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Controlling *Lasioderma serricorne* F. (Col.: Anobiidae) by Fumigation and Packaging

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Abstract: Determining the most resistant packaging polymers against insect's penetration, from storage to consumption by consumers is first goal of this study. In this study, the best polymer that could inhibit insect penetration was determined and then, fumigation with phosphine was carried out for preventing the re-infestation in storages. Different polymers (PE, PP, PVC and Cellophane) with two thicknesses of 16.5 µm and 29 µm were used. The penetration ability of an important species of stored-pest insects (*Lasioderma serricorne* F.) adults and larvae to polymeric packages was investigated. Results showed there was a significant difference ($p \leq 0.05$) in the penetration of insects to those polymers. Cellophane packages with thickness of 16.5 µm were the worst polymer as the products into them had complete infestation and there were a lot of punctures on the packages while foods into polypropylene packages with 29 µm thickness were safe. Moreover, Packaging for disinfestation is effective when healthy foodstuffs would pack. For this purpose, packaged products must fumigate to ruin the developmental stages of pest into packages and so, prevent re-infestation. Infested foodstuffs with three developmental stages of mentioned pests (eggs, larvae and adults) were placed inside those packages and placed in air-tight tanks. The permeability of the packaging polymer to PH_3 is evaluated by calculating percentage insect mortality after fumigation with aluminum phosphide tablets. Finally, results showed that the best polymer for packaging cereal products and tobacco, without permeability to insects but with to gas, is polypropylene (PP) with 29 µm thickness.

Key words: Cereal products · Fumigation · Contamination · *Lasioderma serricorne* · Packaging · Phosphine gas

INTRODUCTION

Stored product insects are a perennial problem in retail stores, where they damage and contaminate susceptible merchandise such as food products and animal feed. In stored grain, insect damage may account for 10-40% of loss worldwide [1]. Although finished products can be shipped from production facilities uninfested, stored product insects can enter packaged goods during transportation, storage in the warehouse, or in retail stores. The packaging of products is the last line of defense for processors against insect infestation of their finished products [2]. When a packaging containing one of insect's life stages enters into storages (infested packaging), it could cause the prevalence of infestation. In addition to reducing food quantity, insects annihilate quality, too. Today, there are several popular types of polymers for foodstuffs packagings. Some may offer virtually no resistance to insects while others may be extremely resistant [3]. The ability of species to penetrate materials may vary between life different stages [4]. Therefore, while one product is packaged, the probability of contamination in products is forecasted that the insects penetration diminishes by kind of the packaging and so with selecting of best materials for packaging. Most researches have been done in order to determine penetration abilities of various species of stored-product insects into packaged agricultural products, recently [4-7]. So, information of polymers permeability to stored pest insects for selection of the best of them for packaging foods is necessary. Moreover, it is accepted that fumigation is the most universal and the less hazardous method for maintaining of agricultural products under storage conditions. So, the passage of gas through these polymers to lower layers for eradicating the contamination into packaged foodstuff is one of the other goals of this study. Phosphine is the most fumigant frequently used because of its simple application [8]. Polymers with

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various thicknesses have different permeability to fumigants [9-15]. Phosphine is characterized as a slow acting fumigant to which insects can develop resistance. So, an imperfect fumigation increases the risk of development of resistance by the insects. The packaging material, its thickness and the manner of its placing in storage should be correct to prevent serious damage in the products. So, the concentrations of phosphine gas for packaged products that could pass through the polymer and devastated the different developmental stages of pest insect determined. Finally, our study proved how polymer and thicknesses could be effective in permeability to gas and insects for improving safety and quality of foodstuffs industry.

MATERIALS AND METHODS

The Test of Polymers Permeability to Insects: In this study, we compared permeability of four kinds of transparent and flexible polymers against stored-insect pests that these are the same current polymers for foodstuffs packaging, including Polyethylene (PE), Polypropylene (PP), Polyvinylchloride (PVC) and Cellophane. These polymers were prepared in two thickness of 16.5 µm and 29 µm. Tab. 1 shows some important properties of these polymers [15,16]. These flexible packaging polymers were cut into 15×22-cm pieces with the aid of a template and after that cutting of these polymeric sheets, we prepared 8×10-cm pouches by the sealed polymeric pieces the aid of a press plastic machine for packaging 15-gr foodstuff of flour. These packages were completely without any pores. The test insects (all obtained from laboratory cultures) were including the different developmental stages of *Lasioderma serricorne* F. (Col.: Anobiidae) adults and first and last instar larvae. First age larvae obtained by collection the pest's eggs from the cultures and placing them into petri dishes at 25±1°C, 75±5% RH and a 12L: 12D h cycle. Then, first instar larvae hatched and tested. The last instar larvae obtained from laboratory culture directly. We tested these insects on packaging polymers in two states viz, without and with food. The prepared packages were without any pores and each one of them with one thickness placed in a ca. 150 cc container vertically. We applied 20 insects (larvae or adult insect) in two mentioned states for examination a thickness of each polymer [7] Each container capped with a filter fine lace-mesh lid to confine the potential escape. Then, the containers incubated at 27±1°C, 65±5% RH and a 12L: 12D h cycle. The packages were extracted from the jars and examined for penetration daily. This pest penetrated in from <4 days and finally most of insects` penetration was occurred as usual at 15 days. When a puncture created on or one insect observed inside packaging, it would be as beginning of penetration and the data recorded. Each hole was made by the insects on the packaging polymers was counted as penetration but only way for determining penetrate percentage was counting of penetrated insects number of punctures because sometimes several insects could penetrate from one break. When insects` number reached maximum and no penetration accomplished later counting were stopped. In these tests, each thickness of each polymer was as one treatment. In addition, each treatment replicated 5 times.

