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Resistance in *Tribolium castaneum* (Hbst.) against phosphine and deltamethrin

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ABSTRACT

The red flour beetle *Tribolium castaneum* (Hbst.) is the most destructive pest of the stored grains and responsible for causing the tremendous loss to stored grains depends upon the storage structures and conditions. It deteriorates the quality and quantity of the stored grains. The current study was carried out to monitor the level of resistance in red flour beetle against phosphine and deltamethrin. Six concentrations of phosphine (0.006, 0.007, 0.008, 0.009, 0.01 and 0.011 ppm) and deltamethrin (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 ppm) were used against this test insect. The mortality data of this insect against phosphine was recorded after 7, 14 and 21 days of treatment. The data regarding mortality (%) was recorded after 24, 48 and 72 hours. The results showed that the maximum mortality of resistant and susceptible strain of *T. castaneum* against deltamethrin at 0.06% was 38.78% and 72.52% respectively. The maximum mortality of resistant and susceptible strain of *T. castaneum* against phosphine at 0.011 ppm was 74.46% and 76.81% respectively. All the data showed that *Tribolium castaneum* was 4.86 times fold resistant against deltamethrin after 72 hours' time interval than over susceptible strains. The LC₅₀ value showed that *T. castaneum* was 0.75 times fold resistant against phosphine after 7 days' time interval than over susceptible strains.

KEYWORDS: Red flour beetle, *Tribolium Castaneum*, resistance, deltamethrin, phosphine

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INTRODUCTION

The most important stored grain insect pest which is severely prompt the infestation in storage structures is *Tribolium castaneum* (Hbst.) [1]. The stored grain insect pests cause the major profitable losses like reduction of nutrients and grain losses [2]. Insect pests also cause the health hazards by the production of aflatoxic material like uric acid [3]. During favorable environment, the infestation rates of the insects are increase which makes the goods unpalatable for human consumption [4]. The insect pest like *T. castaneum* which feed in the internal side of the grains cause the quantity loss and damage the kernel of the grains [5]. The *T. castaneum* represents a serious threat to the farmers by causing the grain losses [6]. Emekci [7] observe that these species are well established in hot and humid environment which is suitable for their proper growth and development. In all the storage beetles, *T. castaneum* has ability to move from one godwon to other and easily bring

about the infestations in stored commodities [8]. Eggs are laying on the surface of the grains by the females which hatch and larvae enter into the kernels of the grains [9].

Lorini reported resistance in *T. castaneum* to deltamethrin [10,11]. Fumigation is also done by the application of Phosphine gas which is an important fumigant for the control of the stored products pest's in through of the world since 1930. It is used to protect the stored products pests. It is comparatively cheap, multipurpose and easy to apply. It is most toxic to those organisms which are aerobically respiring in the storage structures but nontoxic to metabolically dormant and anaerobic organisms [12]. The primary fumigant which is particularly used to protect the greater part of grains and wide diversity of other storage merchandise from insect pests is Phosphine [13,14]. Fumigation of Phosphine is an effective method to manage insect pests of stored product, but the repeated applications and injurious use of this resulted in development of resistant in

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populations [15]. Stored products pests develop the resistance against phosphine has become worldwide concern.

Therefore, the present research was conducted to evaluate the efficiencies of different concentrations of deltamethrin and phosphine for highest mortality and to examine that which concentrations of deltamethrin and phosphine shows more resistance against *T. castaneum*.

MATERIAL AND METHODS

Collection and Rearing of *T. castaneum*

The research trials were carried out in the Grain Research Training and Storage Management Cell (GRTSMC), Department of Entomology, University of Agriculture, Faisalabad during the year 2016-17. The material was comprised of *T. castaneum* deltamethrin, phosphine, filter paper, petri dishes, jars, camel hair brush, micropipette and desiccators chambers. Adults of *T. castaneum* was collected from Godown of the Punjab Food Department and grain market of District Muzaffargarh and was reared in the laboratory to develop homogenous population.

