CO_2 was 40% and in the final step, we injected O_3 gas daily at a specified time and every day on reaching the specified concentration, ozone injection was stopped. A total 7 injections with equal doses during 7 d performed. During CO_2 injection and until 1 hour after O_3 injection, the system was circulated. During experiments, upper surface of packages exposed chamber atmosphere. Exposure period was considered 7 d at 25±2°C, 35±5% r.h. After exposure period, the specimens were transferred to a clean jar containing 3 gr. of food with the same condition. Mortality rates of the insects were recorded 6 h after termination of the treatment. Each test was replicated 3 times on different days, and results were pooled.

Bioassay

In this experiment, we used adults *Rhyzopertha dominica (F.)* 7±2 days old. Preliminary dose-mortality tests were carried out prior last experiment to determine a range of doses that produce 25 to 75% mortality at the lowest and the highest doses, respectively (Robertson et al., 2007). In ultimate experiment compared average mortality in 3 foodstuffs separately by independent sample's t-test and also, analyzed mean mortality in gas mixtures and wrapper treatments together by factorial experiment in the completely randomized design. Comparison of the average mortality rates performed by Tukey's test separately. All of data were analyzed with the Statistical Package for the Social Science (SPSS) software (SPSS Inc., 2007). First, mortality rates of various treatments were adjusted with Abbott's formula and then, for normalizing of residuals variance, the data were transformed to $\text{Arcsin}\sqrt{x}$.

RESULTS

The initial factorial experiment showed that all three factors including foodstuff, gas mixture and wrapper have a significant effect on mortality at 0.05 level. ANOVA test in the first analysis explained 89% of the variance of mortality based on the independent variables. In this test, the foodstuff factor attained the largest effect size (ω^2= 0.72.7) and two other factors created much smaller effect size (Tab. 1). Initial observations showed that wheat treatment from foodstuff factor because of behavior difference with other treatments (rolled oat and flour wheat) made this very large effect size (Fig. 1). This opinion was confirmed by removing wheat treatment and ANOVA re-test. In this analysis, effect sizes of interactions decreased and Foodstuff * Wrapper and Dose * Wrapper not significant at 0.01 and 0.05 levels respectively. The effect sizes three factors were also close to each other (Tab. 1) and the maximum effect size was related to wrapper factor (ω^2= 0.32.5). As a result, it should be stated wheat treatment has very different characteristics with rolled oat and wheat flour treatments. By removing wheat from analysis, foodstuff factor was more homogeneity and consequently effect of ozone concentration on foodstuff increased and conversely, the effect of this gas on wrapper factor was not significant at the 0.05 level (Tab. 1).

Grouping mean mortality performed for wrapper, foodstuff and ozone dose factors separately by Tukey's test. The result without wheat treatment showed that the lowest and highest mortality was in Bopp film 80µm and Nonwoven fabric respectively (Tab. 2). It should be noted that the two wrappers mentioned have the lowest and the highest permeability to gas. Therefore, by increasing the permeability of wrapper in rolled oats and wheat flour foodstuffs, the mortality also increased. The result in the presence of wheat treatment showed that there is no direct relationship between the permeability of wrapper with the mortality rate and the lowest and highest mortality obtained on woven PP (L/P) and BOPP film 40µm respectively (Tab. 3).

Mortality of beetles located in all of foodstuffs showed a significant decrease in 40% CO_2+150 ppm O_3, 40% CO_2+100 ppm O_3 and 40% CO_2+50 ppm O_3 in the level of 0.05 respectively (Tab. 2,3). The most mortality was observed in wheat treatment and then rolled oats and wheat flour were by large margins in the level of second and third (Fig. 2). Table 4 showed percentage mortality rate for treatments (wrapper-Ozone mixed CO_2 -foodstuff) without data transformation and accordingly, the lowest of mortality level was in the BOPP 80µm- 50 ppm O_3-wheat flour treatment.

CONCLUSIONS

Three types of foodstuffs used, are from the main stored products. Wheat grains are alive and breathing and other foodstuffs aren't alive. Research shows that the seed respiration led to an increase in the CO_2 concentration within sealed packages, and this is an important factor influencing mortality (Moreno, 1991). And so, wheat respiration increased CO_2 concentration in the packages, and this condition led to elevation of pest mortality. Accordingly, it can be concluded that in live products, packaging films with low permeability is proper for fumigation. Our results confirmed this assumption and BOPP film with thickness of 40 µm created the highest mortality and therefore, was the most appropriate in wheat (Tab. 3). On the other hand, in non-alive products such as rolled oats and flour wheat, because there is no respiration and no bio-increasing in the mount of gases within the packages, pest mortality was almost exclusively influenced by chamber atmosphere and with elevating CO_2 and O_3 concentrations, increased mortality was followed. Therefore, non woven polypropylene fabric was the most suitable

(Tab. 2). BOPP film and non-woven polypropylene fabric showed the highest and lowest penetration resistance to insects, respectively.

About ozone gas, the results indicate that decomposition it on the grain surface occurs in 2 phases. The first phase, due to the high interaction with the grains surface, the penetration rate is low and in the second phase, movement through the grain is rapid with very little impedance (Kells et al., 2001; Dos Santos et al., 2007). For this reason, we used low doses of O_3 intermittently to achieve minimum damage to the product and maximum performance on the pest control. In this study, for control of beetles within foodstuffs packages in the chamber, in the case of rolled oats and wheat flour was applied only O_3 and CO_2 gases injected into chamber and in the case of wheat apart from the injected gases, an additional CO_2 gas produced by the respiration of wheat grains within the packages influenced on the pest control.

