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RESISTANCE TO PHOSPHINE IN RICE WEEVIL *SITOPHILUS ORYZAE* (L.) FROM SOUTH INDIA

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ABSTRACT

Rice weevil *Sitophilus oryzae* (L.) is one of most serious insect pests of stored grains. Management of this is thus vital and fumigation with phosphine gas is the most common method. However, overreliance on this has resulted in the development of heritable resistance in *S. oryzae*. The detection of frequency and distribution of phosphine resistance is crucial for the development of resistance management strategy. This study assesses the frequency, strength and distribution of phosphine resistance in *S. oryzae* populations from four southern states of India viz., Tamil Nadu, Kerala, Andhra Pradesh and Telangana. The bioassay results revealed that all these survived at both the discriminating concentrations of 0.04 and 0.25 mg/l. There were no susceptible and weak resistant populations, and exhibited varied frequency of strong resistance ranging from 13.33 to 80.90%. The populations from Pudukottai (80.90%) recorded high frequency of strong resistance followed by Trichy (68.97%) and Tirunelveli (67.82%) ones; the one from Thiruvarur exhibited lowest (13.33%). The results indicated that the resistance to phosphine in *S. oryzae* was common and prevalence of strong resistant individuals was prevalent across the sampled regions.

Key words: *Sitophilus oryzae***,** phosphine resistance, frequency and distribution, South India, strong and weak resistance, susceptible, discriminating doses, resistant management strategy

Rice weevil *Sitophilus oryzae* (L.) (Curculionidae; Coleoptera) is one of the most serious insect pests of stored grains and other commodities including processed foods, tobacco, sweet potato and seeds of avocado (Koehler, 1994; Stejskal et al., 2004; Hagstrum et al., 2013). Its larvae mainly feed on internal content of the whole grain, whereas the adults prefer to consume the outer portion of the grain, embryo and endosperm. The heavy infestations produce substantial amount of grain dusts (Hardman, 1977), attracting the secondary feeders. The movement and activity of *S. oryzae* enhances the temperature and relative humidity of the stored grains, which facilitates the microbial contamination (Cotton, 1920; Jian et al., 2012). Thus, it causes significant quantitative and qualitative grain losses (Agarwal et al., 1979; Park et al., 2003). Thus, the management of this weevil species is crucial for maintaining the food security. Fumigation with phosphine gas is the most widely used method for disinfestation of stored grains (Bell, 2000). It is due to its effectiveness against all the life stages besides easy to apply, inexpensive, residue free without affecting the viability of seed (Nayak and Collins, 2008). However, indiscriminate and prolonged use of phosphine gas has resulted in the development of heritable resistance in several stored grain insect pests including *S. oryzae* (Athie et al., 1998; Zeng, 1998; Benhalima et al., 2004; Holloway et al., 2016).

The first detailed survey on the susceptibility of stored product insect pests to insecticides and possible fumigants demonstrated that 8 of 135 samples of *S. oryzae* (5%) tested contained resistant individuals (Champ and Dyte, 1976). Later, high level of phosphine resistance in *S. oryzae* has been reported in India (425 folds) and China (337 folds) (Rajendran, 1999; Zeng, 1998). Resistance to phosphine in *S. oryzae* is increasing in both frequency and strength in several countries (Schlipalius et al., 2014). In India, a detailed nationwide survey on phosphine resistance in stored grain insect pests demonstrated the prevalence of phosphine resistant phenotypes at high frequencies of 72.00% (Rajendran, 1999). Sonai Rajan et al. (2017) studied the spatial distribution of phosphine resistance in *S. oryzae* and reported diverse frequencies of phosphine resistance ranged from 21.21 to 93.38% across the sampled regions. Though, these surveys did not discriminate strong resistant phenotypes from weak. Thus, there is a need for more information on the current status of phosphine resistance phenotypes in *S. oryzae*. There are two levels of resistance to phosphine, weak (White and Lambkin, 1990) and strong resistance (Collins, 1998) based on the ability to survive in the discriminating concentrations. This information on weak and strong resistance to phosphine in stored grain insect pests is crucial for the development of resistance management strategies.

