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Management of *Tribolium castaneum* (Coleoptera: Tenebrionidae) with Phosphine Fumigation in Relation to Packaging Materials and Food Types

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Abstract.- Management of *Tribolium castaneum* was studied in relation to packaging materials and food types with phosphine fumigation. Penetration ability of the beetles was checked for three packaging types' *viz.*, polyethylene (PE), polypropylene (PP) and polyvinylchloride (PVC). Out of the 3 packaging materials PE was a susceptible packaging as compared to the other two types. The efficacy of phosphine was tested against *T. castaneum*in four commodities *viz.*, wheat, rice, whole-wheat flour and semolina, which were packed in four different packaging types namely PE, PVC, PP and jute bags. Experiments were conducted to check the comparative mortality of *T. castaneum* with phosphine fumigation as affected by the above two factors. Efficacy of phosphine was evaluated at 1.5 g/m^{3}dose measured after 2 and 4 days exposure period. Maximum mean mortality was generated after 4 days of treatment than after 2 day exposure period. This was irrespective of the other two factors *i.e.* packaging material and food types. The data are extremely useful for the management of *T. castaneum* with phosphine fumigation while using tin type storage containers.

Key words: *Tribolium castaneum*, phosphine fumigation, packaging material, commodity types, stored grain pests' management.

INTRODUCTION

Annual post-harvest losses of stored cereals range from 10-20% of the overall production, in which insect damage, microbial deterioration and other factors play the major role (Phillips and Throne, 2010). A primary factor in these losses is due to ravages of stored product insect pests that can reduce the quality and quantity of grains (Weaver and Subramanyam, 2000). In Pakistan, estimates of storage losses of food grains due to insects have been reported to vary 4-10% (Huque *et al.*, 1969), about 5.08% (Chaudhary, 1980), 5% (Ahmad, 1984) and 3.5-25.5% (Irshad and Balouch,1988).

Stored grains insect pests make grains unpalatable and unmarketable due to depletion of specific nutrients (Jood and Kapoor, 1994). Among these stored grains insect pests, the red flour beetle *T. castaneum* is very common and most destructive pest throughout the world and is generally found in granaries, mills, warehouses and stored grains. The presence of this pest in stored products results in contamination, economic damage and also decreases its nutritive value (Burkholder and Faustini, 1991). The red flour beetle not only affects the quality and quantity of grains but it also attacks the germ part or embryo portion of grains (Mondal, 1994).

Fumigants are the most potent weapons in managing stored grain insect pests, due to their broad spectrum of activity and minimal or no residues on the treated products and among the fumigants phosphine has largely replaced most of the other fumigants and has got a great potential for management of stored grain insect pests of vast majority in developing countries. Efficacy of a fumigant depends upon the factors like the fumigant used, temperature, dose rate, exposure period and sorption of gas, which in turn depends upon the commodity in which fumigant is being applied and the packing materials that should represent the gas

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holding capacity for a given period.

Grains and the by-products are packed frequently in jute or plastic bags. Packaged foods face many challenges before they are finally consumed. The development of insect resistant packing is of an increasing importance to both the consumer and the manufacturer. The effective packing methods ensure that the packed food remain insect free until these are consumed which is another important future concern (Mullen *et al.*, 2012). The packaging pests are of two categories; penetrators and invaders. Invaders have weaker mouth parts and they enter into the bags by openings which are made by other means. Penetrators on the other hand, chew the bags, make holes by themselves and enter into the commodities (Highland, 1984). Sorptive abilities of food commodities vary, and commodity sorption can be a major factor in determining whether a lethal concentration of fumigant is achieved or not under sufficiently airtight conditions (Banks 1993, Sinclair and Lindgren, 1958).

