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Phosphine Resistance in Turkish Populations of *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae)

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ABSTRACT

In this study, the status of phosphine resistance in *Sitophilus oryzae* (L.,1763) (Coleoptera: Curculionidae) populations collected from Şanlıurfa, Adana and Kahramanmaraş province in Turkey were investigated by conducting the discriminating concentration tests and the concentration–mortality bioassays. Low (0.04 mg/L) and high (0.20 mg/L) discriminating concentration tests indicated that there was phosphine resistance of *S. oryzae*. The survival rates of field population of *S. oryzae* in Şanlıurfa, Adana and Kahramanmaraş ranged from 0 to 99%, 0 to 90% and 0 to 89% at the low discriminating concentration while it ranged from 0 to 83%, 0 to 46.5% and 0 to 28.5% at the high discriminating concentration respectively. Based on 50% mortality level (LC_{50}), the Şanlıurfa 4, Adana 7 and Kahramanmaraş 8 populations were 57.5, 28.9, and 16.3 times more resistant, respectively, than the susceptible population (Kahramanmaraş 4). In conclusion, this study revealed that phosphine resistance in *S. oryzae* is high in the examined areas of Turkey and some populations have levels of resistance that may pose challenges to the continued use of phosphine for their management.

Key words: Discriminating concentration, fumigation, phosphine resistant, rice weevil, *Sitophilus oryzae*, Turkey.

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INTRODUCTION

Total global grain production is around 2.978 billion tons in 2019 (FAO, 2022). Turkey's total grain production was nearly 31.9 million tons in 2021 (TÜİK, 2022), representing 1.29% of total global grain production. During storage, grains are attacked by a numerous pest, particularly insect species, which cause very serious quantitative losses and qualitative degradations. Among stored-grain insect pests, the rice weevil, *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), is one of the most destructive primary pests attacking many common stored cereals including rice, wheat and maize, and has a worldwide distribution (Longstaff, 1981; Gomes, Rodriguez, Poneleit, Blake, & Smith, 1983; Grenier, Mbaiguinam, & Delobel, 1997). Stored and milled rice grains are prone to attack by *S. oryzae*, causing heavy economic losses, and both the adults and larvae feed on the carbohydrates in rice and wheat grains causing weight loss and contamination (Park, Lee, Choi, Park, & Ahn, 2003). Adams (1976) reported that *S. oryzae* causes substantial losses to stored grain amounting 18.30%. Phosphine (PH_3) is the primary fumigant used to protect the majority of grain and a variety of other stored commodities from insect pests (Chaudhry, 2000; Wang, Collins, & Gao, 2006). Attributes that contribute to wide-spread use of PH_3 are that it is relatively cheap, easy to apply, leaves no or minimal residues and can be used in many storage types and commodities (Nayak & Collins, 2008).

The lack of ideal airtight conditions for fumigation in leaky structures increases the frequency of control failures and thus increases the frequency of PH_3 fumigation (Pacheco, Sartori, & Taylor, 1990; Chaudhry, 2000; Benhalima, Chaudhry, Mills, & Price, 2004; Lorini, Collins, Daglish, Nayak, & Pavic, 2007). Phosphine fumigation is a long establishedlong-established effective method to control stored product insects, but its continuous and discriminate use has resulted in the evolution of resistant populations (Chaudhry, 2000; Collins, Daglish, Pavic, & Kopittke, 2005; Lorini et al, 2007; Pimentel, Faroni, Totola, & Guedes, 2007). Repeated applications of PH_3 in poorly sealed storage unit have been considered as the cause of the development of strong resistance (Friendship, Halliday, & Harris, 1986; Zeng, 1999). The resistance of stored-grain insect pests to PH_3 was first reported following a worldwide survey carried out by the Food and Agriculture Organization of the United Nations in 1972-73 (Champ & Dyte, 1976). In this study, PH_3 resistance was detected in 33 out of the 82 countries they surveyed involving 82 of the 849 populations tested. Strong PH_3 resistance in all key species of stored grains has been reported in a number of countries (Lorini et al, 2007; Pimentel, Faroni, Silva, Batista, & Guedes, 2010; Opit, Philips, Aikins, & Hasan, 2012; Daglish, Nayak, & Pavic, 2014; Sağlam, Edde, & Phillips, 2015).

