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EVALUATION OF DIFFERENT PLASTIC PACKING MATERIALS AND FOOD SUBSTRATES ON EFFICACY OF PHOSPHINE FUMIGATION AGAINST LARVAE OF *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE) AND *TROGODERMA GRANARIUM* (EVERTS) (COLEOPTERA: DERMESTIDAE)

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ABSTRACT

Plastic packaging provides a barrier to insects and micro-organisms from getting access to food besides maintaining the internal temperature. In order to see the effect of different packing materials and food substrates on efficacy of phosphine fumigation against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae), an experiment was conducted at the Entomological Laboratory of the University College of Agriculture and Environmental Sciences, The Islamia university of Bahawalpur. Insects were reared on natural diet at optimum conditions of 30±2 ºC and 65±5 % relative humidity. Three packing materials i.e. polyethylene, polypropylene, polyvinylchloride and four food substrates were used i.e. wheat flour, rice flour, corn flour and oat flour for *T. castaneum* while wheat, rice, mung bean and black gram for *T. granarium.* The effect of different packing materials on percent mortality of *T. castaneum* and *T. granarium* was significant at 24 and 48 hours after the treatment while the effect of food substrates on percent mortality was significant at 24 hours for *T. castaneum* and 48 hours for *T. granarium.* The maximum percent mortalities of 83% and 98% were recorded in *T. castaneum* at 24 and 48 hours in all the food substrates packed in polyethylene and polypropylene. Similarly, the maximum percent mortalities of 55% and 88% were recorded in *T. granarium* at 24 and 48 hours in all the food substrates packed in polyethylene and polypropylene. It was concluded that polyethylene and polypropylene are the most suitable packing materials for various food substrates in order to control *T. castaneum* and *T. granarium*.

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INTRODUCTION

Pakistan ranks sixth in the world with regard to its population which is estimated at 199 million with annual growth rate of 1.86% (GOP, 2017). Pakistan produces 20 Mt (casually) of cereal grains annually with wheat, rice and maize being the most important ones (Salam, 2012). Considerable (i.e. 10-20%) stored grain losses are reported to occur in Pakistan due to insect pests (Ahmad and Afzal, 1984; Ahmedani et al., 2011; Jilani, 1981). Similar trends have been reported from other regions of the world i.e. 5-10% in temperate and 20-30% in tropical regions (Nakakita, 1998; Shaaya et al., 1997). The post-harvest losses due to insect pests also encompass the quality parameters of stored grains in terms of nutritional values, taste and odor (Boxall, 2001; Padín et al., 2002; Shankar and Abrol, 2012).

Fumigation under properly sealed conditions i.e. warehouses, shipping containers and other storage structures is a wieldy adopted technique to control stored grain insect pests. The commonly used fumigants are phosphine (Aluminium phosphide) and methyl bromide while others are selectively used including carbonyl sulfide, sulfuryl fluoride and ethylene dichloride-carbon tetrachloride (EDCT) (Rajendran, 2016a, b; Shankar and Abrol, 2012). The rate (concentration) of application of fumigants vary with types of storage structure and kinds of grain commodity to be stored e.g. 3 tablets of aluminum phosphide are recommended for one ton of paddy rice (Rajendran et al., 2001).

Variety of storage structures are being used in different countries and cultures i.e. mud, plastic, wooden or metal bins, hessian cloth, jute bags, bamboo or brick structures and concrete rooms (Bakhtawar, 2013; Baloch et al., 1994; Shankar and Abrol, 2012). More than thirty types of both flexible and rigid forms of plastics are being used across the world as packaging materials i.e. polyethylene, polypropylene, polyvinyl chloride, polyvinylidene chloride, polyethylene terephthalate (PET), polyethylene naphthalate (PEN), polystyrene, polycarbonate, polybutene and polyamide etc. (Baner and Piringer, 2008; Marsh and Bugusu, 2007).