Although many aspects of stored grains insect pests management have been studied in the previous studies but the relationship of packing material and commodities types in relation to fumigation efficacy have not been seen. Present research is a part of an ongoing research project dealing with effect of packaging materials on penetration ability and fumigation efficacy due to phosphine for major stored grain insect pests and their immature stages. The current study is about the red flour beetles. This study will be investigating the effect of different plastic films on penetration ability by red flour beetle adults and efficacy of fumigation by phosphine shall be evaluated against adult red flour beetles in relation to four packaging materials and four food types administered at 1.5 g/m^3 kept for two lengths of time *i.e.* 2 and 4 days.

MATERIALS AND METHODS

Collection and rearing of insects

Tribolium castaneum was collected from different regions of Punjab. The collected populations were reared in the laboratory of Entomology, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur. Insects were fed on a diet comprising of whole wheat flour and yeast (95: 5 by weight) in plastic jars of 1 kg volume, at optimum conditions of 30 ± 2 °C and 65 ± 5 R .H.

Obtaining homogenous age adults

From the culture, adults of *T. castaneum* were taken and allowed to lay eggs in separate plastic jars containing sterilized whole wheat flour for a period of two weeks. After that adults were sifted out and flour containing eggs of *T. castaneum* was left for egg development inside the laboratory. These jars were kept for about one month for adult emergence. After one month from the date of sifting parent adults, emerging adults were about one week older because according to Rees (2001) it takes them 25 days from egg to adult at 30°C.

Experimental materials

Three types of plastic films *i.e.*, PE or Polyethylene, PP or Polypropylene and PVC or Polyvinylchloride were purchased from a plastic factory outlet in Faisalabad, Punjab, Pakistan at Rs. 250 Pak Rupees/KG with homogeneous thickness of 0.02 mm measured with a Digital Micrometer Mitotoyo Corporation. These three types of plastic films were used to evaluate the penetration ability by the red flour beetles. For fumigation test, jute bags of same size were also prepared and comparative efficacy of phosphine was checked against red flour beetles as affected by four types of packaging materials *viz*., PT, PP, PVC and Jute and four types of foods *viz*., wheat, rice, wheat flour and semolina.

Penetration test

Wheat flour was filled in to bags made up of PE, PP and PVC plastic types of 8 x 10 cm size which were placed in 1 kg volume plastic jars. Thirty homogenous age red flour beetles were released outside the bags of plastic films. In all there were 4 jars to replicate the experiment four times. Data were recorded after 3 days since the time adults were released in to jars to check the comparative penetration by the beetles in to wheat packed in different plastic films. Any penetrated adults were counted by sieving the flour and by visually observing holes made on plastic films.

Similar procedure was adopted to measure the penetration after 6 days of treatment.

Fumigation test

For fumigation experiments four types of packaging materials were used *viz.*, Polyethylene, Polypropylene, Polyvinylchloride and jute. Bags of 8x10 cm size were prepared to pack different commodities *viz.*, wheat, rice, whole wheat flour and Semolina. A tin box of 0.7 m^3 volume capacity was prepared for phosphine fumigation. We used 0.7 m^3 tin box because it ensured the phosphine dose of 1.5 g/m^3. A 3g tablet releases 1 g phosphine gas after contact with ambient air. Therefore using 3 g of phosphine tablet in 0.7 m^3 tin box ensured 1.5 g/m^3 phosphine gas.

Experimental procedure

Tin box was prepared with 4 chambers of equal size at the base for treatment replication & a central support was created to hold phosphine tablet. Every packaging material contained 4 types of commodities and it was replicated 4 times in different chambers of tin box. In all there were 64 combinations for packaging and commodities. In every packaging 30 adults of *T. castaneum* of one week age were released and mortality was checked after 2 days since the time of phosphine application e.g. 3 g tablet wrapped in muslin cloth was placed on central support. Same procedure was repeated for checking mortality of *T. castaneum* after 4 days of phosphine fumigation application.

Data analysis

Statica 6.1 analyzed data statistically with Completely Randomized Design (CRD) 2 factor factorial for penetration test and (CRD) 3 factor factorial for fumigation test. Means were separated using LSD at 5 % (Steel *et al.*, 1979).