Resistance is a threat to effective management of *S. oryzae*, with its PH_3 resistance reported from many countries around the world (Champ & Dyte, 1976; Price & Mills, 1988; Herron, 1990; White & Lambkin, 1990; Rajendran & Narasimha, 1994; Benhalima et al, 2004). Strong resistance to PH_3 was first recorded in *S. oryzae* in China in a 1995-1997 survey (Zeng, 1999). In this survey, a resistance level 337 times that of a susceptible strain was detected. The resistance level of *S. oryzae* in

India was reported in 1998 to have increased to 425 times that of a susceptible strain (Rajendran, 1999). Weakly resistant *S. oryzae* was found at a high frequency in most regions of Australia, with strong resistance occurring sporadically in field-collected strains (Emery, Collins & Wallbank, 2003). To date, there are few studies on the status of PH_3 resistance in stored grain insect pests in Turkey, Koçak, Schlipalius, Kaur, Thuck, Ebert, Collins, & Yilmaz, 2015; Koçak, Yilmaz, Alpkent, & Ertürk, 2018). Koçak et al (2015) reported that four Turkish population of *Tribolium castaneum* (Herbst, 1797) were tested through bioassays for determining PH_3 resistance phenotypes and all population exhibited high level of PH_3 resistance. However, it is obvious that there is very limited information on the status and monitoring of PH_3 resistance in stored-grain insect pests in Turkey. The lack of scientific information on the status of PH_3 resistance in stored-grain insect pests from different geographic locations in Turkey justifies the study of long-term monitoring of PH_3 resistance in these insect pest populations in all regions of Turkey. The objective of the present study is to determine resistance status and levels of PH_3 resistance in *S. oryzae* adults collected from grain storage facilities in Şanlıurfa, Adana and Kahramanmaraş province of Turkey.

MATERIAL AND METHODS

Test insects and insect culture

Sitophilus oryzae adults collected from three provinces of Turkey were used in the bioassays and cultured in the laboratory on whole wheat at 26 ± 1ºC and 65 ± 5 % relative humidity (RH). Insect populations were taken in Şanlıurfa, Adana and Kahramanmaraş provinces, which are located in the Eastern Mediterranean and South-eastern regions. For Şanlıurfa, Adana and Kahramanmaraş province, 9, 10 and 10 *S. oryzae* populations were collected respectively. Wheat grains were sampled in bulk with a spiral grain probe from nine to ten different wheat storage units per province. Additionally cylindrical plastic probe traps (STORGARD WB Probe II trap, TREECE, USA) were also used to collect *S. oryzae* adults in bulk stored-grain. A total of 29 different *S. oryzae* populations were collected from Şanlıurfa, Adana and Kahramanmaraş provinces from different grain storages and cultured in the laboratory after species identification according to Boudreaux (1969). Soft wheat (*Triticum aestivum* L., cultivar. Elbistan Yazlığı) with 11 ± 0.5 % moisture content used in insect culture was kept at -20 °C for approximately one week before being used for disinfestation, since it may be contaminated with other storage pests. The insects from infested wheat sampled taken from the grain storages were separated by using a 2 mm metal sieve (Retsch Brand, Retsch GmbH, Germany). After adding 250 g of wheat into 1 l glass jars, unsexed adults were added and the mouths of the jars were covered with a muslin cloth. The culture jars were kept at 26 ± 1 ºC and 65 ± 5% RH for 1 week in a fully dark environment and the adults were allowed to lay eggs. After 1 week, the insects were removed and the jars returned to above conditions. After 40-45 days, a new generation of adults was obtained. In the bioassay tests, the F_2 generations of the insects from field populations were used to determine the frequency of PH_3 resistance.

