Effect of modified atmosphere on obvious and hidden contamination to control of *Plodia interpunctella* (Hubner) and *Tribolium confusum* Jacquelin Du Val inside highly permeable packages

Mohammad Nateq Golestan* and Hassan Rahimi

ABSTRACT

The development of stored products pests in foodstuffs is one of the most important challenges in the food packaging industry. In this research, the control of two species of the most important stored product pests placed inside the packages composed of two types of permeable wrappers under modified atmospheric conditions was studied. The experiments were performed at 25 ± 2 °C and 35± 5% r.h. with exposure period 7 days. Two pest species (*Tribolium confusum* Jacquelin Du Val and *Plodia interpunctella* (Hubner)), three foodstuffs (wheat, wheat flour and rolled oats) with two wrappers (Woven-polypropylene/laminated-perforated and Non-woven-polypropylene fabric) were placed inside the chamber with three levels of O_3 gas (50, 100 and 150 ppm) in base atmosphere of 70% CO_2. The results of obvious and hidden contamination experiments showed that the mortality rate of confused flour beetle was lower than that of the Indian-meal moth at 0.05 level. Among the foodstuff treatments, the lowest mortality rate was observed in wheat flour. Also, wheat treatment had different effects on two species of pests. The NWPP wrapper showed a higher mortality rate than the WPP/LP. The highest mortality rates were obtained at concentrations of 100 and 150 ppm of O_3 gas.

Keywords: Ozone, Stored product pest, Packaging, Foodstuff, Wrapper.

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INTRODUCTION

Stored products of agricultural and animal origin are attacked by more than 600 species of beetle and 70 species of moth pests (Rajendran and Sriranjini, 2008), among which the confused flour beetle, *Tribolium confusum* Jacquelin Du Val is one of the most serious pests of stored products worldwide. It causes damage to an extremely large variety of foodstuffs, and it is a very important pest on wheat flour and broken grain kernels. (Abd El-Aziz *et al.,* 2009; Rossi *et al.,* 2010). The Indian meal moth, *Plodia interpunctella* (Hubner) is a major economic insect pest of stored products and is found on every continent except Antarctica (Mohandass *et al.*, 2007). Fumigation as a pest control method plays a key role in the control and the 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 practised for centuries and has been promoted in recent years as a bio-rational substitute for chemical fumigations (Sadeghi *et al.*, 2011). Ozone 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). Also, CO_2 as an insecticide is efficient only when concentrations are maintained higher than 40% 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 to food security. Food packaging provides not only a method for transporting food safely, but extends product's self-life by from harmful bacteria, contamination and degradation (Chin, 2010). Despite the fact that packaging can ensure security for food products, insect can enter goods during transportation, storage in the warehouse and also in retail stores. It is possible that these initial contaminants develop and destroy foodstuffs (Allahvaisi *et al.*, 2010).consequently the use of the type of packaging to eliminate probable contamination of the food and to prevent re-contamination is one of the basic issues in packaging industry (Nateq Golestan *et al.*, 2015a). Many studies have been conducted on resistance of polymeric films to insect penetration (Highland & Wilson, 1981; Bowditch, 1997; Allahvaisi *et al.*, 2010; Lazic *et al.*, 2010; Nateq Golestan *et al.*, 2015). Also some studies have examined the effect of O_3 gas mixed with CO_2 gas on the mortality stored pest inside food packaging (Nateq Golestan *et al.*, 2015a; Nateq Golestan *et al.*, 2015b; Nateq Golestan *et al.*, 2016). Food packaging with non-woven wrappers is developing and is now used for packaging products such as rice. This wrapper has a high permeability to gases and can be a good option to serve the purpose better. Non-woven products made by using the spun bond process are used in different industries such as packaging. Among various polymers, isotactic polypropylene (PP) is the most widely used polymer for spun bond non-woven production (Lim, 2010). 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 those made from jute (Kennedy & Devereau, 1994). When a product is packaged, contamination or initial contamination may develop. Also, it is known that percentage of insect's penetration and contamination development can depend on the type of packaging material. Therefore, finding the best wrapper for packaging is inevitable. This study examines the simultaneous effects of mixture of ozone and carbon dioxide gas and type of foods on mortality of two stored product pests located inside two highly permeable packages.

MATERIAL AND METHODS

This study was carried out at the Department of Plant Protection Research, Khorasan Razavi Agricultural and Natural Resources Research and Education Center, during the years 2012-2013. Mixed concentration of 50, 100 and 150 ppm O_3 along with 70% CO_2 gas was tested on packages made of woven polypropylene wrapper/laminated perforated (WPP/LP) and non-woven polypropylene fabric (NWPP) filled with wheat, wheat flour and rolled oats foodstuffs.