RESULTS AND DISCUSSION

Efficacy of phosphine against T. castaneum

The overall analyses of variance for penetration ability of *T. castaneum* inside plastic jars shows that, there was significant difference in penetration by beetles as effected by different plastic packaging (Table I; P<0.05). However there was no significant difference observed in penetration with regard to time (3 and 6 days) and the interaction between time and packaging material (Tables I, II; P>0.05). Comparative mean penetration ability of *Tribolium castaneum* as affected by different packaging shows that flour beetles were only able to penetrate through polyethylene packaging and there were no holes and beetles recorded in the remaining packaging types. Analysis of variance also shows significant difference in penetration of different packaging (Tables I, II; P<0.05). The maximum % penetration was recorded in polyethylene (16.25%) while others had no penetration (Table III). Highland (1984) classified the major stored grain insect pests as invaders or penetrators of plastic packaging

Table I.- Overall Analysis of variance of 3 and 6 day data regarding penetration ability of *Tribolium castaneum*(Herbst) on different packaging types.

Source	*df*	*SS*	*MS*	*F*	*P*
Time	1	167.11	167.112	1.17	0.2940
Packaging	2	1408.12	704.058	4.92	0.0197
Time x Packaging	2	334.22	167.112	1.17	0.3334
Error	18	2574.73	143.041		
Total	23	4484.19			

Table II.- Overall comparison of mean penetration ability (%) of *Tribolium castaneum*in polyethylene for 3 and 6 day time interval in plastic jars.

Time	% Penetration ability (Mean ± S.E)
3 days	8.05 ± 1.70a
6 days	2.78 ± 0.54a

Table III.- Overall Comparison of mean penetration ability (%) of *Tribolium castaneum* in different packaging types at 3 and 6 day interval in plastic jars

Packaging	% Penetration ability (Mean ± S.E)
PT	16.25 ± 8.17a
PP	0.00 ± 0.00b
PVC	0.00 ± 0.00b

PT, Polyethylene; PP, Polypropylene; PVC, Polyvinylchloride.

however *T. castaneum* has been reported as an invader as well as a penetrator. Nevertheless, the packaging material having existing openings or holes can help in penetration by both penetrators and invaders. Allahvaisi *et al.* (2010) conducted a study of polymers permeability by some major species of stored grain insects. According to their results there was significant difference among the polymers permeability to different stored product pest. From the four kind of used polymers polypropylene had the least permeability against insects. They showed that penetration of polymers by stored grain insect pests is related to bilateral effects of both type and thickness of the polymer being tested. They also showed that penetration % age at first day is very quick but later on it decreases with the passage of time. Our results are also in agreement with these findings because there was no significant difference in penetration by red flour beetles in different packaging as affected by exposure period however penetration reduced with the passage of time (Table IV). Cline (1978) reported that polymers like polyethylene and cellophane could be penetrated by certain stored grain insects pest. Highland and Wilson (1981) reported that the adults of *T. castaneum* can enter through openings less than 1.3 mm diameter. Our results are in agreement with the finding of above mentioned studies in which *T. castaneum* was unable to penetrate through plastic films like polypropylene and polyvinylchloride however polyethylene was comparatively a susceptible packaging for this pest.

Table IV.- Overall comparison of mean (%) penetration ability in plastic jars against time and packaging type at 3 and 6 day interval on *Tribolium castaneum.*

Time	Packaging	% Penetration ability (Mean ± S.E)
3 days	PT	24.17 ± 5.10a
6 days	PT	8.33 ± 1.61ab
3 days	PP	0.00 ± 0.00b
3 days	PVC	0.00 ± 0.00b
6 days	PP	0.00 ± 0.00b
6 days	PVC	0.00 ± 0.00b