Phosphine fumigation procedure and gas analysis

One hundred thirty ml fumigation glass vials with the insects and 5 gr wheat, which are closed with aluminum cap with septa by using the crimper, were used in biological tests. Phosphine gas was obtained from a 10 l steel gas cylinder (volume; 1% PH_3 + 99% N_2) supplied from Linde Industrial Gases Company (Germany). The latex rubber ball with the septum was used for collecting PH_3 gas from the steel as cylinder. Gas-tight syringes (Hamilton, Bonaduz, Switzerland) were used for collecting PH_3 gas from gas sampling ball and injection of PH_3 gas into the fumigation glass vials (9.45 cm in height x 1.25 cm in diameter) for dosing. Target concentrations of gas were achieved by adding the required amount of the 1% PH_3 to the vial, following removal of the same volume of air, which was determined to meet the target concentration based on vial volume. Analysis and quantification of PH_3 concentrations achieved in laboratory fumigation jars generally followed the methods reported earlier in similar work (Sekhon, Schilling, Phillips, Aikins, Hasan, Corzo & Mikel, 2010, Opit et al, 2012). The gas chromatography (Shimadzu GC-2010 model, Shimadzu Corporation, Japan) with Flame Photometric Detector (FPD) and Rt®-Q-BOND capillary column (30 m length * 0.32 mm inner diameter * 10 µm film thickness, RESTEK GC Columns, USA) was used to determine the concentrations of PH_3 gas exposed to *S. oryzae* adults in biological tests. The injection temperature was set to 250 ºC, the column temperature to 120 °C and the detector temperature to 250 ºC. Nitrogen (N_2) was used as the carrier gas. The amount of PH_3 gas injected into the device was 25 µl. The retention time was 1.56 minute. All analysis of PH_3 gas were preceded by establishing a quantitative standard curve using known PH_3 concentrations that could then be used to derive the concentration in a jar via a regression equation derived from the standard curve. The PH_3 concentration for any given fumigation vial represented the average concentration for that vial measured 30 min after adding the gas and the concentration measured at the end of the exposure period just before venting the vial. There was no reduction of phosphine concentrations at the start and at the end of the measurements.

Phosphine resistance tests

Discriminating concentration tests

Discriminating concentration tests were used by FAO Method No. 16 (FAO, 1975) with the following modifications reported by Daglish & Collins (1999) to determine whether *S. oryzae* field populations had detectable resistance and their frequency of resistance. Each of *S. oryzae* field populations was exposed to low and high discriminating concentrations of 0.04 mg / l (29 ppm) and 0.20 mg / l (1436 ppm) respectively for 20 hours at 25 ± 1 ºC and 60 ± 5% RH by method modified by Daglish & Collins (1999). The results of discriminating concentration test were evaluated and resented by using the in Table 1 classification. Forty *S. oryzae* adults (1-2 week old) were added to each glass vial with a small quantity of diet (5 g of wheat) and the lids of the glass vial were closed with a rubber septum capped with aluminum crimp caps. Target concentrations were applied to each jar using small amounts of 1% PH_3 followed by quantitative GC-FPD as described above. For each insect population, five

fumigation vials with 40 *S. oryzae* adults and food media were separately exposed to the low and high discriminating concentrations. Test insects were held under these concentrations in a growth chamber for 20 h at a controlled temperature of 25 ± 1ºC, and then all vials were removed and held in fresh air for 14 d at 25 ± 1ºC and 60 ± 5 %RH, after which they were assessed for mortality. The beetles were removed from the vials and counted as live, moribund, or dead. Moribund and dead adults were placed in a 9-cm Petri dish containing a piece of filter paper moistened with 0.5 ml of water. These adults were then re-evaluated after 24 h for recovery. Five small vials (200 adults) were held under air in a fumigation jar to serve as experimental control beetles for inclusion in the concentration-mortality analysis as those exposed to 0 ppm PH_3, and were held and evaluated in the same way as fumigated beetles.

Table 1. Interpretation of discriminating concentration test results.

Low concentration 0.04 mg/l*	High concentration 0.20 mg/l**	Resistance classification
No survivors	No survivors	Susceptible
Survivors	No survivors	Weak resistance
Survivors	Survivors	Strong resistance

*FAO (1975) **Modification of Daglish & Collins(1999)

Lethal concentration tests

In order to determine the levels of PH_3 resistance in *S. oryzae* adults collected from grain storage facilities in Şanlıurfa, Adana and Kahramanmaraş provinces, one population for each province, which had the highest survival rate and one of PH_3-susceptible population was used for concentration-mortality tests. For concentration-mortality tests, each selected population was exposed to at least 6 to 8 different concentrations of PH_3 ranging from 0.014 to 0.76 mg/l for 20 h at 25 **±** 1°C and 65±5 % RH. Mortality assessments were conducted 14 days after PH_3 fumigation. Bioassay procedures were similar to discriminating concentration tests. In the determination of the most susceptible *S. oryzae* population, three susceptible field populations of *S. oryzae* which had no survival in both low and high discriminating concentrations (Şanlıurfa 7, Adana 1 and Kahramanmaraş 4) were exposed to concentration-mortality tests. Each selected population was exposed to 6 to 8 different concentrations of PH_3 ranging from 0.005 to 0.025 mg/l for 20 h at 25 **±** 1 °C and 65 **±** 5 % RH. Mortality assessments were conducted 14 days after PH_3 fumigation. According to the results of probit analysis, Kahramanmaraş 4 field population which had the lowest LC_{99} value (0.022 mg/l) was considered a PH_3-susceptible population.