The Test of Polymers Permeability to PH_3 Gas: Following preliminary tests, major concentrations of phosphine as a useful gas in store houses for controlling the pests' three different developmental stages were determined. For this test, little infected nuts to one of the different developmental stages of tested pest was placed into selected polymer from previous stage and then the openings of the prepared bags were sealed with a plastic press machine. The packages containing infected foodstuffs placed in the center of air-tight tanks with a volume of 31 m^3 per tank. Ten packages related to any developmental stage were placed inside each tank. The polymeric packages of different thicknesses were placed inside the tanks randomly. These tests carried out in the tanks empty space. According to FAO recommendations (Phostoxin® at 1g/m^3 for empty and closed warehouses), some phosphine was placed into each tank. Three gram Phostoxin tablet emits one gram phosphine gas. True doses selected from primary testes. Therefore, calculated doses for tobacco beetles larvae and eggs were (530.63, 115.32, 25.104, 5.49 and 1.17) mg/lit. Either, these quantities determined (378.92, 84.24, 18.73, 4.12 and 0.98) mg/lit for adults of the pest. In all these tests, a tank was as control test that in it packages were kept under normal environmental conditions without fumigation. The laboratory temperature was 20 °C. After 72 hours the tanks were opened and the packages were removed from the tanks. Each treatment replicated 10 times.

Egg Stage: In this stage a package was containing of five gram infected foodstuffs with 30 eggs 2-3 days-old of tobacco beetles. After 72 hours fumigation, the foodstuff inside bags held in an incubator (29 ± 1°C, 75 ± 5% R.H.) for 10 days until the eggs hatch. Then the dead and hatched, eggs in both treatment and control experiments were counted under a stereomicroscope. Then, with regard to the percentage mortality upon which its statistical analysis determined the best dose for controlling the pest eggs inside the packaging foodstuffs.

Larva Stage: The experiment procedure for larvae was similar to the egg stage. For this stage five gram nuts heavily infested with 30 last instar larvae were placed into polymer packages. After 72 hours fumigation the containers were removed and the percentage mortality registered.

Adult Stage: The process was similar to the previous stages. In each package containing 2 to 3 days-old adults were released. After 72 hours fumigation the packages were removed from the tanks and after aeration the live and dead insects were counted.

In this study, statistical analysis of data carried out with MSTATC, $SPSS_{10}$ and EXCEL software and Randomized Complete Design (RCD) and the means were compared with Duncan's mean test and T-test.

RESULTS

Analysis of variance showed significant differences ($P \leq 0.05$) among the polymers. From four kinds of used polymers, polypropylene had the least permeability against the pest insect as most of the pests unable to penetrate this polymer and if penetration was occurring, it was very less. There was a significant difference between permeability of thicknesses of 16.5 and 29 µm and consequently, contaminated products inside the polymers with thickness of 29 µm was very less than 16.5 µm ($P \leq 0.05$).

Table 2 and 3 indicate average of penetration percentage of insects while they were without food or with food on various packaging polymers. In these tables, penetration percentage of insect's life stage is different as last instar larvae penetration was more that first age larvae. In addition, larvae had more penetration than adult. More of species of insect's penetration occurred in 16.5 µm thickness of polymers at less than 48 hours but permeability of polymers with 29 µm thickness was occurring slowly. The results show permeability percentage of some packaging polymers against insects is related to both type and thickness bilateral effects. Fig. 1 shows *L. serricorne* penetration percentage in both with and without food in two thicknesses of packaging polymers in penetration's difference times.