Studies demonstrated that the weak resistance to phosphine was common across the grain growing regions of Australia and ranged from 56 to 99%, whereas the strong resistance reported to be appeared sporadically with the level of 52 folds (Nayak, 2012; Nguyen et al., 2015). Survey on resistance to phosphine in Vietnam demonstrated the prevalence of both weak and strong resistant phenotypes (Duong and Bui, 2004). However, the information on phenotypic characteristics particularly weak and strong resistance to phosphine in Indian population of stored grain insect pests is limited. There is a need for more information on the current status of resistance phenotypes. Considering these, the present study assessed the frequency (i.e. weak and strong resistance) and distribution of resistance to phosphine in *S. oryzae* populations collected from four southern states of India using the discriminative dose assay method (FAO, 1975; Daglish and Collins, 1999).

MATERIALS AND METHODS

A total of 25 samples of *S. oryzae* were collected from bulk grain storages of Food Corporation of India (FCI), Central Warehousing Corporation (CWC), and State Civil Supply Corporation located across four major southern states of India including Tamil Nadu, Kerala, Andhra Pradesh and Telangana using standard sampling procedure (Semple, 1992; Muralidharan and Rajendran, 2001) (Table 1). The collected samples were mass multiplied in whole wheat grain (FAO, 1975). The whole wheat grain was disinfested at - 20^{o}C for 48 hr in deep freezer to kill the existing insect pests and their immature stages and then the field collected adults were released individually in 2.0 kg plastic container along with adding 500 g of whole wheat grains and maintained under laboratory conditions (30± 2^{o}C, 60± 5% RH). Phosphine gas was generated from commercial formulation of aluminium phosphide tablet (56% w/w) in a gas generation chamber. Aluminium phosphide tablet of 0.6 g was kept in muslin cloth and placed into the lower vessel of gas generation chamber and an inverted glass funnel was positioned over the tablet which would trap and channel the gas through the neck and led to the central receptacle of the upper vessel. The lower vessel was filled with approximately 1.0 l of 5.0% solution of sulphuric acid. A metal ring was then positioned on the flanges of the lower vessel and the upper vessel was placed on top such that the central receptacle was directly over the funnel neck. A screw thread adaptor containing a silicon septum was used to seal the inner receptacle. Generation of phosphine gas commences immediately and the phosphine gas was observed as gas bubbles. The volume of desiccators is crucial for the calculation of the accurate volume of phosphine gas, which would be injected into each desiccator. For measuring the volume of desiccator, fully assembled desiccator was filled with water completely. The weight of water (g) is relatively equals the volume of desiccator (ml). The volume of the phosphine gas was calculated as per the FAO (Anonymous, 1975) Method No. 16.

$$d_1(\mu l) = \frac{298x\ x_1(mg/l)x\ v_1(l)x\ 22.414x\ 1000x\ 1000x\ 100}{273x\ 1000x\ 33.9977(GMW\ phosphine)x\ 86}$$

Where, $X_{1\ (mg/l)}$ = Required dose of phosphine in desiccator; $V_{1\ (l)}$ = Volume of the desiccators

The uniform aged adult insects of *S. oryzae* was fumigated with two discriminating concentration of phosphine gas *viz*., the low concentration (0.04 mg/ l) (White and Lambkin, 1990) and high concentration (0.25 mg/ l) (Daglish and Collins, 1999); over 20 h exposure period for detecting the frequency of weak and strong resistance to phosphine. The resistance bioassay was performed at 30± 2^{o}C and 60± 5 per cent relative humidity. Each bioassay test was replicated thrice along with control for each population and fifty adult insects were released per replication. After exposure, the insects were provided with small quantity of culture medium for a week and moved to recovery room. Adult mortality was determined after seven days from the end of the exposure period as fixed by modified FAO Method (FAO, 1975). The observation on numbers responding i.e. dead insects was taken and insects showing movement were considered to be alive. Mortality response data were corrected using Abbot's formula (Abbot, 1925), to eliminate the influence of mortality, which was not >10% in these experiments.

$$\text{Corrected \% mortality} = \frac{P_t - P_c}{(100-P_c)} x\ 100$$

Where, P_t = Observed mortality in treatment and P_c = Observed mortality in control. The resistance % was worked out by the formula R = (100-CM)± SE; where CM= corrected mortality; SE = standard deviation. Pooled binomial standard error was calculated by using

Table 1. Geographical details and frequency of phosphine resistance in *S. oryzae* collected from southern states of India