Data analysis

In all biological tests, numbers of dead and live insect obtained on the 14th day after the fumigations were counted and the survival rates were calculated. Analysis of variance (ANOVA) was used to analyze percentage survival data after arcsine transformation to normalize the data. Percentage survival for each discriminating concentration was also adjusted for natural survival in controls using Abbott formula

before analysis and was then analyzed using one-way analysis of variance (factor; insect population). Differences between the means were determined using the Tukey Multiple Range Test at the 5% significance level. In order to calculate the lethal concentration values (LC_{50} and LC_{99}) of the PH_3-resistant *S. oryzae* populations, the concentration-mortality data obtained were subjected to probit analysis using the POLO-PC (LeOra Software, 1994) program. The resistance factor (RF_{50}) for each PH_3-resistant population was obtained by dividing the LC_{50} value estimated for each insect population by the LC_{50} value of the PH_3 susceptible population.

RESULTS

Discriminating concentration tests showed that survival rates at both low and high discriminating concentrations varied significantly among field populations of *S. oryzae* in Adana province (Table 2). Survival data at the low and high discriminating concentrations indicated that there was clear evidence of resistance in nine out ten field populations of *S. oryzae* in Adana province. Survival rate (%) of field population of *S. oryzae* ranged from 0 to 98.5% at the low discriminating concentration while it ranged from 0 to 46.5% at the high discriminating concentration. Numerically, Adana 3 and Adana 6 populations of *S. oryzae* had the highest survival rate, with 98.5 and 46.5% at the low and high discriminating concentrations, respectively. No survivor in the Adana 1 population of *S. oryzae* was obtained at both the low and high discriminating concentrations, which had no detectable resistance, referred as the susceptible population. Two out of ten populations of *S. oryzae*, Adana 2 and Adana 3, had weak resistance, which had survivors ranging from 95.5 to 98.5% at the low discriminating concentration, but no survivors were detected at the high discriminating concentration. Seven out ten populations of *S. oryzae*, Adana 4, 5, 6, 7, 8, 9, and 10, had strong resistance, with survival ranging from 10 to 89% and 7.5 to 46.5% at both the low and high discriminating concentrations respectively.

The survival rates at both the low and high discriminating concentrations varied significantly among field populations of *S. oryzae* in Kahramanmaraş province (Table 3). Discriminating concentration tests showed that there was resistance in eight out of ten field populations of *S. oryzae* in Kahramanmaraş province. Survival rate (%) of the field population of *S. oryzae* ranged from 0 to 90% and 0 to 28.5% at the low and high discriminating concentrations respectively. The Kahramanmaraş 5 population of *S. oryzae* had the highest survival rate, with 90 % at the low discriminating concentration while Kahramanmaraş 10 population had the highest survival rate, with 28.5% at the high discriminating concentration. There were no survivors in Kahramanmaraş 2 and Kahramanmaraş 4 populations of *S. oryzae* at either low or high discriminating concentrations, which demonstrates no detectable resistance. One out ten populations of *S. oryzae*, Kahramanmaraş 3 had weak resistance, with 23% survival at the low discriminating concentration, but no survivors were detected at the high discriminating concentration. Seven out ten populations of *S. oryzae*, Kahramanmaraş 1, 5, 6, 7, 8, 9 and 10, had strong resistance, with survival ranging from 21 to 90% and 4.5 to 28.5% at both the low and high discriminating concentrations, respectively.

Table 2. Survival rates (%) and phosphine resistance classification of field populations of *Sitophilus oryza*e adults collected from Adana province after 20-h exposure to discriminating concentrations (0.04 and 0.20 mg/L) of phosphine.