The results showed that in the wheat foodstuff (alive), the mortality of the adult beetles in the packaging with low permeability was more compared with packaging with high permeability. Conversely, in the rolled oat and wheat flour foodstuffs (non-alive), this mortality in the packaging with low permeability was less compared with packaging with more permeability (Fig. 1). Accordingly, we can hypothesize that suitable model for packaging of live foodstuffs is Low Gas-permeable Packaging (LGPP) and for packaging of non-live foodstuffs, the High Gas-permeable Packaging model (HGPP) is an appropriate option.

Overall, it can conclude that mixture of CO_2 and O_3 gases was appropriate treatment for control of beetles and use of O_3 gas with safe concentrations intermittently with specified interval can reduce the CO_2 concentrations used in modified atmospheres. Furthermore, obtained mixture due to the use of 2 controlling agents can reduce development of pest resistance compared to use of them separately. In addition, respiration of foodstuffs and permeability of wrapper can be factors influenced mortality.

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Table 1. Two factorial experiments for three factors of foodstuff, ozone concentration and wrapper.

Source	presence of wheat in foodstuff factor						absence of wheat in foodstuff factor					
	SS	df	MS	F	ρ	ω^2	SS	df	MS	F	ρ	ω^2
Foodstuff	5.387	2	2.693	3271.4	.00	0.727	0.390	1	0.390	465.86	.00	0.293
Dose	0.792	2	0.396	480.88	.00	0.107	0.386	2	0.193	230.28	.00	0.290
Wrapper	0.452	3	0.151	183.10	.00	0.061	0.435	3	0.145	173.15	.00	0.325
Foodstuff * Dose	0.074	4	0.019	22.578	.00	0.009	0.031	2	0.016	18.764	.00	0.022
Foodstuff * Wrapper	0.447	6	0.075	90.549	.00	0.060	0.009	3	0.003	3.447	.02	0.005
Dose * Wrapper	0.051	6	0.008	10.228	.00	0.006	0.005	6	0.001	0.970	.45	-
Foodstuff * Dose * Wrapper	0.148	12	0.012	14.988	.00	0.018	0.080	6	0.005	5.592	.00	0.055
Error	0.059	72	0.001				0.040	48	0.001			
Total	7.410	107					1.324	71				

ρ is significant at 0.05 level — Adjusted R^2 = 0.988 — Adjusted R^2 = 0.990

ω^2 is the effect size of factors in ANOVA analysis

Table 2. Grouping arcsin $\sqrt{x}$ mean of mortality for three factors in the absence of wheat treatment.

Wrapper	N	Grouping	Dose of O_3	N	Grouping	Foodstuff	N	Grouping
BOPP film 80μm	18	0.5858 a	50 ppm	24	0.6041 a	Wheat flour	36	0.6100 a
BOPP film 40μm	18	0.6565 b	100 ppm	24	0.6660 b	Rolled oats	36	0.7572 b
Woven PP (L/P)	18	0.6911 c	150 ppm	24	0.7808 c			
Nonwoven fabric	18	0.8011 d						
Std. Error		.00965			0.00676			0.00835

Table 3. Grouping arcsin $\sqrt{x}$ mean of mortality for two factors in the presence of wheat treatment.

Wrapper	N	Grouping	Dose of O_3	N	Grouping
Woven PP (L/P)	9	1.0126 a	50 ppm	12	1.0404 a
BOPP film 80μm	9	1.0826 b	100 ppm	12	1.0835 b
Nonwoven fabric	9	1.1483 c	150 ppm	12	1.2959 c
BOPP film 40μm	9	1.3162 d			
Std. Error		0.01329			0.01151

Table 4. Percentage mortality rate for treatments (wrapper-Ozone mixed CO_2 -foodstuff) without data transformation.

	BOPP film 40µm			BOPP film 80 µm			Woven PP (L/P)			Nonwoven fabric		
	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm
Wheat	0.887± 0.007	0.930± 0.009	0.975± 0.007	0.758± 0.004	0.776± 0.012	0.804± 0.008	0.600± 0.007	0.613± 0.015	0.902± 0.007	0.698± 0.008	0.754± 0.011	0.970± 0.012
Rolled oats	0.317± 0.011	0.400± 0.019	0.598± 0.006	0.279± 0.011	0.350± 0.022	0.535± 0.013	0.371± 0.022	0.462± 0.025	0.565± 0.012	0.521± 0.011	0.596± 0.011	0.679± 0.019
Wheat flour	0.263± 0.007	0.304± 0.015	0.367± 0.015	0.212± 0.015	0.221± 0.018	0.262± 0.013	0.275± 0.019	0.333± 0.015	0.442± 0.011	0.367± 0.018	0.408± 0.017	0.521± 0.018

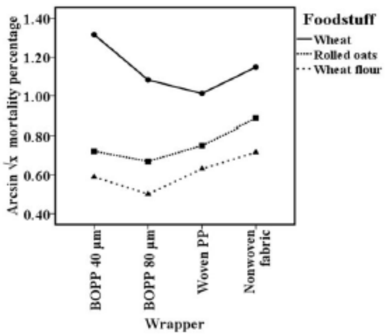

Figure 1. Comparison of arcsin $\sqrt{x}$ mean of mortality in three foodstuffs located in different concentrations of ozone along with 40% carbon dioxide

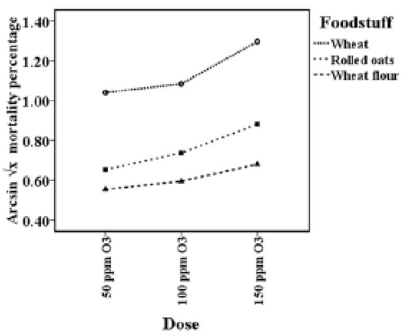

Figure 2. Comparison of arcsin $\sqrt{x}$ mean of mortality in three foodstuffs located in different wrappers