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Screening of Phosphine Resistance in *Sitophilus oryzae* (L.) (Rice Weevil) Populations in Turkey

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In this study, the status and prevalence of phosphine resistance in *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) populations collected from Mersin and Konya Province in Turkey were investigated by conducting the discrimination concentration tests and the concentration–mortality bioassays. Discriminating concentration tests showed that 89.9 and 83.3 % populations of tested total *S. oryzae* populations collected from Mersin and Konya province respectively were resistance to phosphine, which reveals high prevalence of phosphine resistance in the insect sampling locations of both provinces. Moreover, discrimination low concentration (0.04 mg/l) tests indicated that 62.5 and 33.3% of total *S. oryzae* populations collected from Mersin and Konya province respectively had 90% or above survival rate, which showed that the frequency of high phosphine resistance in *S. oryzae* populations collected from Mersin province was higher than that in *S. oryzae* populations collected from Konya province. The concentration–mortality bioassays indicated that there were significant differences in resistance levels of *S. oryzae* populations collected from different provinces. Based on the resistance factors (RF) calculated by LC_{50} values *S. oryzae* populations from Mersin and Konya province were 102- to 104-fold and 38- and 81-fold resistance to phosphine compared with susceptible *S. oryzae* population, respectively. The highest level of phosphine resistance was determined in *S. oryzae* populations from Mersin province, followed by those from Konya provinces, respectively. These results indicated that *S. oryzae* populations from Mersin province had higher phosphine resistance than those from Konya Province. In conclusion, this study showed that high levels of phosphine resistance in *S. oryzae* populations collected from different grain storages in Mersin and Konya province of Turkey were prevalent.

Key Words: Phosphine resistance, *Sitophilus oryzae*, populations, discrimination concentration, Turkey

Introduction

Total global grain production is around 2.569 billion tons in 2015-2016 (FAO 2016). Turkey's total grain production is nearly 33.2 million tons in 2015-2016 (FAO 2016), presenting 1.29% of total global grain production. During storage, grains are attacked by a numerous pests, particularly insect species, which cause very serious quantitative losses and qualitative degradations. Pest control in any storage system depends on fumigation with either methyl bromide or phosphine. Phosphine is the primary fumigant used to protect the majority of grain and a variety of other stored commodities from insect pests (Chaudhry 2000; Wang et al. 2006). Attributes that contribute to wide- spread use of phosphine are that it is relatively inexpensive, easy to apply, leaves minimal residues and can be used in a wide range of storage types and commodities (Nayak and 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 phosphine fumigation (Pacheco et al. 1990; Chaudhry 2000; Benhalima et al. 2004; Lorini et al. 2007). Phosphine fumigation is a long 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 et al. 2005; Lorini et al. 2007; Pimentel et al. 2007). Repeated application of phosphine in poorly sealed warehouse resulting in under closing have been cited as the cause of the development of strong resistance (Friendship et al. 1986; Zeng 1999). Strong phosphine resistance in all key species of stored grains has been reported in a number of countries (Lorini et al. 2007; Pimentel et al. 2010; Opit et al. 2012; Daglish et al. 2014). The resistance of stored grain insect pests to phosphine was reported following a worldwide survey carried out by the Food and Agriculture Organization (FAO) of the United Nations in 1972-73 (Champ and Dyte, 1976) which detected resistance in 33 out of the 82 countries they surveyed involving 82 of the 849 population tested.

Strong resistance to phosphine 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 fully susceptible strain was observed. The resistance level of this species in India was reported in 1998 to have increased to 425 times that of a susceptible reference strain (Rajendran 1999). Weakly resistant *S. oryzae* is found at a high frequency in most regions of Australia, with strong resistance occurring sporadically in field collected strains (Emery et al. 2003). To date, there is only one study on the status of phosphine resistance in stored grain insect pests in Turkey, published by Koçak et al. (2015). In this study, four Turkish population of *T. castaneum* were tested through bioassays for determining phosphine resistance phenotypes and all population exhibited high level of phosphine resistance. There is very limited information on the status of PH_3 resistance in stored grain insect pests in Turkey. The objective of present study to determine resistance frequencies and the levels of phosphine resistance in *S. oryzae* adults collected from grain storage facilities in South and South-eastern region of Turkey.

