PHOSPHINE RESISTANCE OF RUSTY GRAIN BEETLE *Cryptolestes ferrugineus* (*Coleoptera: Laemophloeidae*) POPULATIONS IN TURKEY

Erhan KOÇAK[1], Abdullah YILMAZ[2], Y. Nazım ALPKENT[2], Serdar BİLGİNTURAN[1]

[1]SDU Department of Agricultural Biotechnology, Isparta, Turkey
[2]Plant Protection Central Research Institute, Ankara, Turkey

Corresponding author email: erhankocak@sdu.edu.tr

Abstract

Rusty grain beetle Cryptolestes ferrugineus (Coleoptera: Laemophloeidae) is one of the most important pests of stored grains. It is known that long term use of phosphine against the pests causes to resistance development in the pests. In this study, 16 C. ferrugineus populations from grain warehouses of 11 provinces were collected. The resistance to phosphine of the populations was determined by modifying the FAO method to obtain LC_{50} values. Resistance ratios were calculated by comparing the LC_{50} values of the populations to the LC_{50} value of the sensitive population. The C. ferrugineus population obtained from İzmir province died at 36 ppm discriminating dose and LC_{50} value was calculated as 16.82 ppm/20 hrs. This population was considered susceptible and used in LC_{50} comparisons to determine the resistance ratios. The LC_{50} was not calculated for the six populations which died at the discriminating dose. In the 10 populations which LC_{50} were calculated, resistance ratios were found between 3.0 and 392.6 times. According to the results, phosphine resistance of C. ferrugineus was common and generally high in Turkish populations.

***Key words**: Cryptolestes ferrugineus, phosphine resistance, Turkey.*

INTRODUCTION

Among the 23.9 million hectares of arable land, 49% is the largest share of grains. In total cereal fields, wheat is the first with 67% share. Wheat is followed by barley with 24% share, maize with 6%, rice, rye and oat with 1%. Turkey's grain productions in the year 2016 have been realized as 225 thousand tons; 20.6 million tons of wheat, 6.7 million tons of barley, 6.4 million tons of corn, 920 thousand tons of rice and 300 thousand tons of rye, oats (Anonymous, 2016).

Cereals are subjected to weight, technological value and seed loss due to body residues, scum and nettings and similar substances that have been secreted by many harmful pests. One of the most important pest of the stored grain in our country is the Rusty Grain Beetle *Cryptolestes ferrugineus* (Stephens) (*Coleoptera: Laemophloeidae*). *C. ferrugineus* larvae and adults are fed on seeds and endosperm in wheat, barley, rye, triticale, oats and sometimes milled crops, causing fungal spores to spread throughout the warehouse (Canadian Grain Comission, 2014). This pest is known commonly in Turkey (Aydın, Soran, 1987; Coşkuncu, 2004; Bağcılar et al., 2014). Cereals should not be consumed in a short time and special conditions such as natural disasters and war should be stored on suitable conditions for the preparation. It is important to combat insect species that lead to product loss in the prevention of quality and weight losses that can occur during storage of cereals.

In the world, methyl bromide and phosphine gas were widely used to fight against stored product pests. 157 tons of aluminum phosphide and 12 tons of magnesium phosphide imports were carried out to Turkey in 2011 (Anonymous, 2012). Only phosphine (PH_3) is used as a fumigant by removing it from the use except methyl bromide quarantine applications.

Phosphine mechanism of action on insects; reducing the effect of the enzyme acetylcholinesterase in the nervous system, inhibiting the enzyme glycerophosphate dehydrogenase, and neutralizing the Complex IV region in mitochondria. Furthermore, because of a direct redox relationship between phosphine and cysteine in reactive disulfide, phosphine disrupts the enzyme glutathione reductase and breaks down sulphide bonds in cysteine (Nath et al., 2011). It is important to determine the resistance status of this pest to phosphine when taking into consideration that it is a long-lasting and intensely single fumigant phosphine used in the challenge with *C. ferrugineus*. The World Health Organization defines resistance as the development of a specific dose of a toxic substance found to have killed a majority of individuals in a normal population, the ability to gain tolerance in other affected individuals. The major disadvantage is that the ability of the pests to develop resistance to this chemical but phosphine has many advantages such as ease of use, low cost and no significant residue problem.

Warehouse pests have been found to develop resistance to phosphine in more than 45 countries (Benhalima et al., 2004; Zettler and Keever, 1994; Pimentel et al., 2010). Phosphine is preferred because of its low cost, rapid spreading in air and low residue (Champ and Dyte, 1976; Benhalima et al., 2004).

Therefore, it is important to develop resistance management models by protecting the efficacy of phosphine against stored product pests, the sustainability of its use, and the rate of resistance to phosphine in the pests.