Insect population	Survival rate (%) ± S.E		Phosphine resistance status
	0.04 mg/l PH_3	0.20 mg/l PH_3	
Adana 1	0 ± 0 E	0 ± 0 E	Susceptible
Adana 2	95.5 ± 0 AB	0 ±0 E	Weak resistance
Adana 3	98.5 ± 0 A	0 ± 0 E	Weak resistance
Adana 4	37 ± 4 D	18 ± 3.9 BCD	Strong resistance
Adana 5	10 ± 4.4 E	7.5 ± 1.5 DE	Strong resistance
Adana 6	89 ± 3.4 AB	46.5 ± 6.5 A	Strong resistance
Adana 7	85 ± 2.9 AB	34 ± 4.2 AB	Strong resistance
Adana 8	75.5 ± 3.4 BC	17.5 ± 1.7 CD	Strong resistance
Adana 9	41 ± 9.2 D	21.5 ± 5 BCD	Strong resistance
Adana 10	64 ± 4.7 C	28.5 ± 2.8 BC	Strong resistance
F and P* value	$F_{9,40}$=71.04 P<0.0001	$F_{9,40}$=21.83 P<0.0001	

*One-way ANOVA was applied to the data. Means within a column with the same upper-case letter are not significantly different (Tukey test at 5% level).

Table 3. Survival rates (%) and phosphine resistance classification of field populations of *Sitophilus oryzae* adults collected from Kahramanmaraş province after 20-h exposure to discriminating concentrations (0.04 and 0.20 mg/L) of phosphine.

Insect population	Survival rate (%) ± S.E		Phosphine resistance status
	0.04 mg/l PH_3	0.20 mg/l PH_3	
Kahramanmaraş 1	12.5 ± 3.7 DE	4.5 ± 2.4 CD	Strong resistance
Kahramanmaraş 2	0 ± 0 E	0 ± 0 D	Susceptible
Kahramanmaraş 3	23 ± 3.9 CD	0 ± 0 D	Weak resistance
Kahramanmaraş 4	0 ± 0 E	0 ± 0 D	Susceptible
Kahramanmaraş 5	90 ± 2.8 A	20.5 ± 2.1 AB	Strong resistance
Kahramanmaraş 6	43.5 ± 4.1 BC	12 ± 1.6 BC	Strong resistance
Kahramanmaraş 7	21 ± 7.2 D	5.5 ± 2. 4 CD	Strong resistance
Kahramanmaraş 8	49.5 ± 6.4 B	12 ± 1.2 BC	Strong resistance
Kahramanmaraş 9	24.5 ± 8.3 CD	7 ± 1.4 CD	Strong resistance
Kahramanmaraş 10	71 ± 4.3 A	28.5 ± 3.2 A	Strong resistance
F and P* value	$F_{9,40}$=46.41 P<0.0001	$F_{9,40}$=27.17 P<0.0001	

*One-way ANOVA was applied to the data. Means within a column with the same upper-case letter are not significantly different (Tukey test at 5% level).

The survival rates at both the low and high discriminating concentrations varied significantly among field populations of *S. oryzae* in Şanlıurfa province (Table 4). Discriminating concentration tests showed that there was resistance in eight out of nine field populations of *S.oryzae* in Şanlıurfa province. Survival rate of the field population of *S. oryzae* ranged from 0 to 99% and 0 to 83% at the low and high discriminating concentrations, respectively. Şanlıurfa 2 population of *S. oryzae* had the highest survival rate, with 99 % at the low discriminating concentration while Şanlıurfa 4 population had the highest survival rate, with 83% at the high discriminating concentration. No survivor in Şanlıurfa 7 populations of *S. oryzae* was obtained at both the low and high discriminating concentrations, which had no detectable resistance. Eight out of nine populations of *S. oryzae*, Şanlıurfa 1, 2, 3, 4, 5, 6, 8, and 9, had strong resistance, with survival ranging from 57.5 to 99% and 11.5 to 83% at both the low and high discriminating concentrations, respectively.

Table 4. Survival rates (%) and phosphine resistance classification of field populations of *Sitophilus oryzae* adults collected from Şanlıurfa province after 20-h exposure to discriminating concentrations (0.04 and 0.20 mg/L) of phosphine.

Insect population	Survival rate (%) ± S.E		Phosphine resistance status
	0.04 mg/l PH_3	0.20 mg/l PH_3	
Şanlıurfa 1	97.5 ± 0.7 A	23.5 ± 3.9 D	Strong resistance
Şanlıurfa 2	99 ± 0.6 A	22 ± 5.7 D	Strong resistance
Şanlıurfa 3	91.5 ± 2.5 AB	78.5 ± 4.2 AB	Strong resistance
Şanlıurfa 4	94.5 ± 2 A	83 ± 2.5 A	Strong resistance
Şanlıurfa 5	88.5 ± 3.2 AB	63.5 ± 5.6 B	Strong resistance
Şanlıurfa 6	57.5 ± 10 C	11.5 ± 2DE	Strong resistance
Şanlıurfa 7	0 ± 0 D	0 ±0 E	Susceptible
Şanlıurfa 8	93 ± 2.9 A	61.5 ± 4.4 B	Strong resistance
Şanlıurfa 9	73 ± 2.5 BC	42.5 ± 2.6 C	Strong resistance
F and P* value	$F_{8,36}$=63.66 P<0.0001	$F_{8,36}$=59.93 P<0.0001	

*One-way ANOVA was applied to the data. Means within a column with the same upper-case letter are not significantly different (Tukey test at 5% level).