In these curves, it found that penetration percentage at first days is very quick but whatever number of the insects into packages would be more, insect penetration percentage decreased. It was interesting that number of insects after the maximum penetration dwindled in next days and some exited from the packages for the reason of crowded. The holes created on packages usually were characterized by excess frass and webbing from larvae for pupating and moving and also by fragmented pieces of polymer around the holes. Those last age larvae that do not able penetrate, became changed to pupa on packages. Some larvae are unable to penetrate into any polymers while their adults are capable. Adults and larvae of the

Table 1:L Some properties of used different polymers for packaging foodstuffs

Properties		Polyethylene	Polypropylene	Polyvinyl chloride	Cellophane
Max. heat tolerance (°C)		82-93	132-149	66-93	90-140
Min. heat tolerance (°C)		-57	-18	-46 to -29	-77
Sun light resistance		moderate to good	moderate	good	good
Gas transmission (mm/100 cm^2 in 24 hand 25°C)	O_2	500	160	8-160	122-480
	N_2	180	20	1-70	33-90
	CO_2	2700	540	20-1900	2220
H_2O Absorption %		<0.01	<0.05	0	<0.03
H_2O Vapor transmission (g/100 cm^2 in 24h & 37.8°C & R.H. 90%)		1-1.5	0.25	4-10	0.2-1

Table 2: Average permeability percentage of different polymers to tobacco beetles in state of without food

		Pest insect's penetration in polymeric packaging (Average±SE)							
Polymer		Polyethylene		Cellophane		Polyvinyl chloride		Polypropylene	
Thickness (µm)		16.5	29	16.5	29	16.5	29	16.5	29
L. serricorne	A	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	F	8.4±0.22	5±0.31	16.2±0.6	12.6±0.22	6±0.54	1.2±0.36	0.0±0.0	0.0±0.0
	[1]	b	c	a	a	c	d	e	e
	L	4.6±0.22	3±0.31	11.4±0.22	4.6±0.4	3±0.002	0.0±0.0	0.8±0.5	0.0±0.0
	[1]	b	c	a	c	c	e	d	e

([1]: Being Bilateral Effect and Duncan's Test Grouping, A: Adult, F: First Instar Larvae, L : Last Instar Larvae)

Table 3: Average permeability percentage of different polymers to major stored-product insects in state of with food

Pest insecs`s penetration in polymeric packagings (Average±SE)									
Polymer		Polyethylene		Cellophane		Polyvinyl chloride		Polypropylene	
Thickness (µm)		16.5	29	16.5	29	16.5	29	16.5	29
L. serricorne	A [1]	1.4±0.85	0.0±0.0	3.8±1.61	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	F	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	L [2]	6±0.45	2.8±0.2	7.8±0.36	3.4±0.22	1±0.63	0.0±0.0	0.0±0.0	0.0±0.0

([1] : Being Bilateral Effect, [2] : Disbilateral Effect of Polymer and Thickness, A : Adult, F : First Instar Larvae, L : Last Instar Larvae)

Table 4: The probit analysis of *P. interpunctella* mortalities through PP packagings against determined concentrations of phosphine gas

Developmental	LC_{50} (mg/L)	LC_{95} (mg/L)	χ^2	P	Slope (b)	Intercept (a)
Stage Adult	12.68 (9.7-16.43)	782.8 (462.13-1537.31)	1.91	0.59	0.92	3.99
Larvae	23.71 (18.49-30.35)	1133.81 (611.66-2113.73)	4.02	0.26	0.98	3.65
Egg	40.76 (31.66-52.97)	2330.88 (1343.13-4693.33)	2.88	0.41	0.94	3.49

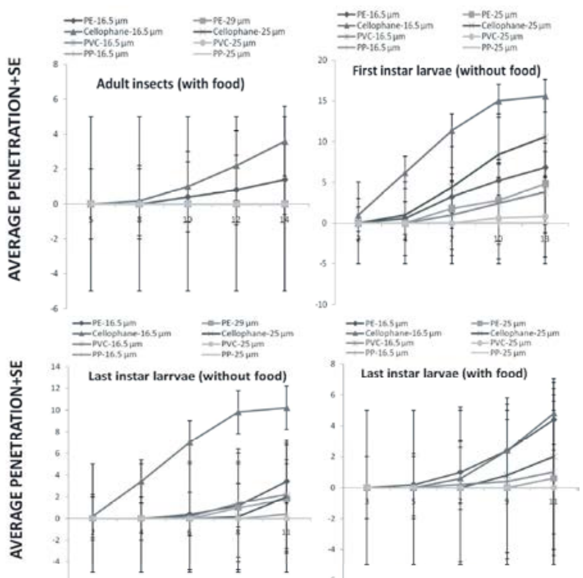

Fig. 1: Number of L. serricorne insects with and without food that penetrated tested polymeric packages (PE=polyethylene, PP=polypropylene and PVC=polyvinylchloride)

used species showed a much greater inclination for penetrate when released without food on polymer packages. According to results of this study, the penetration ability of insects is various based on insects` specie and life stage and polymer's kind and its thickness. On one part, fumigation of different products is frequently carried out under nylon covers where it is important for the polymer to be gas-permeability and transmit enough concentration of the fumigant inside. Fumigation by phosphine gas performed and then, comparison of the mean percentage mortalities of the developmental stages of the tested pest into packages was showed in the Table 4. These LC_{50} and LC_{95} determined the fumigant doses in the storages of maintaining foodstuffs packagings.

