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Evaluation of Phosphine and Cypermethrin resistance in field collected strains of *Rhyzopertha Dominica* (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) under laboratory conditions

Aqsa Arshad[1*], Asim Munawar[2], Muhammad Ishaque Mastoi[3], Samar Sohail[4], Chengjuan Liang[5]
[1, 2] Department of Entomology, University of Agriculture Faisalabad, Pakistan
[2] Institute of Insect Sciences, College of Agriculture and Biotechnology, Zhejiang University, China
[3] Department of Plant and Environmental Protection, NARC, Islamabad, Pakistan
[4] Department of Horticulture, University of Agriculture Faisalabad, Pakistan
[5] Institute of Applied Ecology, Fujian Agriculture and Forestry University, China

Abstract
Storages pests presenting a major threat to storage commodities throughout the world from recent decades with causing severe economic losses to a variety of food products at large scale. Cypermethrin and phosphine are becomes major insecticides and fumigants as a grain's protectants against *Tribolium castaneum* and *Rhyzopertha Dominica*. Unfortunately, due to repeated applications of these both control agents most of the major insect pests of storages developing resistance speedily. Under such circumstances current study was focused on to evaluate the resistance mechanism of field collected strains of these two major insects' pests. Our results showed that both pests showed maximum resistance against cypermethrin, while in case of phosphine more mortality was observed which was indicated as less resistant to *T. castenum* and *R. Dominica*. Maximum mortality was observed (26.86±2.89) and (38.23±3.65) after 72 h of exposure of phosphine against *T. castaneum* and *R. Dominica*, cypermethrin showed minimum which was (25.41±2.73) and 926.60±3.28) respectively. The LC_{50} value of both collected strains showed that phosphine was more resistant against *T. castenum* and *R. Dominica* while cypermethrin was detected less resistant. We hope this research could provide a greater-understandings for practical application of insecticides and fumigants for the control of storages pests.

Keywords: Cypermethrin, *T. castenum*, Phosphine, Mortality, Resistant, *R. Dominica*

Introduction
Storage of grains commodities has been an important tactics for storing many years. Storage of commodities have a significant role in reserves, as emerging issues about food security (Miffre and Joelle, 2016) [16]. Due to increasing world population day by day at the rate of 1.14 % annually its becomes mandatory for farmers and storages holder to reserve for longer period of time to feed the population as agriculture resources are not enough that can directly fulfill food security (Flinn *et al*., 2010) [8]. Barley, wheat, maize, rice and sorghum are directly coped with human hunger in many countries to fulfill nutritional requirements (Proctor, 1994) [21].

Wheat grains which are staple food of Pakistan and most other countries in the world have a vital source of energy and proteins (Bostan and Naeem, 2002) [4]. Unfortunately, grain losses due to storages pests increasing day by day due to certain environmental conditions and factors (Chaturvedi and Anilraj, 2015). Under storages grains effected by different factors as like temperature, moisture, geographical locations and most important insects and microorganisms (Govender *et al*., 2007) [10]. According to previous investigations around one third percent food production is loosed due to many storages' insect pests' species (Ahmed and Grainage, 1986) [2]. Rather than other species about 60 % of storages insect's pests belong order coleopteran, while only 10 % are lepidopteron (Atwal and Dhaliwal, 2008) [3]. Red flour beetle, *T. castenum* and lesser grain borer, *R. Dominica* are considered most important pests of storages commodities (Chimoya and Abdullahi, 2011) [6].

Both pests are widely distributed, polyphagous in nature and have wide hosts range. These pests causing severe economic losses at larger scale to a variety of food commodities (Hamed and Khattak, 1985) [11]. Control of these two pets mostly depends on fumigants and insecticides (Opit *et al*., 2012) [19]. There low cost, less residual substances, rapid dispersal are important components that make them major usage against *T. castenum* and *R. Dominica*. These favorable components are leading to increasing reliability on phosphine and insecticides (Wang *et al*., 2006). Unluckily due to repeated application of these two treatments the strain of *T. castenum* and *R. Dominica* are rapidly developing resistance against insecticides and fumigants (Opit *et al*., 2012; Chaudhry, 2000) [19, 5]. Due to these ongoing threats current research was focused to check the resistance of two important strain of storage commodities *T. castenum* and *R. Dominica* against fumigant, phosphine and insecticide, cypermethrin. We hope this research will give operational as well as practical approach to storages holders for the effective managements of major storage pests especially *T. castenum* and *R. Dominica*. Our research can also prove much helpful to tackle with food security issues.