As concentrations of PH_3 discriminating concentration tests also indicated that the frequencies of PH_3 resistance in *S. oryzae* populations varied according to sampling locations (Fig. 1). Six and seven populations of *S. oryzae* in Şanlıurfa and Adana had high survival ranging from 61 to 100% at the low discriminating concentration respectively, whereas only one population in Kahramanmaraş had high survival. In the case of the high discriminating concentration, only Şanlıurfa had five populations with high survival while Adana and Kahramanmaraş had no populations with high survival. These findings indicated that the high resistance frequencies in *S. oryzae* populations were more common in Şanlıurfa than those in Adana and Kahramanmaraş while Kahramanmaraş had the lowest number of *S. oryzae* populations with high resistance frequency.

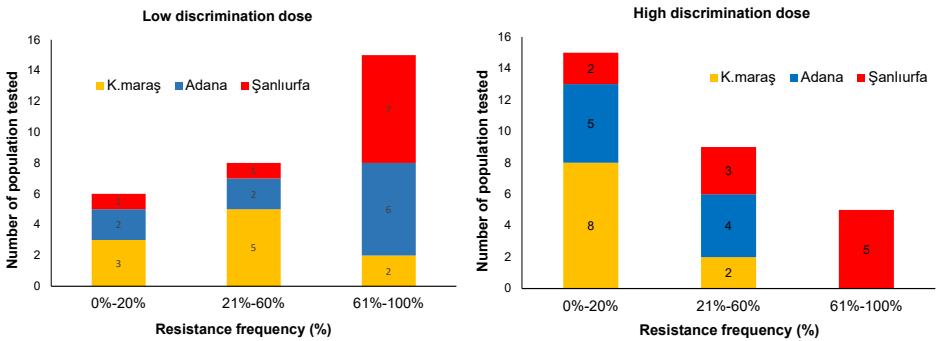

Fig. 1. Resistance frequency distribution for field populations of *Sitophilus oryzae* collected from Şanlıurfa, Adana and Kahramanmaraş province after 20-h exposure to (a) low (0.04 mg/L) and (b) high (0.20 mg/L) discriminating concentration of phosphine.

As concentrations of PH_3 required to kill 50 and 99% of the susceptible population (Kahramanmaraş 4) were compared with Adana 7, Şanlıurfa 4, Kahramanmaraş 8 population of *S. oryzae*, 95 % CLs of the susceptible population did not overlap those of Adana 7, Şanlıurfa 4, Kahramanmaraş 8 population, so the LC_{50} and LC_{99} values in each case were different. Therefore, in relation to LC_{50}, the Adana 7, Şanlıurfa 4, and Kahramanmaraş 8 populations were 28.9, 57.5, and 16.3 times more resistant than the susceptible population (Kahramanmaraş 4) respectively. Similarly, 95 % CLs of Şanlıurfa 4 population did not overlap those of Adana 7 and Kahramanmaraş 8 population, so the LC_{50} and LC_{99} values in each case were different. Based on LC_{50} values, Şanlıurfa 4 population was 2 and 3.5 times more resistant than Adana 7 and Kahramanmaraş 8 populations respectively Table 5. Finally, 95 % CLs of Adana 7 population did not overlap those of Maraş 8 population, so the LC_{50} and LC_{99} values in each case were different. Based on LC_{50} values, Adana 7 population was 1.77 times more resistant than Kahramanmaraş 8 population. It is important to note that the significant differences found in all comparisons existed despite the fact that heterogeneity values in all cases were >1, which implies the differences between the populations compared were quite pronounced.

Table 5. Probit analysis data of mortality for phosphine-resistant and susceptible field populations of *Sitophilus oryzae* resulting from 20-h laboratory fumigations at 25±1 °C and 65±5 % relative humidity.

