CONTROL OF *CALLOSOBRUCHUS MACULATUS* (COLEOPTERA: BRUCHIDAE) IN INDUSTRY OF PACKAGING FOODSTUFFS

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The contamination of various varieties of stored cowpea to *Callosobruchus maculatus* Fabr. (Coleoptera: Bruchidae) was a subject for our study to determine the most resistant packaging polymer against insect's penetration, from storage to consumption of these products. Different polymers (PE, PP, PVC and Cellophane) with two thicknesses of 16.5 µm and 29 µm were used. There was a significant difference ($p \leq \leq 0.05$) in the penetration of insects to those polymers. Cellophane packages with thickness of 16.5 µm were the worst polymer as the products into cellophane packages had complete infestation and there were a lot of punctures on the packages while foods into polypropylene packages with 29 µm thickness were safe. Also, fumigation after packaging is effective for preventing re-infestation. For this purpose, infested cowpea with two developmental stages of mentioned pests (eggs and adults) were placed inside the packages in air-tight tanks. After fumigation, the penetration of PH_3 through the packages was investigated by calculating percentage insect mortality. The results showed that the best polymer for packaging cowpea and grains-based products, resistant to insects penetration but permeable to gas, is polypropylene (PP) with 29 µm thickness.

Key words: *Callosobruchus maculatus*, cowpea, penetration, packaging, contamination, phosphine gas.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp (Fabaceae family) is attacked by pests both in the field and during storage. *Callosobruchus maculatus* Fabr. (Coleoptera: Bruchidae) insects are the most serious pest threat to stored cowpea and the family of Fabaceae, at present, are mainly controlled by fumigation. Pods of cowpea stored for 8 months could have as much as 50 % of the grains damaged by *C. maculatus* (Caswell, 1984). The response of *C. maculatus* is different to various varieties of cowpea (Dick & Credland, 1986). Although, finished products could be shipped from production facilities uninfected, stored product insects can enter 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 (Hou *et al*., 2004).

When a packaging containing one of insect's life stages enters into storages (infested packaging), it could spread the contamination to other packages. 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 (Highland, 1981). The ability of species to penetrate materials may vary between life different stages (Cline, 1978). Therefore, while one product is packaged, the probability of contamination in products is forecast that the insects penetration diminishes by kind of 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 (Domenichini & Forti, 1975; Fletcher & Childs, 1976; Cline, 1978; Bowditch, 1997). So, information of polymers permeability to stored pest insects for selecting 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 frequently used fumigant because of its simple application (Ferizli *et al*., 2004). Polymers with various thicknesses have different permeability to fumigants (Stout, 1983; Appert, 1987; ACIAR, 1989; Iqbal *et al.,* 1993; Valentini, 1997; Hall, 1970; Marouf & Momen, 2004). 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 were 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.

MATERIAL 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 thicknesses of 16.5 μm and 29 μm. Table 1 shows some important properties of these polymers (Odian, 2004; Marouf & Momen, 2004). 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 with the aid of a press plastic machine for packaging 15 g cowpea. These packages were completely without any pores. The insects (all obtained from laboratory cultures) included *Callosobruchus maculatus* Fabr. adults 5 days-old. We placed them into petri dishes at 25±1 °C, 75±5 % RH and a 12L: 12D h cycle. 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 in two mentioned states for examination of thickness of each polymer (Bowditch, 1997). In the first state, the starved insects were released during the test around the packages, which were containing foodstuffs. In this experiment, we specified the abilities of the stored-product insects to enter into packages. In second state, the insects with very little cowpea (2 gr) were placed inside packages in order to determine the ability of the species to puncture the packages and the creation of contamination into other packages. Each container was 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. These insects penetrated in from < 2 days and finally most of insects' penetration has occurred as usual at 9 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 made by the insects on the packaging polymers was counted as penetration but the only way for determining penetration 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 was accomplished later counting was 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 cowpea to one of the different developmental stages of tested pest including egg and adult 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 were placed in the center of air-tight tanks with a volume of 31 m^3 per tanks. Fumigation was performed in the tanks with aluminum phosphide tablets, which emit PH_3 when exposed to air. 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 were carried out in the tanks empty space. According to FAO recommendations (Phostoxin® at 1 g/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 were selected from primary testes. Therefore, calculated doses for cowpea weevil eggs were (530.63, 115.32, 25.104, 5.49 and 1.17) mg/l. Either, these quantities were determined (378.92, 84.24, 18.73, 4.12 and 0.98) mg/l for adults of the pest. In all these tests, a tank was used as control test as packages were kept into it under normal environmental conditions without fumigation. The laboratory temperature was 20 ºC. 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 contained five gram infected foodstuffs with 30 eggs 2-3 days-old of cowpea weevil. After 72 hours fumigation, the foodstuff inside bags was held in an incubator (29 ± 1 ºC, 75 ± 5 % R.H.) for 7 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 its statistical analysis determined the best dose for controlling the pest eggs inside the packaging foodstuffs.