Ahmed (1983) regarded 7 out of 18 listed stored grain insect pests as the most destructive species in Punjab, Pakistan. These include *Trogoderma granarium*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Sitotroga cerealella*, *Corcyra cephalonica*, *Tribolium castaneum* and *Callosobruchus chinensis*. *Trogoderma granarium* is amongst one of the notorious primary pests of stored grains across the world (Tayoub et al., 2012), causing 2%-10% losses to stored grain products (Hafiz and Hussain, 1961; Irshad and Iqbal, 1994; Khan and Cheema, 1978). Being primary pest, it consumes the whole grains; leaves husk and decreases seed germination ability (Ahmedani et al., 2007; Jood and Kapoor, 1993).

Tribolium castaneum is also one of the most devastating stored grain pests of cracked cereal grains and their byproducts in tropical and sub-tropical regions of the world. Both, the adults and the larvae cause damage (Nadeem et al., 2012). Red flour beetle may cause carcinogenic effects by releasing, benzoquinones in the flour (El-Mofty et al., 1989; Lis et al., 2011).

Irshad and Iqbal (1994) regarded substandard practices as one of the important reasons for development of resistance against phosphine in *T. granarium*. The resistant strains of *T. granarium* are reported to have very low mortality i.e. 5-12% (Borah and Chahal, 1979). Previous studies suggest that most of the populations of *T. granarium* are resistant against phosphine in Pakistan (Alam et al., 1993). Similar trends have been reported from Bangladesh (Tyler et al., 1983) and India (Rajendran et al., 2001).

Few recent studies have shown some interesting interactions between packing materials (e.g. Polyethylene, polypropylene, polyvinyl chloride and cellophane) and food substrates in terms of mortality as resulted by phosphine gas (Allahvaisi et al., 2010; Qasim et al., 2013). However, larval stage of *T. castaneum* has not been studied so far as previous literature suggests the use of only adults in interaction with packing material and food substrates. On the other hand, *T. granarium* has been subjected to such sort of studies except on mung bean and black gram.

Current study was planned to investigate efficacy of phosphine tablets at their recommended dose against *T. castaneum* and *T. granarium* larvae in interaction with different packing materials and food substrates. We used larvae of *T. castaneum*, mung bean and black grams for the first time in our study.

MATERIAL AND METHODS

Collection and rearing of insects: The adults and larvae of *Tribolium castaneum* and *Trogoderma granarium* were collected from local markets of district Bahawalpur, Punjab, Pakistan during January, 2017. The collected populations were shifted to the laboratory of Department of Entomology, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur. Adults of *T. castaneum* were reared on the artificial diet comprising of wheat flour and yeast with the ratio of 95:5, respectively. Larvae of *T. granarium* were reared on whole wheat grains. Both the species were reared in plastic jars of 182 cubic inches under the optimum temperature of 30±2 ºC and relative humidity of 65±5 %. To obtain homogenous larvae of similar age, both the species were allowed to produce F1 generation under controlled conditions. One month after the inoculation, the adults were sieved out and diet containing eggs were separated for obtaining F1 generation.

Packaging materials and tin box preparation: Three types of packaging materials were used i.e. polyethylene, polypropylene and polyvinylchloride with homogenous thickness of 0.02 mm. Plastic pouches of 8×12 cm i.e. width x length was prepared from these packing materials with the help of an electrical impulse heat sealer. Each plastic pouch was filled with four different types of food substrates (50 g each) for experimental insects i.e. wheat flour, rice flour, corn flour and oat flour for *T. castaneum* while wheat, rice, mung bean and black gram for *T. granarium.* There were three replications for each substrate under each plastic packing material. Twenty larvae of each insect were released into their respective plastic pouches and then sealed with electrical impulse heat sealer.

A fumigation chamber of 0.7 m^3 -made up of tin- was used for phosphine fumigation. A phosphine tablet was placed in the center of the chamber while all the 72 plastic pouches (i.e. comprising of four substrates, three packing materials, two insect species in three replications). Each plastic pouch was labeled for their respective food substrate, packing material used, insect species and replication number. Since for effective pest control, the recommended dose of phosphine tablet is 1.5 g/m^3, we used 1 phosphine tablet for our fumigation chamber of 0.7 m^3. One set of each treatment (24 pouches i.e. comprising of four substrates, three packing materials and two insect species) –regarded as control treatments- was placed in another fumigation chamber without phosphine tablets.