Insect

Confused flour beetle, *Tribolium confusum*, and Indian-meal moth, *Plodia interpunctella* were collected from a flour silo and silver berry (*Elaeagnus angustifolia*) warehouse in city of Mashhad (36°20'N 59°35'E) respectively in Iran. Cultures were established and maintained on healthy uncontaminated food at 25±2ºC and 35±5% r.h. in plastic bottles and were closed with pieces of muslin cloth fixed by rubber bands. Rearing medium used was composed of wheat and wheat flour. 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 produced a by oxygen generator, LFY-I-5F-W model, provided by Longfei Group Co. Ltd., produce purified oxygen 93 ± 3% with a 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.

Wrapper

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. Woven polypropylene wrapper laminated/perforated (WPP L/P) 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 of 5 mm from each other.

Bioassay

In "obvious contamination test", all the 108 packages 20×30 cm were filled with one kilogram of wheat, wheat flour and rolled oats separately. Then a cage (10×10 cm) containing 40 insects and 3 gr. foodstuff was placed in each package and sealed with a plastic press machine. Subsequently, packages were transferred into chamber 70×120×180 cm and placed horizontally at its bottom 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 70% and in the final step, we injected O_3 gas daily at a specified time until it recorded 50, 100 and 150 ppm concentration and then ozone injection was stopped. A total of 7 injections with equal doses during 7 d performed. During CO_2 injection and until 1 hour after O_3 injection, the system gets 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 foodstuff 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.

In this experiment, we used adults of confused flour beetle 12±4 days old and third instar larvae of Indian-meal moth. Preliminary dose-mortality tests were carried out prior to the last experiment to determine the range of doses that produce 25 to 75% mortality at the lowest and the highest doses respectively (Robertson *et al.*, 2007). In the ultimate experiment, mean mortality was analyzed in gas mixtures, foodstuff and wrapper factors together by factorial experiment in a completely randomized design."Hidden contamination test" was performed to compare immature stages resistance of the *Plodia interpunctella* and *Tribolium confusum*. In this test, 100 g of pest culture medium without adults were poured inside the test cages and their door was closed with paper gluing. Following bioassay tests procedure the cages were placed inside woven and non-woven packets containing one kilogram of foodstuffs and transferred to the gas chamber. Because of the high resistance of egg and pupa stages in storage pests (Krischik *et al.*, 1995), the atmospheric CO_2 and O_3 concentration for this test was 70% and 150 ppm respectively. After the test, the cages were removed from the packages and their content was transferred to a one liter container. Adult emergence of treatment (AET) from treated culture medium was counted weekly and continued until the 12 weeks equivalent to about twice the life cycle of the species (Ahmady *et al.*, 2017 and Mbata & Osuji 1983). Adult emergence of control (AEC) placed under normal conditions in the room was recorded according to other treatments. Emergence rate of adults was calculated with the following formula: ER (%) = (AET/ AEC)*100 "Insect permeability tests in wrapper" were performed in three replications for 14 d. In "penetrate out" test, 20 adults from *Tribolium confusum* was poured into the packages 4×8 cm without foodstuff and then placed in a one liter container containing 10 grams of rolled oats. In "penetrate into" test also 20 adults from *Tribolium confusum* were put in one liter container without foodstuff and packages 4×8 cm containing 10 grams was placed inside containers. Then After 14 d, the amount of penetration in the wrapper was determined by the number of holes formed on the wrapper.

Statistical analysis

All data were analyzed with the SPSS 16.0 software (SPSS Inc., 2007). First, mortality percentages of various treatments were adjusted with Abbott's formula and then, for normalizing of residuals variance, the data were transformed to $\mathrm{Ln}\,(x+0.5)+1$. The effects of treatments were tested with ANOVA based on general linear model (GLM) and Tukey's test ($P<0.05$) was used for the comparison of the average mortality rates, separately.