Material and Methods

Test insects and insect collection

Sitophilus oryzae adults collected from two provinces of Turkey were used in the bioassays and were cultured in the laboratory on whole wheat at 26 ± 1^{o}C and 65 ± 5 % relative humidity (RH). Insect populations were taken in Mersin and Konya provinces of Turkey, which are located in Central Anatolian and South-eastern regions. For Mersin and Konya province, 18 and 6 *S. oryzae* populations were collected respectively. Wheat grains were taken in bulk with a spiral grain probe from ten different wheat storages per each province. Cylindrical plastic probe traps (STORGARD WB Probe II trap, TREECE, USA) were also used to detect adult stage of *S. oryzae* in bulk stored-grain. From June, 2016 up to November 2016, the probe traps were checked for adult beetles every month.

Phosphine fumigation procedures

One hundred fifty ml fumigation glass vials with the insects and 40 gr wheat, which are closed with aluminum cap with septa by using the crimper. Phosphine gas cylindrical tube (volume; %1 PH_3 + % 99 N_2 and latex rubber ball for gas sampling. Collecting phosphine gas from gas sampling ball by the syringe. Injection of phosphine gas into the fumigation glass vials for dosing. A GC with FPD detector for measuring phosphine concentration. Taking phosphine gas concentration in each vial and injection to GC sampling port. Fumigation jars held for 20 hours at 25 ± 1°C. Insects held for 14 d at 25 ± 1°C and 65 ± 5% RH

Phosphine resistance tests

Discrimination concentration tests

Discrimination concentration tests was used to determine whether the samples had detectable resistance and the frequency of resistance (presence of resistance) by FAO Protocol #16 FAO Protocol: *S. oryzae* adults exposed to low discrimination dose (0.04 mg/l-29 pm) phosphine for 20 h, at 25°C and 65% RH. Mortality determined after 2 wk. In order to classification resistance population weak or strong, high discrimination dose (0.20 mg/l-1436 ppm) for 20 h by method of modification of Daglish and Collins (1998) (Table 1). Mortality determined after 2 weeks. FAO Protocol recommends 3 replications for each treatment and each replicate 40 mixed sex adults.

Table 1. Interpretation of discrimination concentration test results

Low dose 0.04 mg/l*	High dose 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 and Collins(1998)

Concentration-mortality tests

In order to determine the levels of phosphine resistance in *S. oryzae* adults collected from grain storage facilities in Mersin and Konya provinces of Turkey, two populations for each province, which had the highest resistance frequency and one of phosphine-susceptible population were used for concentration-mortality tests. For concentration-mortality tests, each selected population was exposed to at least 6 to 8 different concentrations of phosphine for 20 h at 25°C and 65% RH. Mortality assessments were conducted at 14 day after phosphine fumigation. Bioassay procedures were similar to discrimination concentration tests.

Data processing and 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 was also adjusted for natural survival in controls using Abbott formula before analysis and was then analyzed using two-way analysis of variance (factors; application concentration and insect population). Differences between the means were determined using the Duncan's Multiple Range Test at the 5% significance level. In order to calculate the lethal concentration values (LC_{50} and LC_{99}) of the phosphine-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 phosphine-resistant population was obtained by dividing the LC_{50} value estimated for each insect population by the LC_{50} value of the phosphine susceptible population.