Materials and Methods

Collection of insects

Strains of *T. castenum* and *R. Dominica* was collected from various locations, godwons, and grains processing markets as well as different geographical positions of Faisalabad, Pakistan. Collected strains were brings to laboratory for rearing homogeneous population for research experiments.

Insect culture

Collected strains of *T. castenum* and *R. Dominica* were placed separately in an alredy sterilized jars. Clean and sterilized wheat flour was offered for growth and development. Jars was covered with muslin cloth for avoiding the insects to flee away and no other insects can enter. Rearing jars was kept at 35±2°C and 65±5% RH in an incubator. Insect populations were regularly checked, as assuming 50/50 male and females were separated in an individual jars filled with 20 gram flour and 200 gram wheat grains. Beetles were allowed to oviposit at least for three days. Collect eggs with flour was transferred to and clean jars to increase populations and to get different stages of insects.

Cypermethrin resistance against *R. Dominica* and *T. castenum*

Cypermethrin used for experiment was brought from insecticidal markete. Cypermethrin was diluted into different concentration 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 with water. Water was used to male dilution was sterilized. Whole experiment was repeated three times.

Phosphine resistance against *R. Dominica* and *T. castenum*

Different concentration of phosphine (0.007, 0.008, 0.009, 0.01, 0.011 and 0.012) in ppm was applied. These concentration was obtained from phosphine tablets collected by acidified water. Micro syringe was used to inject fumigants of phosphine gas into desiccators closed with rubber septum which was fitted on desiccator as a lid. Desiccator was placed at 35±2°C and 65±5% RH and regularly inspected to check the mortality rates. Whole experiment was repeated three times.

Bioassay

Efficacy of cypermethrin was checked against *T. castenum* and *R. Dominica* at different time intervals with different concentration as described above. Petri dishes with size (140x25cm) was used, placed with filter papers. With the help of micropipette prepared dilution of cypermethrin was poured into each petri dish, and was placed in an open environment for one hour for evaporation of solvent. Twenty adult insect of *T. castenum* and *R. Dominica* were placed in petri dishes for 24, 48 and 72 hours, while control get on water treatment. Same method was applied for phosphine treatments.

Statistical analysis

To determine the resistance level of adult *T. castenum* and *R. Dominica* collected data was subjected to analyze with log probit analysis. Tucky HSD test was applies for comparisons among means at 5% significant level. Excel sheet (2016) was used to draw tables. The LC_{50} values was computed by Log Probit analysis while resistant factors was calculated by using following formula.

$$Resistant\ factor = \frac{LC50\ of\ susceptible\ strains}{\text{LC50 of resistant strains}}$$

Results

Current research was carried out to evaluate the resistance mechanism of field collected strains of red flour beetle, *T. castaneum* and lesser grain borer, *R. Dominica* against phosphine and Cypermethrin. Concluding results by factorial analysis showed that *T. castaneum* show highest resistance to cypermethrin and Phosphine as compared to *R. Dominica* respectively. *Tribolium castaneum* was found more resistant to two both checked as compared to *R. Dominica*. According to previous researches phosphine is a poisonous gas which act very slowly. At physiological level it's no chance for stored pests to retrieval after phosphine fumigation, while in case of cypermethrin who's mode of action to act on insect nervous system if they come intact with insecticide.

Phosphine mode of action is oxidative and give metabolic stress tangled, while cypermethrin have direct impact on insect cuticle. Previous research reported that the mortality of stored pests due to phosphine fumigation is depends on initial and subsequent exposures. Stored pests which are already exposed to phosphine develop less resistant to phosphine. In our reported results we observed that phosphine resulted more mortality of *T. castaneum* and *R. Dominica* as compared to cypermethrin. Cypermethrin showed moderate mortality of these two stored pests which may be due to less intact with grains or other physiological matter.