Data recording and analysis: We maintained this experiment in two splits. In one experiment we recorded percent mortality after 24 hours and in other experiment after 48 hours. For recording mortality, we simply touched the individuals with camel hairbrush; they were considered as dead if remained motionless. We used two-way ANOVA to see significance among three packing materials in interaction with four food substrates in terms of percent mortality, separately for both the insect species. Means were compared using Tuckey test at 5% level of significance. Computer software XLSTAT was used for the analysis (XLSTAT, 2008).

RESULTS

In case of *T. castaneum*, the effect of different packing materials on percent mortality was also significant at 24 and 48 hours after the treatment (d.f.=2, f=88.44, p=0.0001; d.f.=2, f=234.35, p=0.0001, respectively). However, the effect of food substrates on percent mortality was significant (d.f.=3, f=3.69, p=0.02) only at 24 hours after the treatment. The interaction between packing material and food substrates in terms of percent mortality was not significant at 24 and 48 hours after the treatment (Table 1).

Table 1. ANOVA results of percent mortality of *T. granarium* and *T. castaneum* in interaction with different packing materials and food substrates at alpha 0.05.

Source of variation	d.f	Mean square values			
		Trogoderma granarium		*Tribolium castaneum*	
		24h	48h	24h	48h
Packing materials	2	19728*	28341*	6186*	3053*
Food substrates	3	131^{ns}	85*	258*	15^{ns}
Packing material-Food substrate	6	132*	37^{ns}	98^{ns}	17^{ns}

*difference significant at 0.05 level, ^{ns}Non significant

The maximum and statistically similar percent mortalities of >83% and >98% were recorded at 24 and 48 hours after treatment, respectively in all the four food substrates (i.e. wheat flour, rice flour, corn flour and oat flour) packed in polyethylene and polypropylene. On the other hand, the minimum percent mortalities (i.e. <58% and <77% at 24 and 48 hours, respectively) were recorded in food substrates packed in polyvinylchloride (Figure 1).

The effect of different packing materials on percent mortality of *T. granarium* was significant at 24 and 48 hours after the treatment (d.f=2, f=387.46, p=0.0001; d.f=2, f=1069.5, p=0.0001, respectively). However, the effect of food substrates on percent mortality was significant (d.f.=3, f=3.23, p=0.04) only at 48 hours after the treatment. The interaction between packing material and food substrates in terms of percent mortality was significant only (d.f.=6, f=2.61, p=0.04) at 24 hours after the treatment (Table 1).