Results and Discussion

Discriminating concentration tests showed that 89.9 and 83.3 % populations of tested total *S. oryzae* populations collected from Mersin and Konya province respectively were resistance to phosphine, which reveals high prevalence of phosphine resistance in the insect sampling locations of both provinces. Moreover, discrimination low concentration (0.04 mg/l) tests indicated that 62.5 and 33.3% of total *S. oryzae* populations collected from Mersin and Konya province respectively had 90% or above survival rate, which showed that the frequency of high phosphine resistance in *S. oryzae* populations collected from Mersin province was higher than that in *S. oryzae* populations collected from Konya province.

The concentration–mortality bioassays indicated that there were significant differences in resistance levels of *S. oryzae* populations collected from different provinces. Based on the resistance factors (RF) calculated by LC_{50} values *S. oryzae* populations from Mersin and Konya province were 102- to 104-fold and 38- and 81-fold resistance to phosphine compared with susceptible *S. oryzae* population, respectively. The highest level of phosphine resistance was determined in *S. oryzae* populations from Mersin province, followed by those from Konya provinces, respectively. These results indicated that *S. oryzae* populations from Mersin province had higher phosphine resistance than those from Konya Province.

Conclusion

This study showed that high levels of phosphine resistance in *S. oryzae* populations collected from different grain storages in Mersin and Konya province of Turkey were prevalent.

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Co-fumigation with phosphine and sulfuryl fluoride: Potential for managing strongly phosphine-resistant rusty grain beetle, *Cryptolestes ferrugineus* (Stephens)

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Abstract

Populations of rusty grain beetle, *Cryptolestes ferrugineus*, have developed a very high level of resistance (1300×) to the fumigant phosphine (PH_3) in Australia. Resistant insects triggered control failures, threatening the country's annual grain market worth AU$8 billion. Although PH_3 protocols were amended to manage this new resistance, fumigation requires lengthy exposure periods which has practical difficulties. While there is no suitable replacement for PH_3, the current study explores potential approaches to enhance the efficacy of this fumigant. One possibility is co-fumigation of PH_3 with another complementary fumigant, sulfuryl fluoride (SO_2F_2 or SF), with the dual goals: enhanced efficacy and minimise use of both fumigants. A cohort of mixed age eggs and adults of PH_3-resistant *C. ferrugineus* was fumigated with PH_3 and SF individually, as well as in combination inside desiccators at 25°C and 60%RH for 168 h. Two doses below the maximal registered rates for SF (8.9 mg L^{-1}, equivalent to 1500 g hm^{-3}) and PH_3 (1.0 mg L^{-1}) were tested. Co-fumigation was performed simultaneously for 168 h. Our results revealed that, the mixture of 1.1 mg L^{-1} or 2.2 mg L^{-1} of SF and 0.5 mg L^{-1} of PH_3 over 168 h achieved complete control against resistant *C. ferrugineus* eggs and adults, whereas each of the tested doses failed individually. Our study confirms that SF and PH_3 enhance the efficacy of each other when used in combination, which holds great potential for managing resistant *C. ferrugineus*.

Key words: stored grain, phosphine resistance, sulfuryl fluoride, co-fumigants, resistance management

1. Introduction

Phosphine (PH_3), an effective fumigant is commonly used to disinfest stored grains and processed products from insect pests. However genetic resistance to this fumigant in insect pests is widespread and increasing (Schlipalius et al., 2012). For example, in Australia, populations of rusty grain beetles, *Cryptolestes ferrugineus* (Stephens), have developed a high level of resistance (1300×) to PH_3 and resistant insects require high concentrations (1 mg L^{-1}) and long exposure periods up to 14 days (Nayak et al., 2013). Thus, resistant insects of this species are a threat to grain industry as live insects of this species can jeopardy the country's access to international grain export markets worth of AU$ 8 billion annually. Although, new PH_3 protocols were developed (Kaur and Nayak, 2015) with higher PH_3 rates, there is an urgent need to find alternative pest control strategies that can enhance the efficacy of PH_3, specifically to shorten the fumigation period. One of such approaches is co-