RESULT AND DISCUSSION

In order to compare the mortality percentage of the two studied species, the factorial experiments with 4 factors including insect, foodstuff, wrapper and ozone gas concentration was initially performed. The result showed that mortality rate of the third instar larvae of Indian meal moth was higher than that of the adult of confused flour beetle ($P<0.05$). Next variance analysis tests were performed for two species separately. The results showed that effect of all three factors including foodstuff, wrapper and ozone concentration in two insect species were significant at 1% level. In both species was found interaction between foodstuff × ozone concentrations (a×b) and it seems to be due to different levels of gas adsorption in foodstuff treatments (Table 1). ANOVA test of *Tribolium confusum* showed that 98.2% of variance changes of mortality rate were explained by the main effects (foodstuff, ozone concentration and wrapper) and interaction effects. The most and least effect size in the foodstuff factor (ω^2= 0.93) and the wrapper factor (ω^2=0.009) were obtained. Also, ANOVA test of *Plodia interpunctella* showed that 97.4% of variance changes of mortality rate were explained by the main and interaction effects. The most and least effect size in the foodstuff factor (ω^2= 0.92) and the ozone factor (ω^2=0.018) were obtained (Table 1). The superiority rate (SR) of foodstuff factor to wrapper factor in mortality of *Tribolium confusum* is the highest (**ω^2a/ ω^2c=** 96.99) while rate in mortality of *Plodia interpunctella* (**ω^2a/ ω^2c=** 45.32) is close to the SR of foodstuff factor to ozone factor (**ω^2a/ ω^2b=** 50.32) (Table 1). Grouping mean mortality was performed for foodstuff, ozone and wrapper treatments separately by Tukey's test. In confused flour beetle, result showed that by adding O_3 gas from 50 to 150 ppm, the mortality rate increased. Also, the lowest and the highest mortality rate were observed in wheat flour and wheat respectively ($P<0.05$). Between the wrappers, NWPP caused more mortality from WPP wrapper ($P<0.05$) (Table 2). In Indian meal moth, the highest mortality was observed in 100 and 150 ppm O_3 at the 0.05 level (Table 3). In hidden contamination test, emergence rate (ER) of confused flour beetle in NWPP was highest in wheat flour and rolled oats treatments while this rate in WPP was the highest in wheat flour alone (Table 4). Also, this rate in Indian-meal moth was the highest in the wheat flour treatment for both wrappers separately (Table 5). Maximum and minimum emergence rate of *Tribolium confusum* and *Plodia interpunctella* adults were shown in wheat flour and wheat respectively. Also similar to obvious contamination, control level of the pest in Non woven PP was more than Woven PP in all three foodstuffs separately. The lowest and highest of emergence rate (ER) obtained are in "*Tribolium* + Wheat + NWPP" and "Wheat flour + WPP" (*Tribolium* = 0.3721 ± 0.00347 and *Plodia* = 0.3673 ± 0.0192) treatments respectively (Tabs.3, 4). In insect permeability tests in wrapper, after 14 days, no species was able to penetrate into and out of the wrappers.

In general, based on factorial experiment with 4 factors, beetle's resistance was greater than that of moth, but when the obvious contamination tests was conducted for species separately, in wheat treatment, the mortality rate of beetle was equal to that of moth . Also when the hidden contamination tests were done, in wheat treatment, the moth's resistance

Table 1. Factorial experiment for three factors at insect treatments separately

	Tribolium confusum				*Plodia interpunctella*			
S. V.	Df	MS	F	ω^2	df	MS	F	ω^2
Foodstuff(a)	2	1.1228	1350.096**	0.926	2	0.7339	950.5163**	0.919
Ozone(b)	2	0.0511	61.45758**	0.041	2	0.0153	19.86832**	0.018
Wrapper(c)	1	0.0240	28.81814**	0.0095	1	0.0331	42.90512**	0.020
a×b	4	0.0023	2.715722*	0.0023	4	0.0054	6.955011**	0.012
a×c	2	0.0024	$2.848536^{n.s}$	0.0013	2	0.0064	8.227584**	0.007
b×c	2	0.0014	$1.662487^{n.s}$	0.0005	2	0.0001	$0.154999^{n.s}$	0
a×b×c	4	0.0011	$1.362211^{n.s}$	0.0005	4	0.0004	$0.538168^{n.s}$	0
Error	36	0.00083			36	0.00077		
C.V.		0.0443				0.0402		
ω^2a/ ω^2b				22.31				50.32
ω^2a/ ω^2c				96.99				45.32
ω^2b/ ω^2c				4.347				0.901

n.s ρ is not significant; * ρ is significant at 0.05 level; ** *P*-value is significant at 0.01 level
ω^2a/ **ω^2**b is the superiority rate of A factor to B factor in mortality

Table 2. Grouping untransformed mortality rate on *Tribolium confusum* for three factors

Grouping	N	**Wrapper**	Grouping	N	**Ozone**	Grouping	N	**Foodstuff**
0.6497^{b}	27	Non woven PP	0.5690^{c}	18	50 ppm	0.8874^{a}	18	Wheat
0.6022^{a}	27	Woven PP	0.6233^{b}	18	100 ppm	0.3537^{c}	18	Flour wheat
			0.6890^{a}	18	150 ppm	0.7044^{b}	18	Rolled oats
0.0056			0.0068			0.0068		S. E.