S. No	Locations	District	State	Source	GPS coordinates		% resistance (Mean± SE)	
					Latitude	Longitude	0.04 mg/ l	0.25 mg/ l
1.	Kollam	Kollam	Kerala	FCI	8.8800 N	76.6000 E	43.33± 3.85	24.44± 2.94
2.	Thiruvarur	Thiruvarur	Tamil Nadu	SCSC	10.7730 N	79.6370 E	45.56± 2.22	13.33± 1.92
3.	Kavali Nellore	Nellore	Andhra Pradesh	FCI	14.9200 N	79.9800 E	46.67± 1.92	22.22± 1.11
4.	Virudhunagar	Virudhunagar	Tamil Nadu	CWC	9.5833 N	77.9500 E	55.56± 2.22	38.89± 2.94
5.	Nagercoil	Kanyakumari	Tamil Nadu	CWC	8.1700 N	77.4300 E	57.47± 2.22	36.78± 2.94
6.	Kottayam	Kottayam	Kerala	FCI	9.5800 N	76.5200 E	57.78± 2.94	30.00± 3.33
7.	Alleppey	Alleppey	Kerala	FCI	9.4900 N	76.3300 E	58.89± 2.22	35.56± 2.22
8.	Kannur	Kannur	Kerala	FCI	11.8667 N	75.3653 E	61.36± 1.92	39.77± 1.11
9.	Cochin	Ernakulam	Kerala	FCI	8.5656 N	76.8747 E	65.56± 1.11	52.22± 2.22
10.	Kazipet	Warangal	Telangana	FCI	17.9667 N	79.5000 E	68.89± 2.22	35.56± 2.94
11.	Avadi	Tiruvallur	Tamil Nadu	FCI	13.1200 N	80.1000 E	69.77± 3.85	50.00± 1.11
12.	Jammikunta	Karimnagar	Telangana	FCI	18.2864 N	79.4761 E	72.22± 2.22	48.89± 2.94
13.	Erode	Erode	Tamil Nadu	CWC	10.8050 N	78.6856 E	72.73± 2.22	46.59± 2.94
14.	Peelamedu	Coimbatore	Tamil Nadu	FCI	11.1000 N	77.0100 E	73.86± 2.94	53.41± 2.22
15.	Tuticorin	Tuticorin	Tamil Nadu	FCI	8.8100 N	78.1400 E	73.33± 3.33	48.89± 2.94
16.	Vellore	Vellore	Tamil Nadu	FCI	12.9202 N	79.1333 E	74.44± 2.22	61.11± 1.11
17.	Jangalapalli	Guntur	Andhra Pradesh	FCI	13.2133 E	79.0948 E	74.71± 2.94	56.32± 2.94
18.	Chalakkudy	Thrissur	Kerala	FCI	10.3000 N	76.3300 E	76.67± 1.92	51.72± 1.92
19.	Palakkad	Palakkad	Kerala	FCI	10.7700 N	76.6500 E	77.78± 2.94	58.89± 2.22
20.	Thanjavur	Thanjavur	Tamil Nadu	FCI	10.7825 N	79.1313 E	78.89± 2.22	60.00± 1.92
21.	Tirunelveli	Tirunelveli	Tamil Nadu	FCI	8.7300 N	77.7000 E	79.31± 1.92	67.82± 1.11
22.	Sanathnagar	Hyderabad	Telangana	FCI	17.4589 N	78.4433 E	80.00± 1.92	65.56± 1.11
23.	Chennai	Chennai	Tamil Nadu	FCI	13.0839 N	80.2700 E	81.11± 1.11	61.36± 3.85
24.	Trichy	Trichy	Tamil Nadu	CWC	10.8050 N	78.6856 E	83.91± 2.94	68.97± 1.92
25.	Pudukkottai	Pudukkottai	Tamil Nadu	TNCSC	10.3800 N	78.8200 E	93.26± 2.94	80.90± 3.33

FCI-Food Corporation of India; CWC- Central Warehousing Corporation; SCSC- State Civil Supply Corporation ; TNCSC- Tamil Nadu Civil Supply Corporation. Low resistance (0-10%), medium resistance (11-50%) and maximum resistance (51-100%).

the formula SE = Stdev $/\sqrt{n}$. Further, the frequency of resistance was categorized as low (0-10%), medium (11-50%) and maximum (50-100%).