After collection, the insect populations were kept in the jars. The capacity of jars was 800 gm in which insect populations was reared. The jars were sterilized at 60°C for 60-90 minutes. The jars were filled with un-infested wheat flour diet for proper growth and development of studied insects. The jars were covered with muslin cloths to prevent escape and the entry of ants or other insects. Insect population was regularly checked to avoid from infestation. The jars were kept in incubator. The temperature of 30 ± 2°C and R.H 70 ± 5% was maintained in laboratory [16]. Each jar was filled with 200 g wheat grains and mixed with 20 g flour. About 100 beetles were added to each jar assuming 50 males and 50 females. Beetles were allowed for 3 days to oviposit in the medium. After 3 days, the insects was removed with the help of sieves using camel hair brushes from the media and was added to another set of sterilized jars filled with 200 g wheat grains and 20 g flour for further infestation for the purpose of fresh culture development. The flour containing eggs of the test insect were placed in other jar to get different larval stages of the test insects and the fresh adults. It was take approximately 28 days for reaching to 6th instars larvae and 39 days to become adult. Homogenous population of equal size and age was sieved out which later on was used for bioassay studies.

Preparation of Test Chemicals

The deltamethrin (Guardian 1.5 EC) was diluted in ethanol to obtain 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 ppm solution to be tested against the test insects. These concentrations were prepared for experimental purpose by talking 0.66ml, 1.33ml, 2ml, 2.66, 3.33ml and 4ml deltamethrin added with 99.34, 98.67, 98, 97.34, 96.67 and 96 ml ethanol respectively.

Test insects were initially be exposed to a discriminating dose of phosphine in accordance with the method recommended

by FAO. Phosphine was generated from aluminum phosphide tablets. Fumigation chamber was used for the application of phosphine against the test insects. Within the desiccators, insects were confined in 50 cc beakers closed with nylon mesh. Six concentrations (0.006, 0.007, 0.008, 0.009, 0.01 and 0.011 ppm) of phosphine were used. The mortality data of phosphine concentrations were recorded after 7 days, 14 days and 21 days. Micro syringe was used for the injection of phosphine gas into desiccators through a rubber septum fitted to a socket in the desiccators' lid. The desiccators were placed in the lab line walk in environment chamber at 25 DC and 65% R.H for 20 h after which observations on mortality was made. Resistant strains were tested at a series of doses to record their "resistance factor" all the tests were triplicate for obtaining accuracy.

The response of deltamethrin at different doses for different time intervals was checked against *T. castaneum*. Circular plastic petri dishes (150×25 mm) were used as testing arena. A filter paper (Fisher Sci., Pittsburg, Penn.) was placed in the bottom of each petri dish. Ethanol solution of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 ppm deltamethrin were applied separately to the filter papers in the dishes through micropipette. The solvent was completely evaporated by the opening of the petri dishes for an hour. Thirty adults of *T. castaneum* were released in each treatment including control for up to 72 hours. The petri dishes were covered with lid to avoid the escape of the test insect. Six concentrations (0.006, 0.007, 0.008, 0.009, 0.01 and 0.011 ppm) of phosphine were used. The data regarding percent mortality was recorded after 7 days, 14 days and 21 days. All the treatments including control were replicated thrice. Though, the mortality of the test insect in each concentration were recorded after 24, 48 and 72 hours, an overall mean percent mortality in each concentration in the mentioned three time intervals were analyzed with Analysis of Variance (ANOVA), Tuckey HSD and Probit analysis. The resistance factor was calculated by using the following formula:

$$\text{Resistant Factor} = \frac{\text{LC}_{50} \text{ of susceptible strain}}{\text{LC}_{50} \text{ of resistances train}}$$