Table 1: Factorial analysis for mortality of field strains of *Tribolium castaneum* and *Rhyzopertha Dominica* against different concentrations of Cypermehtrin.

Source	*DF*	*Tribolium castaneum*		*DF*	*Rhyzopertha Dominica*	
		F	*P*		*F*	*P*
Concentration	5	66.607**	0.0000	5	100.004**	0.0003
Exposure time	2	67.596*	0.0001	2	36.939*	0.0102
Concentration × Exposure time	10	2.260*	0.0352	10	2.30*	0.0371
Error	36	—	—	36	—	—
Total	53	—	—	53	—	—

Table 2: Factorial analysis for mortality of field strains of *Tribolium castaneum* and *Rhyzopertha Dominica* against different concentrations of Phosphine.

Source	*DF*	*Tribolium castaneum*		*DF*	*Rhyzopertha Dominica*	
		F	*P*		*F*	*P*
Concentration	5	71.51**	0.0004	5	150.007*	0.02471
Exposure time	2	83.58*	0.0157	2	166.180*	0.01425
Concentration × Exposure time	10	2.51*	0.0318	10	2.245**	0.0017
Error	36	—	—	36	—	—
Total	53	—	—	53	—	—

Mean mortality caused by different concentration of two checked insecticides phosphine and cypermethrin showed that the mortality of both pest gradually increased as concentration increased respectively. Highest mortality was found 48.29±3.14 against *R. Dominica* when these pests treated with 0.012 ppm concentration of phosphine. While in case of cypermethrin highest mortality was found

37.71±2.80 against *R. Dominica* when these pests treated with 0.06 % concentration. *Tribolium castaneum* showed maximum mortality 31.54±3.14 at 0.06 % concentration of cypermethrin, while it showed maximum mortality 32.91±3.25 when these pests was treated with 0.012 ppm concentration of phosphine. Other concentrations showed similar increased mortality as these war increased.

Cypermethrtin is most effective when there is a highest outbreak of stored products, as well as for largest products holding areas, while in case of phosphine fumigation which is only effective for small holding products areas. There are more chances for to escape fumigation gas, but cypermethrin sprayed which have very less non-target effects. Cypermethrin remain effective for longer time which also cause hazardous effects on food as well as on human health. Phosphine stability remained for very short period of time but it give highest results and as well as show less toxicity to food and human health. In our research phosphine showed highest control rate for both checked stored pests as compared to cypermethrin. Cypermethrin showed lowest control rate because it cause only mortality when pests feed on grains and come intact to them. Most of the stored pests develops resistance against sprayed insecticides, because they smell them and hide themselves in walls while phosphine cause mortality even inside the walls or others hiding places. We use different increased concentration of both checked insecticides against these two stored pests so that we can check the resistance level at different time's intervals, which could be helpful for gaps studies.

Table 3: Mean mortality (mean±SE) of *Tribolium castaneum* and *Rhyzopertha Dominica* strains treated with different concentration of Cypermethrin and Phosphine. Means sharing different letters are significantly different at 5% level ($P < 0.05$, Tucky HSD test).

Concentrations (%)	*Tribolium castaneum*		*Rhyzopertha Dominica*	
	Cypermethrin	Phosphine	Cypermethrin	Phosphine
0.01	8.03±1.26 e	—	6.48±0.97 e	—
0.02	12.06±1.81 cd	—	12.83±0.98 d	—
0.03	14.32±1.40 c	—	18.62±1.49 c	—
0.04	21.21±2.09 b	—	23.25±1.86 c	—
0.05	25.81±2.79 b	—	29.62±2.40 b	—
0.06	31.54±3.14 a	—	37.71±2.80 a	—
0.007 ppm	—	6.45±1.12 d	—	10.31±1.85 f
0.008 ppm	—	11.08±2.42 cd	—	16.76±2.85 e
0.009 ppm	—	15.08±2.07 c	—	22.60±3.14 d
0.010 ppm	—	20.86±2.97 b	—	27.84±2.09 c
0.011 ppm	—	25..44±2.62 b	—	36.01±2.79 b
0.012 ppm	—	32.91±3.25 a	—	48.29±3.14 a