ADULT STAGE

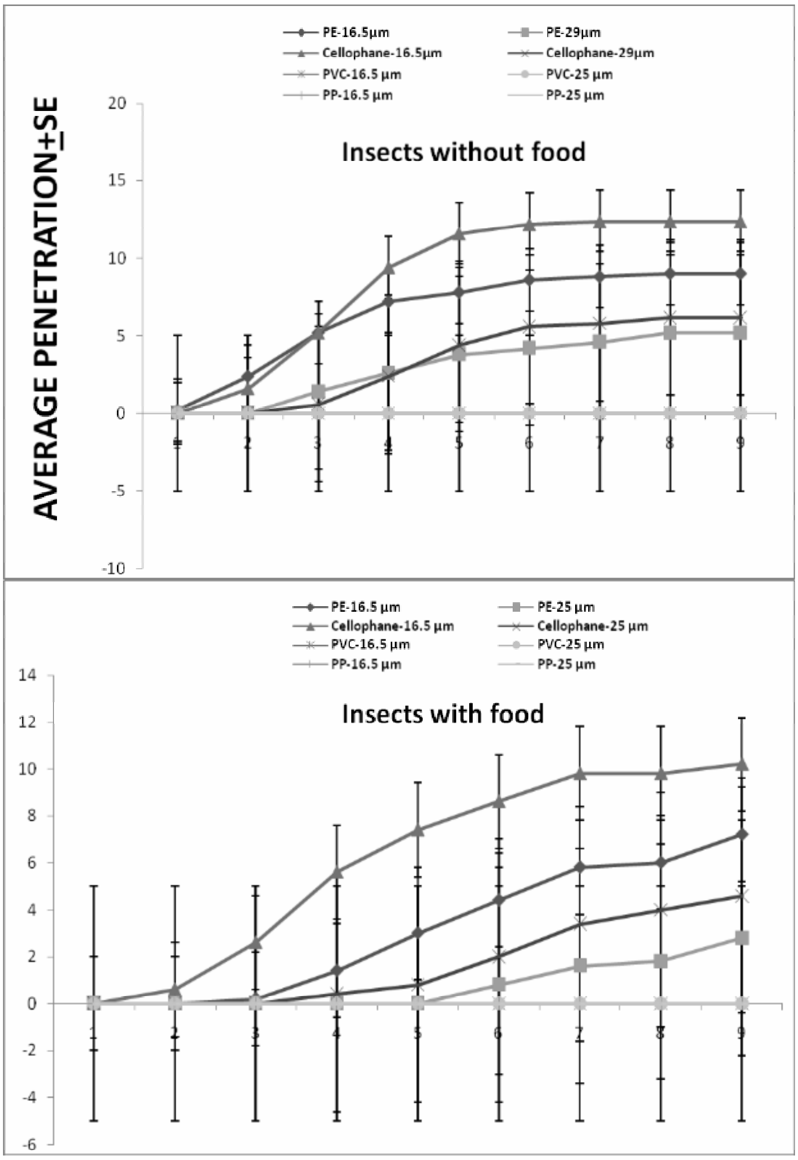
The process was similar to the previous stage. In each package 2 to 3 days-old adults were released. After 72 hours fumigation the packages were removed from the tanks and after aeration the live and dead insects were counted.

STATISTICAL ANALYSIS

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 were unable to penetrate this polymer and if penetration occurred, it was less. There was a significant difference between permeability of thickness of 16.5 and 29 µm and consequently, contaminated products inside the polymers with thickness of 29 µm were less than in polymers at 16.5 µm ($P \leq 0.05$).



Days of exposure

Fig. 1. Number of *C. maculatus* insects with and without food that penetrated tested polymeric packages (PE = polyethylene; PP = polypropylene; PVC = polyvinylchloride).

Table 2 indicates average of penetration percentage of insects while they were without food or with food on various packaging polymers. More insect's penetration occurred in 16.5 µm thickness of polymers at less than 48 hours but permeability of polymers with 29 µm thickness occurred slowly. The results show permeability percentage of some packaging polymers against insects is related to both type and thickness bilateral effects. Fig. 1 shows *C. maculatus* penetration percentage in both with and without food in two thicknesses of packaging polymers in penetration's difference times.