In this study the phosphine resistance levels against *C. ferrugineus* populations collected from grain storage in various regions of Turkey was determined.

MATERIALS AND METHODS

In the experiments, 16 *C. ferrugineus* populations obtained from grain warehouses in Adana, Burdur, Konya, Isparta, İzmir, Samsun and Şanlıurfa (Figure 1). *C. ferrugineus* were cultured in 500 ml glass jars at 30°C and 60% relative humidity under constant conditions with 5 g yeast in 100 g oatmeal.

From this culture, in which the adults were kept in the medium for 10 days, the eggs were transferred to a new culture medium daily. Toxicological studies were carried out on 2-3 week old adults (Flinn and Hagstrum, 1998).

In order to determine the resistance ratios of the cultured individuals to the phosphine in the laboratory, discriminating dose applications were performed primarily.

The discriminating dose for *C. ferrugineus* is the application of 0.05 mg/l phosphine for 20 hours. LC_{50} and LC_{99} studies have been carried out for the populations in the case of live individuals. In the absence of live individuals, the population was considered sensitive (FAO, 1975; Anonymous, 2013).

Figure 1. Provinces across Turkey where *Cryptolestes ferrugineus* populations collected

In the case of live individual/s, the LC_{50} and LC_{99} studies were carried out in 5 different doses and 3 replications, determined preliminarily. Live-dead insect counts were subjected to probit analysis to obtain lethal concentration values (LC_{50} and LC_{99}), slope and confidence limits. For probit analysis, POLO-PC 1987 and PoloPlus 2002-2009 (LeOra Software Inc., Berkeley, CA, USA) programs were used. Resistance ratios were obtained by dividing the LC_{50} values of the land population by the LC_{50} values of the sensitive population (FAO, 1975; Pimentel et al., 2008).

The gaseous phosphine (PH_3) used in the studies was produced by mixing aluminum phosphite pellets in water containing 5% H_2SO_4 (FAO, 1975). The PH_3 gas collected at the top of the gas generator was taken from the septum by gas syringe.

In experiments, gas-tight custom desiccants with a volume of 3 liters and ATI-Porta Sense II gas meter to measure the amount of phosphine were used. In each desiccator 25 randomly selected adult individuals were placed in 3 x 3 x 3 cm PVC containers with 2 g nutrients. Various amounts of phosphine gas were injected from the septum of the desiccator and the required gas concentrations in the desiccator were provided and then the gas meter and the gas inlet-outlet valves were closed.

The desiccator was placed into incubator at 25°C and 65±5% humidity (FAO, 1975; Kahraman, 2009; Opit et al., 2012). After 20 hours of fumigation application and transfer of adult individuals into the food containing jars in incubators (25°C and 65±5% humidity) for

14 days, live individuals were counted for both the discriminating dose and the LC_{50}-LC_{99} determinations.

RESULTS AND DISCUSSIONS

The studies were carried out in 2016-2017. In this study, resistance against phosphine in *C. ferrugineus* populations collected from different regions of Turkey was investigated. After reaching enough numbers to be assayed in the laboratory, the cultures were subjected to a 20-hour treatment with a concentration of 0.05 mg/l (35.71 ppm) phosphine, the discrimiating dose for *C. ferrugineus*. It has been determined that populations resist at varying rates. As a result of the application, live individuals were observed in the populations of K1, K2, K5 and E4, and LC_{50} and LC_{99} values were determined by bioassay experiments. The İ5 population was accepted as sensitive population because of the low lethal concentration of phosphine, LC_{50} and LC_{99} values were calculated and assessed for comparison (Table 1).

In the bioassay experiments with K2, K5 and E4 populations, up to 80% deaths were not detected despite the increase the dose of phosphine. The exact LC_{50} values for the three populations mentioned could not be determined because testing of higher doses than our phosphine measurement device limits had the risk of subsequent flammability.

Considering the LC_{50} values, the resistance ratios obtained by proportion of the populations examined to the sensitive population were 11.73 for A3, 43.88 for E11, 19.73 for E12, 75.32 for F3, 3.68 for G2, 3.38 for H3, 66.71 for İ1, 279.62 for E4, 358.9 for K2 and eventually 392.65 times more resistant for K5 populations. The most resistant population was found in samples taken from Adana Mustafabeyli TMO warehouse with 392.65 times.