P-value is significant at 0.05 level

Table 3. Grouping untransformed mortality rate on *Plodia interpunctella* for three factors

Grouping	N	**Wrapper**	Grouping	N	**Ozone**	Grouping	N	**Foodstuff**
0.7033^{a}	27	Non woven PP	0.6372^{b}	18	50 ppm	0.9041^{a}	18	Wheat
0.6452^{b}	27	Woven PP	0.6810^{a}	18	100 ppm	0.4431^{c}	18	Flour wheat
			0.7044^{a}	18	150 ppm	0.7215^{b}	18	Rolled oats
0.0053			0.0065			0.0065		S. E.

P-value is significant at 0.05 level

Table 4. Emergence rate of *Tribolium confusum* adults in hidden contamination test

Wrapper			**Non woven PP**		**Woven PP**	
Adults Emergence	**N**	**Control (AEC)**	**Mixed gas (AET)**	**Emergence rate (ER)**	**Mixed gas (AET)**	**Emergence rate (ER)**
wheat	3	132.33±4.33	7.33±0.88	0.0551 ± 0.0048^{b}	16±1.1547	0.1206 ± 0.00479^{c}
Wheat flour	3	109.33±3.18	32.67±1.76	0.2984 ± 0.0075^{a}	40.67±0.88	0.3721 ± 0.00347^{a}
Rolled oats	3	84.67±2.91	21.67±0.88	0.2558 ± 0.0034^{a}	25.67±0.67	0.3034 ± 0.00461^{b}

Emergence Rate = Adults Emergence of treatment/ Adults Emergence of control
P-value is significant at 5% level

Table 5. Emergence rate of *plodia interpunctella* adults in hidden contamination test

Wrapper			**Non woven PP**		**Woven PP**	
Adults Emergence	**N**	**Control (AEC)**	**Mixed gas (AET)**	**Emergence rate (ER)**	**Mixed gas (AET)**	**Emergence rate (ER)**
Wheat	3	26.00±2.30	2.67±0.67	0.10 ± 0.017^{b}	5±0.578	0.1914 ± 0.0053^{b}
Wheat flour	3	40.33±3.93	6±0.58	0.152 ± 0.024^{a}	14.67±0.67	0.3673 ± 0.0192^{a}
Rolled oats	3	31.33±2.96	3.33±0.88	0.104 ± 0.02^{b}	6.67±1.20	0.2095 ± 0.0174^{b}

Emergence Rate = Adults Emergence of treatment/ Adults Emergence of control
P-value is significant at 5% level

was greater than the beetle's (Tables 4, 5). To explain this, it seems that the interactions between foodstuff and other test factors have changed conditions to the advantage of Indian meal moth (Table 1). It also seems that being alive (wheat) and not alive (rolled oats and wheat flour) as well as gas adsorption level of foodstuffs are important in this change. The lowest mortality rate was found in wheat flour which can be due to very low permeability and a high adsorption level for gas. Although the WPP wrapper was perforated (WPP L/P), the permeability of the NWPP wrapper was much larger than the WPP, and this could be the cause of more mortality in the NWPP wrapper. It should be noted that because of the texture of the NWPP, its gas adsorption level should be greater than the WPP (Tabs.2, 3). Due to limited use of high ozone concentration (Navarro, 2006; Jamieson *et al.*, 2009) and the use of carbon dioxide in the range of maximum effective concentration in atmospheres with delayed resistance (over 21 generations) in the test (Donahaye, 1992), this control method in wheat foodstuff can have acceptable effects. It is therefore advisable to increase the gas exposure time (Mason, 2006), or increase the limited concentration of ozone gas in wheat. Considering the effect of corrosive ozone, by increasing the application time and amount of ozone concentration, it seems that the pests of the packaged wheat product are more effectively controlled.

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Mohammad Nateq Golestan* and Hassan Rahimi
Plant Protection Research Department, Khorasan Razavi Agricultural and Natural Resources Research and Education Center, AREEO, Mashhad, Iran,
Tel.:+985133409137; Fax: +9833409138
***Corresponding author**
E-mail- Nateq1215@yahoo.com