RESULTS

The present study was carried out to determine the resistance level in *T. castaneum* against deltamethrin and phosphine in different strains. The test insect *T. castaneum* was exposed to six concentrations of deltamethrin 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 ppm and six concentrations of phosphine 0.006, 0.007, 0.008, 0.009, 0.01 and 0.011 ppm. The effects of these concentrations were recorded after 24, 48 and 72 hours for deltamethrin and after 7, 14 and 21 days for phosphine. The mean percentage mortality of adults at various concentrations of deltamethrin in Muzaffargarh field and laboratory (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 ppm) is given in the (Figure. 1) which reveals that the mortality of the adult insects was directly related to increase of concentrations. When the concentrations of insecticides were increased then the mortality of the test insects was increased. The results showed that at

the higher concentrations of insecticides, mortality of the insects was increased. The highest mortality was recorded at 0.06% concentration was 38.78% of adult *T. castaneum* in field condition and 72.62% in laboratory condition. The mean mortality of the insects in field at other concentrations (0.01, 0.02, 0.03, 0.04 and 0.05 ppm) was 11.78, 18.25, 26.23, 22.43, and 30.8% respectively and in laboratory was 15.48, 30.56, 50, 58.33 and 66.27% respectively.

The mean percentage mortality of adults of *T. castaneum* at various concentrations of phosphine (0.006, 0.007, 0.008, 0.009, 0.01 and 0.011 ppm) is given in the (Figure. 2) which reveals that the mortality of the adult insects was directly related to increase of concentrations. The maximum mean mortality was recorded at 0.011 ppm concentration was 74.46% of adult *T. castaneum* in field condition and 36.81% in lab condition. The mean mortality of the insects at other concentrations (0.006,

0.007, 0.008, 0.009 and 0.01 ppm) was 9.36, 29.78, 48.93, 58.29 and 63.4% respectively and in laboratory was 2.55, 11.06, 19.57, 16.59 and 25.32% respectively.

The results regarding mean mortality (%) of adults of *T. castaneum* in field conditions after various time intervals (24, 48, and 72 hours) by the application of deltamethrin in the given above (Table 1). The data shows that the maximum mean mortality of the adult insect (30.22%) was observed after 72 hours' time interval by the application of deltamethrin followed by 18.44% and 25.47% after 24 and 48 hours' time intervals respectively. While at the same time interval the application of deltamethrin in laboratory condition shows that the maximum mean mortality of the adult insect (52.78%) was observed after 72 hours' time interval by the application of deltamethrin followed by 44.05% and 49.80% after 24 and 48 hours' time intervals respectively. In both strains the data shows that when

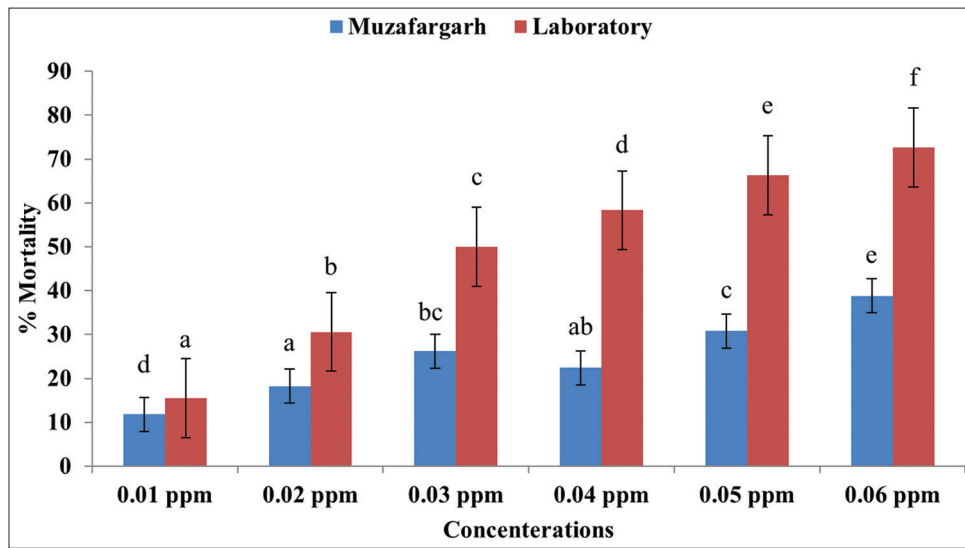


Figure 1: Comparison of mean values data regarding % mortality of Muzaffargarh and laboratory strain of *T. castaneum* at different concentrations of deltamethrin