Insect population	n[a]	Slope±SE	LC_{50} (mg/l) (95 % CI)[b]	LC_{99} (mg/l) (95 % CI)	χ^{2c}	(df)[d]	H[e]	RR[f]
Adana 7	1200	6.57±0.58	0.23 (0.20-0.25)	0.52 (0.47-0.63)	54.97	(28)	1.96	28.9
Şanlıurfa 4	1000	12.92±0.9	0.46 (0.45-0.48)	0.70 (0.66-0.77)	39.08	(23)	1.69	57.5
Kahramanmaraş 8	600	3.45±0.3	0.13 (0.10-0.15)	0.64 (0.46-1.03)	37.86	(18)	2.1	16.3
Kahramanmaraş 4*	1000	4.86±0.3	0.008 (0.007-0.009)	0.022 (0.026-0.033)	50.53	(23)	2.1	-

[a] Number of insects subjected to phosphine bioassay, excluding control, [b] Numbers in brackets give the 95% confidence intervals, [c] Chi square value, [d] Degree of freedom, [e] Heterogeneity value, [f] Resistance Ratio (RR) = Resistance Ratio (LC_{50} of resistant /LC_{50} of susceptible strain). * Susceptible field population.

CONCLUSION AND DISCUSSION

This study of PH_3 resistance in *S. oryzae* involved more extensive sampling than previous work in collecting field insects to assess the frequency and levels of resistance in some geographical locations in Turkey (Koçak et al, 2018). There are more populations with higher frequencies of resistance in our study compared with a similar study carried out in Turkey (Koçak et al, 2018). Low and high discriminating concentration tests indicated that there was clear evidence of PH_3 resistance in nine and eight out of ten field populations of *S. oryzae* (90 and 80%) in Adana and Kahramanmaraş respectively and eight out of nine populations (88.9%) in Şanlıurfa, whereas Koçak et al, (2018) found that only eleven out of 28 populations (39.3 %) of *S. oryzae* sampling from 14 provinces in Turkey had detectable resistance to PH_3. Similar to our study, Tingiş, Işikber, Sağlam, Bozkurt & Doğanay, (2018) reported that 89.9 and 83.3 % populations of tested *S. oryzae* populations collected from Mersin and Konya provinces respectively were resistant to PH_3, which reveals high prevalence of PH_3 resistance in the insect sampling locations of both provinces. In the present study, survival rates within field populations of *S. oryzae* in Adana, Kahramanmaraş and Şanlıurfa ranged from 0 to 98.5%, 0 to 90% and 0 to 99% at the low discriminating concentration while it ranged from 0 to 46.5%, 0 to 28.5% and 0 to 83% at the high discriminating concentration respectively. In the Tingiş et al, (2018) study, the ranges of these frequencies were 0-97.5% and 0-90% at the low and high discriminating concentrations for Mersin province, while they were 0-91.5% and 0-63% for Konya province respectively. These findings indicate that the high frequencies of resistance in *S. oryzae* populations are widespread in the grain storages sampled in Turkey, although the frequencies of PH_3 resistance varied according to the locations. Similarly, the resistance to PH_3 in *S oryzae* populations in many countries is increasing in frequency and strength. Although Champ & Dyte (1976) reported no resistance in Australian samples in 1976, less than two decades later resistance was detected in 35% of samples from the state of Queensland (White & Lambkin, 1990), 18% of samples from New South Wales (Herron, 1990) and 9% of samples from Western Australia (Emery, 1994). In India, the percentage of resistant strains increased from 30% during the early 1970s 10 to 72% in the 1990s (Rajendran, 1999).

PH_3 resistance was detected in 33 out of the 82 countries they surveyed involving 82 of the 849 populations tested (Champ & Dyte, 1976). At the time of that survey, the most resistant populations had 12 times more resistance than the susceptible ones. Reported resistance factors for unselected, or partially selected, field population of *S. oryzae* have increased from a maximum of 2.5× in the early 1970s (Champ & Dyte, 1976) to 52× in Australia (Nguyen, Collins & Ebert, 2015), 337× in China (Zeng, 1999) and 425× in India (Rajendran, 1999). And now, the present study shows that high levels of resistance occur in Turkish populations of *S. oryzae* as well. Based on 50% mortality level (LC_{50}), the Adana 7, Şanlıurfa 4, and Kahramanmaraş 8 populations in Turkey were 28.9, 57.5, and 16.3 times more resistant than the susceptible population (Kahramanmaraş 4) respectively. Tingiş et al (2018) and Koçak et al (2018) reported also a higher level of PH_3 resistance in different provinces in Turkey. In Tingiş et al (2018) study, based on the resistance factors (RF) calculated by LC_{50} values, *S. oryzae*

populations from Mersin and Konya province in Turkey were 102- to 104-fold and 38- to 81-fold more resistant to PH_3 compared with susceptible ones, respectively. Koçak et al (2018) also reported that based on LC_{50} values, *S. oryzae* populations from different provinces in Turkey were 3- to 200-fold more resistant to PH_3 compared with susceptible ones. These findings indicate that there are great variations in levels of PH_3 resistance in *S. oryzae* populations sampled from different provinces in Turkey.