DISCUSSION

The results of this study should be viewed from point of view of using the different polymers for packaging as stored pests are unable penetrate into them. This topic could help to the sanitation of foodstuffs, it should be effective for the consumer's healthy and thus it would prevent spreading of contamination in stores. Permeability of used polymers including PE, PP, PVC and Cellophane to tested insects showed that there are significant differences between them. These polymers rank generally from easiest to most difficult to penetration, Cellophane, polyethylene, Polyvinylchloride and Polypropylene. The least penetration carried out by larvae in polypropylene polymers. Foodstuffs packaged by polypropylene polymers could provide the conditions and so, by suitable packaging the stored pest insects don’t access to food and they without food extinct. Also, remaining constant and subsequently decreasing the slope of the curves at insects' penetration last days (after maximum penetration) prove that insects always attempt to penetrate new food packages and their high activity is for availability to more food sources. In this study, the insects of without food had more penetration into polymers. The findings of our study are agreed with Cline's study [4].

The least penetration carried out by insects (adult or larvae) in polypropylene polymers with 29-µm thickness. Therefore, permeability the polymers with 29-µm thickness take place later. The results of this study agree with findings of previous studies such as Cline [4] that believed penetration of large larvae and adult insect of many species of stored pests to polyethylene and cellophane polymers with thickness of less than 29 µm is possible. Proctor and Ashman [17] suggest using of polyethylene layers with thickness of more than 65 µm in plastic bags and unsuitable using of bags with thickness of less than 40 µm. Highland and Wilson [18] believe that in this case polypropylene has a higher resistance than polyethylene (with equal thickness). Bowditch [7] undertook a study to evaluate the barrier qualities of two flexible transparent films of the same thickness against 1st and 5th ages larvae of *E. cautella* Walker and *P. interpunctella* (Hübner) and *T. confusum* Jacquelin du Val adults. He found that the polypropylene film tested was resistant to penetration by 1st-instar larvae of *E. cautella*. Moreover Fleurat-Lessard [19] reported that *Prostephanus truncates* can penetrate 30-300 µm polyethylene films. In the investigations, *L. serricorne* penetrated two thicknesses of PE, PVC and Cellophane, but did not be able to penetrate 16.5 µm thickness of PP polymers, even. Therefore, it is one of the important results in this study that in insect's penetration what has principle role is following first polymer kind and subsequently its thickness. In addition, fumigation of different products is frequently carried out under nylon covers where it is important for the polymer to be gas-permeability and transmit enough concentration of the fumigant inside. Hall [14] and Stout [9] consider that plastic sheeting (polyethylene and polyvinyl chloride) less than 0.1 mm thick is permeable to phosphine. Appert [10] claims that opaque polyethylene or polypropylene with 300 µm thickness in plastic packages is suitable for conserving fumigated grain seeds. He believes that polyethylene films of 150- 200 µm thickness are suitable for fumigation. Iqbal [12] showed that polyethylene sheetings with 200µm thickness are suitable to retain sufficient concentration of phosphine to kill *Tribolium confusum*. Valentini [13] reported that polyethylene and polyvinyl chloride 210 µm thick prevents phosphine exchange. In addition, ACIAR [11] believed that 200 µm films of polyvinyl chloride and polyethylene have a low permeability to methyl bromide. Marouf and Momen [15] suggested that polypropylene liners of less than 100 µm thickness are suitable as inner liners of jute bags to allow the fumigant to enter the bags. Therefore, with order to the results of this study and other studies, it is suggested that polyvinylchloride with 29 µm thickness could be the best polymer for different products which need to packaging and fumigation and could pass and retain enough concentration of the fumigant through the packages. With regard to bioassay tests, it proved that eggs of *L. serricorne* are the most resistant stage against phosphine gas and the concentration of 40.76 mg/L (31.66-52.97) as LC_{50} is adequate for killing pest eggs into packaged cereals.

It is evident that polypropylene liners with 29µm thickness are suitable for foodstuffs packaging to allow the fumigant to enter the packages (because of their high permeability) and so, to prevent insect pests from entering the packages, thus protecting the products from recontamination. Therefore, the results presented here would lead to a reduction in the economic losses associated with infestation and minimize injury to company image as a manufacture of high quality foodstuffs.

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