Penetration ability of T. castaneum *against different plastics films*

Experiments conducted to check the mortality of *T. castaneum* with phosphine (1.5 g /m^3) for 2 and 4 days (experiments ran independently for two exposure periods) showed that there was no significant difference in mortality of beetles as affected by commodity, packaging and time interval 2 or 4 days. However the overall comparison of mortality as affected by time interval and treatments after 2 days versus treatment after 4 days showed that there was significant difference in mortality as affected by time interval (Table V; P<0.05). Maximum % mean mortality of *T. castaneum* was observed in PVC (98.94) and PP (97.25) after 4 days of treatments. This was significantly different from the percent mean mortality observed in PP (80.69) after 2 days of treatments (Table VI, P<0.05). The only difference in mortality was time interval. Packaging material did not influence mortality of beetles. However mortality was sufficient when treatment interval was increased from 2 to 4 days. The % mean mortality of *T. castaneum* varied significantly in relation to different commodities when tested for 2 and 4 days of phosphine treatments. Maximum % mean mortality in semolina (99.56) was found after 4 days interval and minimum % mean mortality was recorded in rice (79.69) at 2 days interval which differ significantly while other combinations were non-significant (Table VII P < 0.05). Our results are in agreement with Winks (1986, 1987) who reported that the exposure period is a critical factor in determining the effectiveness of low phosphine concentration. The insects respond better to lower concentrations with longer exposure period than to higher concentrations with shorter exposure period. Low concentration may only be effective if the exposure period is long enough to enable insects to develop from a phosphine tolerant stage to a more susceptible stage in their life cycles. It has been demonstrated that poor fumigation practices including leaky and perforated storage premises, sorption of gases by the commodities and differential gas retaining properties of packing and sheeting materials have led to revised target concentrations and the previous fumigation concentration of about 100-150 ppm have now been revised and a target concentration of > 1000 ppm is

Table V.- **Overall Analysis of variance of mortality of *T. castaneum* with phosphine (1.5 g/m^3) in relation to time (2, 4 days), packaging, commodities and their interactions.**

Source	*df*	*SS*	*MS*	*F*	*P*
Time	1	3200	3200	6.444	0.012739
Packaging	3	232	77	0.156	0.925674
Commodity	3	1914	638	1.285	0.284051
Time x Packaging	3	788	263	0.529	0.663313
Time x Commodity	3	1339	446	0.899	0.444704
Packaging x Commodity	9	3488	388	0.780	0.634833
Time x Packaging x Commodities	9	2085	232	0.467	0.893637
Error	96	47670	497		