In the present study, the frequencies of PH_3 resistance in *S. oryzae* populations varied according to sampling locations. The high resistance frequencies in *S. oryzae* populations were more common in Şanlıurfa than in those from Adana and Kahramanmaraş while Kahramanmaraş had the lowest number of *S. oryzae* populations with high resistance frequency. Şanlıurfa is known to have the highest production of wheat among the sampling locations (TUİK, 2020). Information from previous studies shows that *S. oryzae* is one of the most common stored-grain insect species in Turkey, and PH_3 is likely the only fumigant used in the control of stored grain insects in these provinces (Tingiş et al, 2018; Koçak et al, 2018). The high use of PH_3 can serve as an increased selection pressure for the resistance alleles. The adults of *S. oryzae* sampled from Kahramanmaraş had a lower resistance frequency, which could be due to very low frequencies of resistant genes under an environment of regular effective PH_3 use, or from minimal selection pressure of resistant genes due to low use of PH_3 in that province.

The gas concentration and exposure time (C × t product), in addition to temperature and RH, when fumigating with PH_3, plays a crucial role in its effectiveness for the most tolerant life stages of a pest species (Hagstrum et al, 2012). The frequency and level of PH_3 resistance in *S. oryzae* detected in the present study are based on experiments with a 20-h PH_3 exposure period at 25°C using adult beetles, which is the standard FAO method allowing us to discriminate between resistant to susceptible beetles in a simple way. Furthermore, the dose-mortality studies followed for a 20-h exposure time at 25°C to determine weak- and strong-resistant phenotypes based on PH_3 concentration alone. In the present study dose-mortality experiments clearly determined the weak- and strong-resistant phenotypes, but the calculated estimated concentrations required for 99% mortality should not be considered for practical fumigations to control infestations in the field, for which 20-h exposure period would be impractical and never recommended (Hagstrum et al, 2012).

The high level of PH_3 resistance detected among *S. oryzae* populations may be due to the previous selection pressure caused by inadequate fumigations in the storage facilities, from where the insect samples were collected. Other factors for differences in PH_3 resistance observed in the present study could be: fumigation in unsealed silos, lack of monitoring of PH_3 concentration during fumigation, and extensive use of only one fumigant (Benhalima et al, 2004; Wang et al, 2006; Lorini et al, 2007), favorable weather conditions for the insect pest (Collins et al, 2005; Lorini et al, 2007) and little or no insect management practiced in farms and others storage facilities (Pacheco et al, 1990; Lorini et al, 2007). In addition, the movement of insects as a result of the commodity trade is a likely contributor for the spread of PH_3 resistance in stored-product insects. As a result, lots of grains are fumigated frequently, and since the insect populations were

not successfully controlled, very high concentrations of PH_3 were used, increasing the likelihood selection for resistance (Collins et al, 2005; Lorini et al, 2007).

Phosphine gas is an important tool for the management of stored grain pests, and the occurrence of PH_3 resistance in pest populations presents challenges to the continued effective use of this fumigant. In the present study, the findings that PH_3 resistance was detected in nearly all populations of *S. oryzae* studied, with high frequencies of resistance in those populations, and the finding that many of these populations contained insects with the strong-resistant phenotype, point to the challenge pest managers are facing with PH_3 resistance. The levels of resistance in Turkey call for the use of an effective resistance monitoring program that contributes to fumigation operators to make decisions whether or not to use PH_3 in a given situation. Further work is required to continue monitoring for PH_3 resistance in Turkey, to determine strategies to manage resistance to PH_3 and to develop or use alternative fumigants such as sulfuryl fluoride, where PH_3 resistance has developed to very high levels (Opit, Thoms, Phillips & Payton, 2016) and also inert dusts, such as diatomoceus earth can be alternative for resistant strains (Korunic, 1998; Conceição, Faroni, Sousa, Pimentel, & Freitas, 2012; Sakka & Athanassiou, 2021).

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