RESULTS AND DISCUSSION

Knowledge on frequency, strength and distribution of insecticide or fumigant resistance in stored grain insect pests is vital for the development of effective pest and resistance management strategies (Rajendran, 1999; Holloway et al., 2016). This study assesses the strength of weak and strong resistance to phosphine and their frequency in *S. oryzae* populations collected from four southern states based on the response of adult insects to the discriminating concentrations of 0.04 and 0.25 mg/ l over 20 hr exposure period and the results are summarized in Table 1. The discriminating bioassay with the lower concentration of 0.04 mg/ l demonstrated that all the 25 populations were resistant to phosphine and the frequency of resistance varied from 43.33 to 93.26% across the populations. For the individual states, the frequency of phosphine resistance varied from 45.56- 93.26% for Tamil Nadu, 43.33- 77.78% for Kerala, 68.89- 80.00% for Telangana and 46.67 to 74.71% for Andhra Pradesh.

None of the populations were grouped into low resistance group; populations collected from Kavali Nellore (46.67%), Thiruvarur (45.56%), and Kollam (43.33%) were categorized into medium resistance group, whereas the remaining 22 populations from Peelamedu, Chennai, Nagercoil, Virudhunagar, Thanjavur, Tuticorin, Vellore, Tirunelveli, Avadi, Trichy, Pudukkottai, Erode, Alleppey, Chalakkudy, Cochin, Kannur, Kottayam, Palakkad, Jammikunta, Kazipet, Sanathnagar, and Jangalapalli recorded higher % of phosphine resistance ranging from 55.56 to 93.26% and grouped in to maximum resistance group. This clearly demonstrated that the resistance to phosphine was common and prevalent in *S. oryzae* populations collected from Tamil Nadu, Kerala, Andhra Pradesh and Telangana and these findings are in agreement with the earlier report of Sonai Rajan (2015) and Rajendran (1999). The frequency of phosphine resistance recorded in the present study was comparatively higher than the previous reports in *S. oryzae* from India (Sonai Rajan et al., 2017; Rajendran, 1999; Sivakumar, 2009), Australia (Holloway et al., 2016; Emery, 1994), Turkey (Tings et al., 2018), and Brazil (Athi and Mills, 2005) and other stored grain insect pests (Ramya et al., 2018; Muralidharan et al., 2018; Rajesh et al., 2018; Subramanian et al., 2016).

Resistance screening of all the field collected populations of *S. oryzae* with the higher concentration of 0.25 mg/ l over 20 hr exposure period identified that all the 25 populations containing strongly resistant weevils with the survivors of up to 80.90%. Further, grouping of these strong resistant populations demonstrated that none gets grouped in to low strong resistance group, whereas 12 populations from Thiruvarur (13.33%) Nagercoil (36.78%), Virudhunagar (38.89%), Tuticorin (48.89%), Erode (46.59%), Alleppey (35.56%), Kannur (39.77%), Kollam (24.44%), Kottayam (30.00%), Jammikunta (48.89%), Kazipet (35.56%) and Kavali Nellore (22.22%) were grouped into moderate strong resistance group. The remaining 13 populations exhibited high frequency of strong resistance ranged from 50.00 to 80.90% and these populations were categorized under maximum resistance group. Overall, the results revealed that the populations of *S. oryzae* collected from Tamil Nadu, Kerala, Andhra Pradesh and Telangana survived at both the discriminating concentrations, indicating the prevalence of strong resistant populations. Similarly, the prevalence of strong resistance to phosphine in *S. oryzae* was reported in Australia, China, Vietnam and other parts of the world (Nayak et al., 2021; Nguyen et al., 2015; Duong and Bui, 2004), though the frequency of resistance reported varied from the present study.

This varied frequency of resistance to phosphine and prevalence of strong resistant populations across the sampled regions might be the result of continuous selection pressure with higher concentrations of phosphine gas over a long period of time (Benhalima et al., 2004; Collins et al., 2005) and inadequate or inappropriate fumigation practices (i.e. storage facilities not adequately sealed before fumigation and fumigant concentrations not being monitored) in bulk grain storage structures (Rajendran, 1999; Lorini et al., 2007; Pimentel et al., 2007). In addition, high insect populations favoured by climate and little or no insect control in storage godown are also the possible reasons for the development of strong resistant populations (Benhalima et al., 2004; Collins et al., 2005; Lorini et al., 2007). The results of the present study clearly demonstrated the prevalence of medium (22 populations) to maximum (13 populations) frequency of phosphine resistance in *S. oryzae* collected from four major southern states of India and this information would be helpful for the development of region specific phosphine resistance management strategies with optimum dose and exposure period in the future.

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