Table 1. Of phosphine resistance ratios of *C. ferrugineus* populations across Turkey

Population	n	Slope ± SE	χ2(df)	LC50 (ppm) (Confidence intervals)	LC99 (ppm) (Confidence intervals)	RR
İ5 (Susceptible) Samsun	375	5.09 ± 0.45	15.89	16.82 (14.95- 18.65)	48.14 (40.08- 62.91)	1
H3 Burdur	375	3.19±0.31	10.04	56.91 (49.22- 64.06)	303.87 (238.97-429.25)	3.38
G2 Isparta	375	3.33 ±0.35	9.42	62.05 (53.81 -70.28)	309.77 (237.52- 457.78)	3.68
E12 Konya	375	5.79±0.63	4.93	325.87 (291.24-356.72)	821.93 (710.2- 1017.56)	19.73
İ2 Samsun	375	7.47 ±1.56	15.36	459.18 (390.30 - 501.20)	940.64 (762.38 - 1701.57)	27.29
E11 Konya	375	3.23±0.3	35.36	738.22 (603.75- 908.40)	3864.1 (2463.1- 9161.5)	43.88
İ1 Samsun	375	5.43±0.47	7.22	1122.21 (1048.28 -1198.53)	3005.96 (2582- 3708.86)	66.71
F3 Şanlıurfa	375	5.35 ±0.55	18.24	1265.53 (1166.79- 1373.13)	3438.66 (2758.43-4977.97)	75.32
E4 Konya	375	6.68±1.04	1.79	4703.21 (4354.68-5333.53)	10480 (8170.8-16675)	279.62
K2 Adana	375	7.47±2.05	2.16	6036.73 (5173.89-9797.06)	12365 (8313-46013)	358.9
K5 Adana	375	7.09±2.34	2.92	6604.5 (5388±16247.7)	14046 (8587.7- 135995)	392.65

n = number of *Cryptolestes ferrugineus*
RR = Resistance ratio (LC_{50} value of the examined population/LC_{50} value of the sensitive population)

It has been found that 10% of the storage pest samples collected in the world-wide studies conducted within the FAO in 1972-1973 were found to be phosphine-resistant. Considering the cases of phosphine resistance, it was found highest in *R. dominica* (F.), *Tribolium* spp. and *Sitophilus* spp. (Champ, Dyte 1976; Taylor, 1989). There are a number of studies showing phosphine resistance in *C. ferrugineus* in China (Liu, 2004; Lu et al., 2005). Madhumathi et al. (2007) indicate that the resistance rate of the population of *C. ferrugineus* collected from different regions in India between 1995 and 1997 was 210 to 230 times.

Nayak et al. (2010) found that there was a need for 0.5 mg/l dose-30 days and 1 mg/l dose-24 days in their study to provide resistance management in Australia's highly resistant population of *C. ferrugineus*.

In Australia, Nayak et al. (2013), in the *Rhyzopertha dominica* population known to be resistant to phosphine, 100 % mortality was observed at 1.4 mg/l during the 144 hours application period, while in the same application this dose was found to be 3.62 mg/l in the *C. ferrugineus* populations with strong resistance. Same research indicated absolute death at 25°C in the strongly resistant *Sitophylus oryzae* population and 0.56 mg/l at 72 hours of exposure, while absolute death for *C. ferrugineus* was observed at the dose of 0.7 mg/l for 30 days.

Konemann et al. (2017), report that resistance were 7.3, 636.4, and 968.6 ppm, respectively, over a 3-d exposure period. The most resistant population, was 133.5 times that of the susceptible laboratory strain.

Duong et al. (2016) collected 176 populations from 125 regions of Vietnam. High frequency resistance has been detected from *C. ferrugineus* (37 and 58%, n = 19), *R. dominica* (1.5 and 97%, n = 65), *S. oryzae* (34 and 59%, n = 82) and *T. castaneum* (70% and 30%, n = 10), respectively.

Konemann et al. (2017) estimated discriminating dose by examining susceptible *C. ferrugineus* populations under 56.2 ppm PH_3/20 hours exposure. They used this discriminating dose to determine PH_3 resistance in 19 *C. ferrugineus* populations from Oklahoma/USA. They determined that all tested populations were resistant to phosphine. However, they have calculated only five populations LC_{99} values because the resistance frequencies of these populations are greater than 90%. The LC_{99} of the susceptible population was found to be 7.33 ppm/3 days, the highest two populations were 636.4 and 968.6 ppm/3 days. They reported that the resistance level of the most resistant population was 133.5 times more resistant than the susceptile laboratory population.

CONCLUSIONS

As a result of the study, *C. ferrugineus* populations of Turkey is widespread in grain storages, which are key pests and over the years have been able to develop high resistance against phosphine. The presence of high levels of resistance (8 of 10 populations), shows that Turkish *C. ferrugineus* populations have high resistance (Avg. 80%) against phosphine. When it is considered that the sampled granaries are generally commercial deposits, it can be easily seen that *C. ferrugineus* does not have a no resistance zone in the reserves using phosphine. To ensure the use of phosphine of sustainable, phosphine resistance related results showed that, the researchers, phosphine traders, practitioners and managers must get togather to shifting to phosphine-use management in Turkey and the determination of national phosphine resistance management strategies and implementation requirements.

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