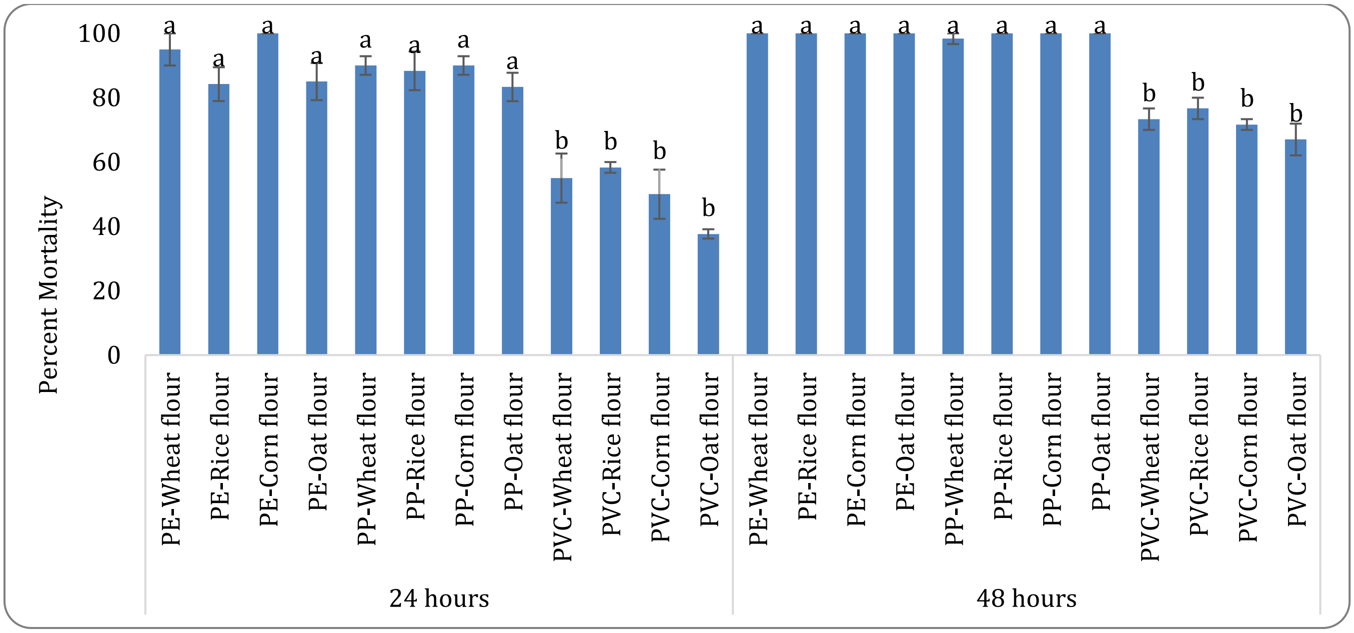

Figure 1. Effect of different packing materials on percent mortality of *T. castaneum* as affected by different food substrates at 24 and 48 hours after phosphine treatment. Error bars show ± standard error. Means sharing similar lettering are statistically non-significant at alpha 0.05. PE= polyethylene, PP= polypropylene and PVC= polyvinylchloride).

At both the observation times (i.e. 24 and 48 hours), the maximum and statistically similar percent mortalities (i.e. >55% and >88%, respectively) were recorded in all the four food substrates (i.e. wheat, rice, mung bean and black gram) packed in polyethylene and polypropylene. On the other hand, the minimum percent mortalities (i.e. <5% and <6% at 24 and 48 hours, respectively) were recorded in food substrates packed in polyvinylchloride (Figure 2).