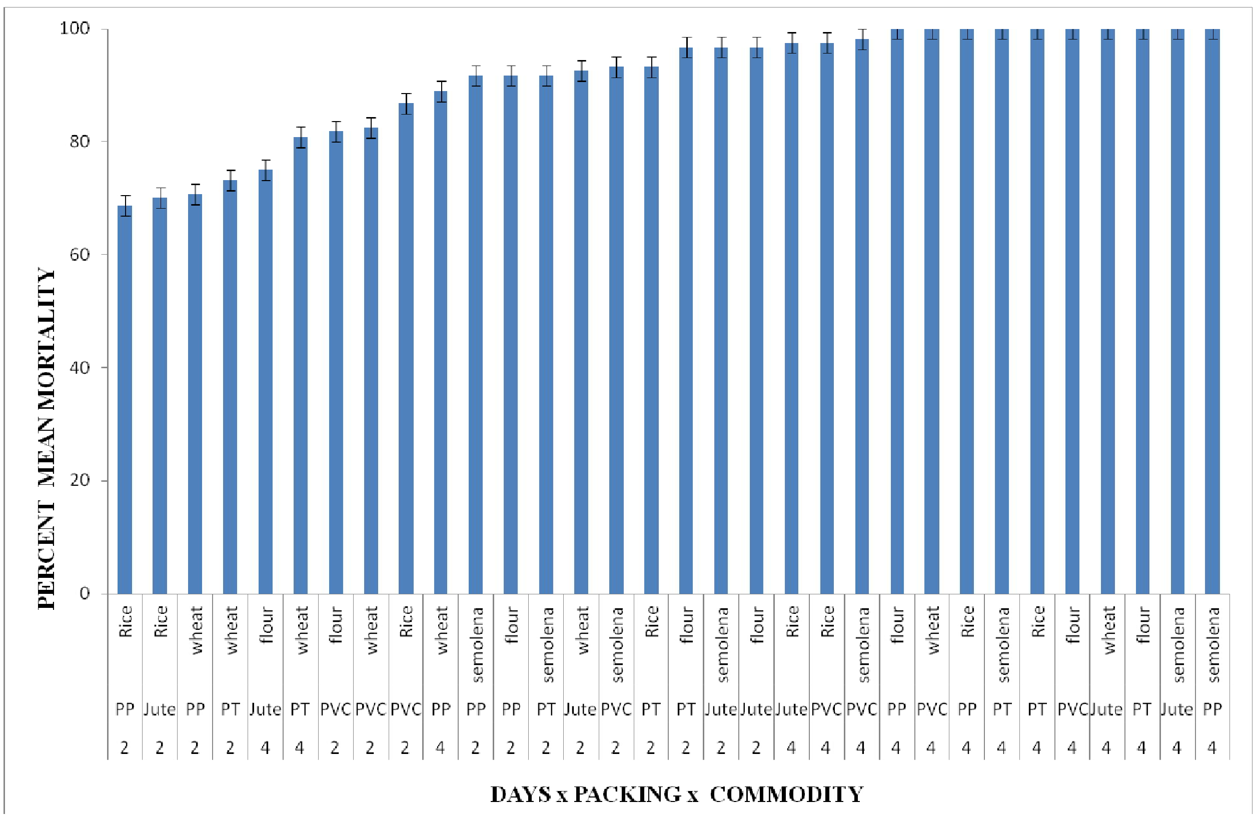

Fig. 1. Mortality (%) of *T. castaneum* due to phosphine in relation to food types, packaging material and time interval

being utilized as an effective phosphine dose (Reddy *et al*., 2007). In our studies we applied 1.5 g/m^3 that are also equivalent to > 1000 ppm of phosphine concentration. All other factors *i.e.* packaging and commodities remained non-significant but only time interval while moving from 2 to 4 days showed a successively higher mortality (Fig. 1). Therefore, it is recommended that in order to manage red flour beetles with phosphine fumigant at a dose of 1.5 g/m^{3}in closed tin containers administered in wheat, rice, wheat flour or semolina treatment time ideally should be more than 4 days. We also noticed differential mortality of red flour beetles in individual replications being some times less or zero in wheat or rice than in flour or semolina which could be due to sorption of gas by these commodities more than flour or semolina. However further studies are needed in this regard. In case of resistance to penetration to beetles, polypropylene and polyvinylchloride were resistant to red flour beetles and polyethylene was a susceptible packaging material for them. Our further studies in the research project are being carried out on other stored grain insect pests adults and the immature of red flour beetles as well as other beetle pests.

Table VI.- Overall comparison of means (%) mortality of *T. castaneum* with phosphine (1.5 g/m^3) in relation to time interval and packaging.

Time	Packaging	% Penetration ability (Mean ± S.E)
2 days	PP	80.69 ± 2.83a
2 days	PVC	86.06 ± 1.18ab
2 days	PT	88.75 ± 2.35ab
2 days	Jute	89.00 ± 2.86ab
4 days	Jute	93.13 ± 2.71ab
4 days	PT	95.19 ± 2.15ab
4 days	PP	97.25 ± 1.23b
4 days	PVC	98.94 ± 0.28b

Table VII.- Overall Comparison of mean (%) mortality of *T. castaneum* with phosphine (1.5 g/m^3) in relation to time and commodity.

Time	Packaging	% Penetration ability (Mean ± S.E)
2 days	Rice	79.69 ± 5.08a
2 days	Wheat	79.75 ± 5.69a
2 days	Flour	91.75 ± 1.56ab
2 days	Semolina	93.31 ± 1.30ab
4 days	wheat	92.44 ± 2.76ab
4 days	Flour	93.75 ± 2.28ab
4 days	Rice	98.75 ± 0.46b
4 days	Semolina	99.56 ± 0.15b

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