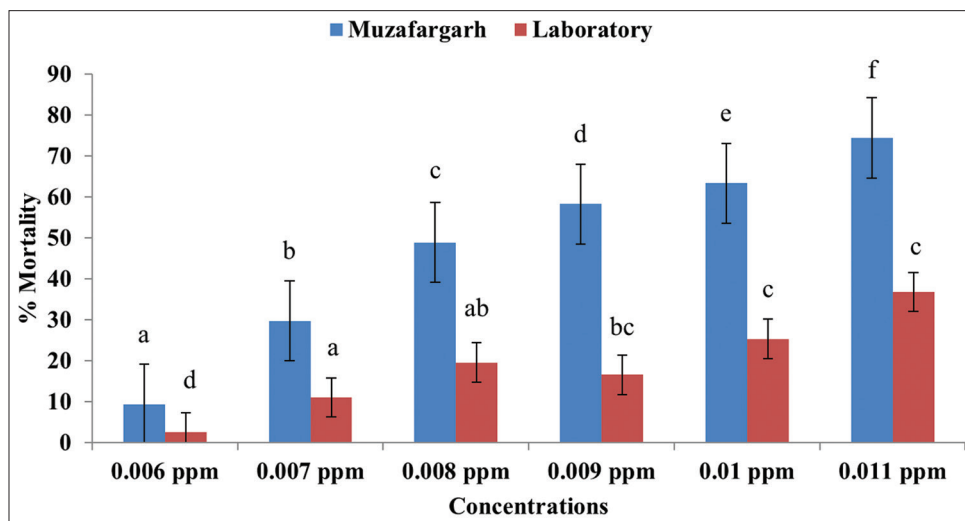


Figure 2: Comparison of mean values data regarding % mortality of Muzaffargarh and laboratory strain of *T. castaneum* at different concentrations of Phosphine

the exposure times of the insecticides was increased then the mortality of the insects was also increased.

The results regarding mean mortality (%) of adults of *T. castaneum* in field conditions after various time intervals (7, 14 and 21 days) by the application of phosphine in the given above (Table 2). The data shows that the maximum mean mortality of the adult insect (52.34%) was observed after 21 days' time interval by the application of phosphine followed by 42.98% and 46.81% after 7 and 14 days' time intervals respectively. While in laboratory conditions the data shows that the maximum mean mortality of the adult insect (24.68%) was observed after 21 day time interval by the application of phosphine followed by 12.34% and 19.57% after 7 and 14 days time intervals respectively. In both strains the data shows that when the exposure times of the phosphine was increased then the mortality of the test insects was also increased.

The log probit analysis was used to evaluate the LC_{50} values at the various concentrations (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 ppm) of deltamethrin after 24 hours' time interval in the above given (Table 3) two strains of *T. castaneum* were collected, one from District Muzaffargarh and the other from laboratory strain which was susceptible and considered as a standard. The

Table 1: Comparison of mean values of the data regarding % mortality of Muzaffargarh and Laboratory strain of *T. castaneum* after different times intervals after treatment various concentrations of deltamethrin

Exposure Time	Mortality±SE in field	Mortality±SE in laboratory
24 hours	18.44±2.69 ^a	44.05±5.33 ^b
48 hours	25.47±2.17 ^b	49.80±4.86 ^a
72 hours	30.22±2.05 ^c	52.78±4.69 ^a

Table 2: Comparison of mean values of the data regarding % mortality of Muzaffargarh and laboratory strain Population of *T. castaneum* after different times intervals after treatment various concentrations of Phosphine

Exposure Time	Mortality±SE in field	Mortality±SE in laboratory
7 days	42.98±5.97 ^a	12.34±3.26 ^a
14 days	46.81±5.23 ^b	19.57±2.69 ^b
21 days	52.34±4.83 ^c	24.68±2.55 ^c

Table 3: Probit analysis for the comparative evaluation of level of resistance in Muzaffargarh and Laboratory strain of red flour beetle, *T. castaneum* against deltamethrin after 24,48,72 hours and phosphine used after 7,14 and 21 days exposure time period