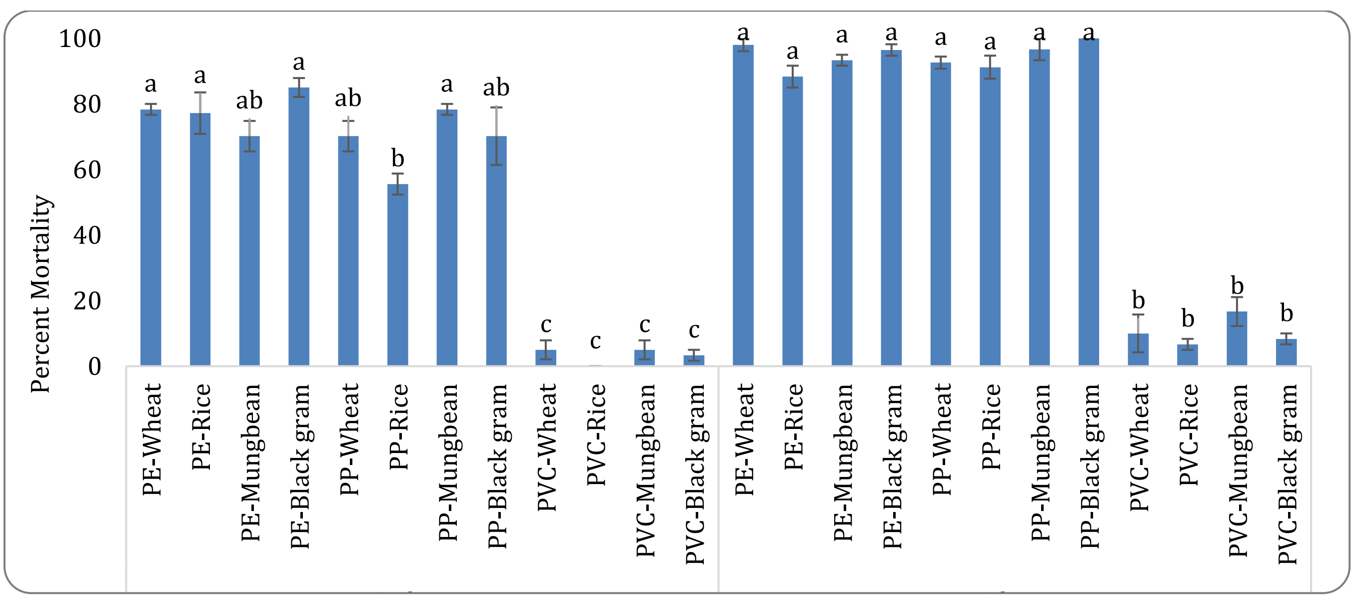

Figure 2. Effect of different packing materials on percent mortality of *T. granarium* as affected by different food substrates at 24 and 48 hours after phosphine treatment. Error bars show ± standard error. Means sharing similar lettering are statistically non-significant at alpha 0.05. (PE= polyethylene, PP= polypropylene and PVC= polyvinylchloride).

DISCUSSION

Results of this study showed higher mortality at 48 hours in both the insects. Similar results have been reported by previous studies. Bell et al. (1984) reported that very few larvae of *T. granarium* survived 120 hours after the phosphine exposure at 0.6 g/m^3 or above. Bell and Wilson (1995) also reported the maximum mortality in laboratory reared larvae of *T. granarium* at 96 hours after phosphine. Similar trend has been reported in case of *T. castaneum*. Qasim et al. (2013) observed the maximum mortality of *T. castaneum* at 96 hours after phosphine application. In our results, *T. castaneum* larvae showed greater mortality than that of *T. granarium* at 24 and 48 hours after the phosphine exposure. This is might be because *T. granarium* prefers feeding in crevices of whole grains while feeding in whole grains has reported to prolong the mortality time (Bell and Wilson, 1995). Moreover, larvae of both the insects have shown to have more susceptibility towards phosphine than pupae and adults (Rajendran, 1992).

In our findings, the minimum mortality of *T. granarium* and *T. castaneum* larvae was recorded in PVC than other packing materials at 24 and 48 hours. Barrier properties and film permeability is influenced by several factors i.e. food, environmental conditions, polymer thickness, structure etc. Permeability is also depends on the shape, polarity, air pressure and size of the molecule as well as polymer crystallinity (Siracusa, 2012). The mortality of *T. granarium* and *T. castaneum* was similar in PE and PP with thickness of 0.02 mm. Packing materials with thickness ranging from 0.01 to 0.15 mm have been tested for their permeability towards phosphine gas against many stored grain insect pests i.e. *T. castaneum*, *T. grararium, Sitophilus granarius*, *Rhyzopertha dominica, Sitotroga crealella, Oryzaephilus surinamensis, Plodia interpunctella, Lasioderme serricorne, Ephestia kuehniella, Batrachedra amydraula* and *Callosobruchus maculates.* Both the packing materials have shown greater mortality due to high permeability of phosphine gas (Allahvaisi et al., 2010; Hassan et al., 2016; Marouf and Momen, 2004; Qasim et al., 2013).

In case of *T. granarium*, all the four food substrates showed different mortality rates while the least was recorded in the rice. This might be because of feeding behavior of *T. granarium* i.e. they bore and hide in crevices (Bell and Wilson, 1995). This offers physical barrier to exposure to phosphine gas. On the other hand, in case of *T. castaneum*, all the four food substrates showed the similar percent mortality which again might be due to their feeding behavior i.e. they are secondary pests (Zettler and Cuperus, 1990) and do not hide in crevices.

In conclusion, because of their high permeability towards phosphine gas, polyethylene and polypropylene are the most suitable packing materials for various food substrates in order to control stored grain insect pests.

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