Stored products pests have different responses to control measure at different times. Some treatments used for the control of stored pests take time to caused mortality but some insecticides like phosphine which always in fumigation form caused direct effect on insect mortality. Phosphine have an oxidative effects on pests due to which stored pests especially beetles die to due to suffocations. In our current research we checked the effect of phosphine and cypermethrin against *T. castaneum* and *R. Dominica* at three different time's intervals to check the best interval for the mortality of stored pests. *Rhyzopertha Dominica* and *T. castaneum* were exposed to treatments as 24, 48 and 72 hours intervals. Statistically analyzed results showed that phosphine showed highest mortality gradually as time period was increased, 24 h (12.39±1.87), 48 h (16.66±2.10) and 72 h (26.86±2.89) against *T. castaneum* but its mortality becomes more significant against *R. Dominica* at 24h (18.38±2.48), 48 (24.29±3.19) and 72 h (38.23±3.65) respectively. Cypermethrin as compared to phosphine caused minimum mortality of *T. castaneum* at 24 h 912.71±1.70), 48 h (18.36±1.84) and 72 h (25.41±2.73) as well as against *R. Dominica* at 24 h (16.95±2.12), 48 h (20.70±2.40) and 72 h (26.60±3.28) respectively.

Both treatments showed gradually increased mortality of *T. castaneum* and *R. Dominica* which confirm that effect of these both insecticides depends on time. After 72 h of exposure treatments caused maximum mortality but in case of phosphine it was greater than cypermethrin, which concluded that phosphine have speedy effects on stored pest's mortality depending on time and concentration. After 24 h of exposure both treatments showed somewhat similar results which come in confirmation that treated insecticides or fumigants need time to become more toxic against stored pests.

Time and concentration both are most important parameters need to keep in mind during stored pests control managements. Pest's outbreaks, life stage, reproducibility and fecundity rate are also considered to be important during stored products insect pest's managements. Long time exposure to treated insecticides and fumigants could also cause some toxic effects on human consumption, as previous researches explains that sprayed insecticides are more hazardous to human health than fumigants.

Table 4: Mean mortality (mean±SE) of *Tribolium castaneum* and *Rhyzopertha Dominica* strains after three different exposure times of Cypermethrin and Phosphine. Means sharing different letters are significantly different at 5% level ($P < 0.05$, Tucky HSD test).

Exposure period (hour)	*Tribolium castaneum*		*Rhyzopertha Dominica*	
	Cypermethrin	Phosphine	Cypermethrin	Phosphine
24 h	12.71±1.70 c	12.39±1.87 c	16.95±2.12 c	18.38±2.48 c
48 h	18.36±1.84 b	16.66±2.10 b	20.70±2.40 b	24.29±3.19 b
72 h	25.41±2.73 a	26.86±2.89 a	26.60±3.28 a	38.23±3.65 a

Table 5: Log probit analysis to compute the LC_{50} value of *Tribolium castaneum* and *Rhyzopertha Dominica* at different time's intervals against Cypermethrin and Phosphine (*P*<0.05%). RF: Resistant factor; LC: Lethal concentration; Cyp: Cypermethrin; Ph: Phosphine

Red flour beetle, *Tribolium castaneum.*								
Variable	**Interval**	**Treatment**	**Coefficient**	**Standard**	**95% fiducial limit**	**LC_{50}**	***P***	***RF***
Conc.	24	Cyp.	1.080	0.252	0.101-0.723	0.282	*<0.01*	0.1067
Conc.	48	Cyp.	0.743	0.189	0.080-0.293	0.131	*<0.00*	0.6966
Conc.	**72**	Cyp.	0.906	0.168	0.052-0.089	0.072	*<0.05*	0.0744
Conc.	24	Ph.	1.305	0.277	0.084-0.300	0.324	*<0.00*	0.8786
Conc.	48	Ph.	1.033	0.217	0.057-0.097	0.170	*<0.02*	0.5686
Conc.	72	Ph.	0.962	0.166	0.054-0.132	0.272	*<0.02*	0.9439
Lesser grain borer, *Rhyzopertha Dominica.*								
Conc.	24	Cyp.	1.202	0.218	0.069-0.162	0.192	*<0.00*	0.3533
Conc.	48	Cyp.	0.985	0.190	0.049-0.069	0.056	*<0.03*	0.7432
Conc.	72	Cyp.	1.138	0.175	0.042-0.058	0.148	*<0.01*	0.7061
Conc.	24	Ph.	1.845	0.2349	0.025-0.023	0.452	*<0.08*	0.9941
Conc.	48	Ph.	1.617	0.199	0.465-0.287	0.331	*<0.00*	0.8461
Conc.	72	Ph.	0.925	0.1368	0.032-0.044	0.248	*<0.00*	0.7854