Table 1

Some properties of different polymers used 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 h and 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 cowpea weevil

Pest insects`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
C. maculatus (without food)	A[2]	9±0.31	2.4±0.22	6.2±0.36	5.4±0.22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C. maculatus (with food)	A[1]	7.2±0.2	2.4±0.22	10.2±0.2	4.6±0.22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

([1]: There is no bilateral effect between polymer and thickness, [2]: There is bilateral effect)

In these curves, it was found that penetration percentage at first days is very quick but whatever number of insects into packages would be, insect penetration percentage decreased. It was interesting that the number of insects after the maximum penetration dwindled the next days and some exited from the packages for the reason of being 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. Adults showed a much greater inclination for penetration when released without food on polymer packages.

Table 3

The probit analysis of *Callosobruchus maculatus* mortalities through PP packagings against determined concentrations of phosphine gas

Developmental Stage	**LC_{50} (mg/L)**	**LC_{95} (mg/L)**	χ^2	**P**	**Slope (b)**	**Intercept (a)**
Adult	6.22 (4.7-8.044)	275.947 (174.26-496.689)	3.89	0.59	0.99	4.21
Egg	32.56 (24.73-42.7)	1730.5 (998.2-3535.11)	4.07	0.25	0.95	3.56

According to results of this study, the penetration ability of insects is various based on insects` species 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 shown in Table 3. These LC_{50} and LC_{95} determined the fumigant doses in the storages of maintaining foodstuffs packages.

DISCUSSION

The results of this study should be viewed from three aspects: 1 – the use of polymers for foodstuffs packaging as stored pest insects unable to penetrate through them, 2 – the use of polymers as gas-permeable covers to pass by bags to fumigate goods in stacks or bulks and minimize cross-infestation, 3 – the use of polymers as gas-proof covers to fumigate products into stacks. These topics could help to the sanitation of foodstuffs, it should be effective for the consumer's health, 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 the easiest to the most difficult to penetration, Cellophane, polyethylene, Polyvinylchloride and Polypropylene. The least penetration is carried out in PP and

PVC polymers. Foodstuffs packaged by PP and PVC polymers could provide the conditions and so, by suitable packaging the stored pest insects do not access to food and without food they become 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 without food had more penetration into polymers. The findings of our study are agreed with Cline's study (1978). The least penetration carried out by insects (adult or larvae) was in polypropylene polymers with 29 µm thickness. Therefore, permeability of polymers with 29 µm thickness takes place later. The results of this study agree with findings of previous studies such as Cline's (1978) that believed penetration of large larvae and adult insects of many species of stored pests to polyethylene and cellophane polymers with thickness of less than 29 µm is possible. Proctor & Ashman (1972) suggest using of polyethylene layers with thickness of more than 65 µm in plastic bags and unsuitable use of bags with thickness of less than 40 µm. Highland & Wilson (1981) believe that in this case polypropylene has a higher resistance than polyethylene (with equal thickness). Bowditch (1997) 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, Fleural-Lessard & Serrano (1990) reported that *Prostephanus truncates* can penetrate 30-300 µm polyethylene films. In the investigations, *C. maculatus* penetrated both thickness of PE and of Cellophane, but it was not able to penetrate 16.5 and 29 µm thickness of PP and PVC polymers. Therefore, it is one of the important results in this study that in insect's penetration what has a principle role is 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-permeable and transmit enough concentration of the fumigant inside. Hall (1970) and Stout (1983) consider that plastic sheeting (polyethylene and polyvinyl chloride) less than 0.1 mm thick is permeable to phosphine. Appert (1987) 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 *et al.* (1993) showed that polyethylene sheetings with 200 µm thickness are suitable to retain sufficient concentration of phosphine to kill *Tribolium confusum*. Valentini (1997) reported that polyethylene and polyvinyl chloride 210 µm thick prevent phosphine exchange. In addition, ACIAR (1989) believed that 200 µm films of polyvinyl chloride and polyethylene have a low permeability to methyl bromide. Marouf & Momen (2004) 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. They believed in comparison to other polymers, polyvinyl chloride could be a suitable polymer (cover) for fumigation products under it. Therefore, from the results of this study and other studies, it is suggested that polyvinylchloride with 29 μm thickness could be the best cover for different products which need fumigation and could retain enough concentration of the fumigant inside the packages. In contrast, polypropylene has high permeablility to gas. With regard to bioassay tests, it was proved that eggs of *C. maculatus* are the most resistant stage against phosphine gas and the concentration of 32.56 mg/L (24.73-42.7) as LC_{50} is adequate for killing pest eggs into packaged cereals.

CONCLUSION

With regard to the results of this study it is evident that from four current polymers for fabaceous grains packaging, polypropylene nylons with 29 μm thickness are the most suitable to prevent penetration of pest insects to the packages and also, to allow the fumigant to pass through packaged grains and protect 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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Received September 14, 2010

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