Locality	Strains (LGB)	Exposure Time	LC_{50}	95% Fiducial limit	R.F
Muzaffargarh	MGS	24 hours	0.0867475	0.0697341-0.128988	2.28
Laboratory	LS	24 hours	0.0379401	0.0345798-0.0414776	2.28
Muzaffargarh	MGS	48 hours	0.116414	0.0764431-0.331931	3.63
Laboratory	LS	48 hours	0.0320686	0.0283490-0.0357373	3.63
Muzaffargarh	MGS	72 hours	0.140826	0.0772148-1.33238	4.86
Laboratory	LS	72 hours	0.0289678	0.0250784-0.0326361	4.86
Muzaffargarh	MGS	7 days	0.0091021	0.0088172-0.0093856	0.75
Laboratory	LS	7 days	0.0121359	0.0114192-0.0134125	0.75
Muzaffargarh	MGS	14 days	0.0087934	0.0084504-0.0091285	0.684
Laboratory	LS	14 days	0.0128387	0.0116188-0.0154874	0.684
Muzaffargarh	MGS	21 days	0.0082237	0.0078336-0.0085696	0.60
Laboratory	LS	21 days	0.0136293	0.0117632-0.0192921	0.60

LGB=lesser grain borer, MGS=Muzaffargarh Gowdon strain, LS=Laboratory Strain

resistance factor (R.F) was calculated to compare the two strains of *T. castaneum*. The data showed that the *T. castaneum* was 2.28, 3.63 and 4.86 times fold resistant against deltamethrin after 24, 48 and 72 hours' time interval than over laboratory susceptible strains. While phosphine showed 0.75, 0.684 and 0.60 times fold resistant after 7, 14 and 21 days' time interval than over laboratory susceptible strains.

DISCUSSION

Research trials was carried out in the Grain Research Training and Storage Management Cell (GRTSMC), Department of Entomology, University of Agriculture, Faisalabad during the year 2016-17. In this experiment, there were firstly checked the mortality of the stored grain insect pest. Different concentrations of insecticides were used against this insect to evaluate that the effective concentration of the both deltamethrin and phosphine which caused the highest mortality of the stored grain insect pest. Among these concentrations, there was indicated that which concentration was the most effective for the controlling of this stored grain insect pest. The main purpose of this experiment was monitoring of the resistance in *T. castaneum* against deltamethrin and phosphine. Resistance is the avoidance of biotic and abiotic factors. In most of the stored grain insect pests, resistance was developed against both the deltamethrin and phosphine.

The main reason for the development of resistance was due to the repeated applications of the same mode of action of insecticides. There were two strains of *T. castaneum* taken, one from field and the other from laboratory which was kept as a standard. *T. castaneum* strains were collected from various places of District Muzaffargarh. The field strains were found to be 11.78, 18.25, 26.23, 22.43, 30.80 and 38.78 times more resistant as compared to the laboratory susceptible strain. These findings of our experiment were similar to those of Ahmad *et al.* [17] who reported that both strains of *T. castaneum* exhibited the diverse level of resistance against deltamethrin. In the case of field population, the highest mortality was occurred at 0.06 % concentration was 41.82 %. The results were almost similar. But due to some uncertain situations the results were incompatible with the previous results was due to different geographical

distribution of the species, Micro-environment of the laboratory and climate change effect the population buildup of the species.

Both the populations of *T. castaneum* were exposed with phosphine fumigation to check the level of resistance against different phosphine concentrations 0.006, 0.007, 0.008, 0.009, 0.01 and 0.011 ppm. The data showed that the field population was gave the highest mortality as compared to the laboratory population. Hence, the laboratory population was more resistant as compared to the field population against different concentrations of phosphine. Those findings of our experiment were similar to Pimentel *et al.* [18] who evaluated the phosphine resistance in 12 populations of *T. castaneum*. The highest mortality of the test insect was done at the highest concentration 0.011 % was 75% in laboratory population.

CONCLUSION

The cross resistance was observed mostly related to these compounds in *T. castaneum* which is the notorious pest of the stored products pest. The results of the current experiment were further showed that, the resistance levels ranging from 4.86 times fold against deltamethrin in *T. castaneum* respectively. The level of resistance against phosphine was 0.60 times fold against phosphine in *T. castaneum*. This leads to the conclusion that maximum resistance exists in *T. castaneum* against deltamethrin followed by the phosphine was minimum resistant.

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