Probit function was applied to check the quantile function associated with standard normal distributed mortality of two important pests of stored products pests *T. castaneum* and *R. Dominica* against two insecticide and fumigant. Different concentration of phosphine and cypermethrin exposed to different time intervals to calculate LC_{50} values, which was performed by log probit analysis. Resistant factor of both strains was computed and compared to evaluate resistant stored pests strain against fumigants and insecticides. Concluded results explains that the phosphine showed maximum resistant folds against *R. Dominica* (0.9941, 0.8461 and 0.7854) and minimum against *T. castaneum* (0.8786, 0.5686 and 0.9439) respectively. Cypermethrin showed somewhat same type of resistant factors against both stores products pests.

Discussions

Stored grains insect pests has been becomes a big challenges for warehouses, factories and godowns holders to eradicate them. Worldwide losses in stored grains due to insect pests has been reached to 10% in serious conditions reached at 30%, on which this part food can feed billions peoples (Utono, 2013) [24]. Most of the storage pests not only cause damage in storages but also cause many harmful impacts at field levels. Due to their short life cycles, fecundity and oviposition rate it's becomes very difficult to develop an effective management tactics for these dangerous storages pests (Suleiman and Rugumamu, 2017) [23]. From the last decades there are lots of developments in insect pest's managements especially to storage pests. Still fumigants and pyrethroids (cypermethrin) are providing a great interest to farmers and storages holder for the control of storage insect's pests (Opit *et al*., 2012; Rajendran, 1992; Manivannan, 2015) [19, 22, 5]. By keeping in view the old and current efforts for the control of stored products insect pests, present research was carried out to check the resistance mechanism of *T. castaneum* and *R. Dominica* against phosphine and cypermethrin. In our research fumigant, phosphine present highest rate of mortality as compare to cypermethrin which are correlated with Primental *et al*. (2008) who checked the resistance level of *T. castaneum* and *R. Dominica* against phosphine and found same type of results.

Doganay *et al*. (2018) [7] also check the efficacy of cypermethrin against *R. Dominica*, and showed results which are somewhat correlated with our study. Nayak *et al.* (2017) [18] stated that phosphine has been in use throughout the world for the control of storage insect's pests, but from the last 20 years resistance to phosphine of storages insect pests becomes big issue. In our results we observed that different concentration that we use presents different mortality rates as concentration increases mortality rate was increased which are same with (Manivannan, 2015) [15] who use different concentration of phosphine and (Naeem *et al*., (2015) [17] who use different concentration of cypermethrin to check the resistance level of *T. castaneum* and *R. Dominica* against these two treatments respectively. In our research we also presents mortality rate of both tested pests against phosphine and cypermethrin at different time intervals (24h, 48h and 72h). We want to compare our results with Khaleqazzaman and Khanom (2006) [12] who also tested the different formulations of cypermethrin at 24 and 48 hour of intervals to evaluate the best time for mortality of *T. castaneum*. Ahmad *et al*. (2013) [1] checked the resistance level of *T. castaneum* and *R. Dominica* strains collected from different regions of Sindh against phosphine. We use log probit analysis to evaluate the resistance factor of both strains against phosphine and cypermethrin. The resistance factor of both strains was found higher against cypermethrin, while against phosphine it was lowest. Which showed that major storages pests have less resistance against fumigants as compared to insecticides (Kocak *et al*., 2015; Khan *et al*., 2015; Gautam *et al*., 2016) [14, 13, 9].

The main purpose of current research was to evaluate the resistance level of major insect pests against currently using control treatments. Our research present that phosphine was prove much better than cypermethrin due to its rapid toxicity and less human health risks. We hope our research will provide a great breakthrough to small and big holding farmers for the control of these two major insects' pests of storage products.

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