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Toxicity and resistance levels of phosphine against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.) populations

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ABSTRACT

The most destructive stored grain pests in India are the rust red flour beetle and the rice weevil. The most common method for controlling these two pests in storage is phosphine fumigation. However, frequent and acute or chronic doses of exposure than the recommended doses lead to the development of genetic and morphological resistance by changing their traits towards phosphine.Our recent study aimed to detect phosphine resistance degreesin T. castaneum and S. oryzae populations from twelve locations in Odisha. According to bioassay analysis, for S. oryzae after 24 hrs exposure period forphosphine gas to all the assessed populations, the LC_{50} value varied from 0.004 mg/l to 0.038 mg/l and the population of one location i.e., Chhata (Kendrapara) was found to have strong resistance to phosphine with 9.50 fold more resistant in comparison with the laboratory population. The phosphine toxicity levels for T. castaneum ranged from the LC_{50} values of 0.130 mg/l (Kendupali, Barghar) to 0.011 mg/l (Durgapali, Sambalpur), i.e., they were 13.00 and 1.10 times more resistant than the laboratory-susceptible population. It is possible that increasing resistance levels is due to storage structures are not tightly sealed during fumigation leading to sub-lethal phosphine concentration and lack of knowledge about fumigation procedures.

Key words: *Rice weevil, rust red flour beetle, phosphine, fumigation, resistance ratio,Odisha*

INTRODUCTION

Cereal grains are the staple food in developing countries such as India, where 60-70% of the population is dependent on agriculture, but the most challenging problem is storing these cereal grains. Unfortunately, for decades, post-harvest losses have accounted for approximately 10% of total food grains due to unscientific storage, insects, rodents, micro-organisms, etc. (Dhuri, 2006). Stored grain insect pests can cause reductions in weight, quality, commercial value, and seed viability by their infestation. Seventy-five percent of the insects are coleopterans, with the most damaging storage insect species belonging to the genera *Sitophilus* and *Tribolium* (Marsans, 1987; Khan and Selman, 1988; Pinto et al., 1997).

The rice weevil and the rust flour beetle are two devastating and cosmopolitan insect pests that cause qualitative and quantitative losses in stored products and food grains in tropical and subtropical regions of the domain (Bell, 2000; Hagstrum et al., 2013). These are considered serious stored grain pests in India and are extensively distributed all over the country. The rice weevil can attack unbroken rice grains directly and consume the internal portion of the grain, which acts as a primary host for this pest (Batta, 2004). The activity of this pest may cause grain weight losses, affect seed viability and vigour, losses of nutritional content, and their presence makes grains susceptible to contamination by microbes (Da Silva Costa et al., 2016; Zakladnoy, 2018). The grain is converted into dust particles as a result of heavy pest infestation

(Hardman, 1977), creating a suitable environment for secondary feeders and accounting for damage of about 6.5% of total food grain losses in India (Raju, 1984). Neither the adults nor the larvae of the rust flour beetle can damage unbroken grains, but they feed on grain products that have already been damaged by primary feeders. This is the most serious pest of cereal products such as wheat and rice flour, maida, and suji and is more common in flour mills, causing losses of up to 40% (Ajayi and Rahman, 2006). Wheat or rice flour turns greyish-yellow or develops red taints as a result of heavy infestation, which later becomes mouldy and emits an offensive pungent smell.

Various control measures are used before the storing of grain commodities, including sun drying, winnowing, removal of grain debris, cleaning, and the application of pesticides (Arthur, 2019). Chemical insecticides are effective and give immediate results in controlling insect pests. These pesticides are not environmentally safe due to their long-term residue action, and grain products are not suitable for human consumption (Phillips et al., 2012; Wijayaratne et al., 2018). The repeated or excessive use of these chemical insecticides may result in the development of insecticide resistance in stored pests (Opit et al., 2012). Because of the frequent application of contact chemical insecticides, the stored grain pests have developed resistance to various classes of insecticides, such as organophosphates and pyrethroids (Daglish and Nayak, 2018; Attia et al., 2020). *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) have already been identified as resistant to several insecticides like fenitrothion, malathion, and pirimiphos-methyl (Guedes et al., 1994; Guedes et al., 1995).

For decades, Asian countries such as India, Indonesia, China, Vietnam, and the Philippines have been using phosphine as a fumigant as a primary grain protectant. It is a desirable fumigant gas that can be easily applied to 80-85% of stored commodities (grains, pulses, tobacco, and dried fruits) (Agrafioti et al., 2019) and storage structures (gunny bags, warehouses, silos, Jute bags, and polypropylene bags) against various stored product insect pests because it is cheap, low-cost, easy to use, and has a relatively lower residual effect than contact insecticides (Kaur and Nayak, 2015; Nayak et al., 2015; Holloway et al., 2016). Nonetheless, prudent and repeated use of phosphine gas has resulted in the development of genetic resistance in a number of stored grain pests (Holloway et al., 2016; Afful et al., 2018).

Champ and Dyte (1976) performed the first worldwide survey corresponding to phosphine fumigation and found that various major stored grain pests had shown phosphine resistance in various countries in the 1970s (Afful et al., 2018). Earlier findings have identified the presence and absence of phosphine resistance in pest populations and also provided information about the presence of two observable resistance traits in insect individuals (*i.e.*, strong and weak) (Nguyen et al., 2015; Afful et al., 2018). When compared to the concentrations reported to be required to keep sensitive insects like *S. oryzae* and *T. Castaneum* under control, pest phenotypes have 10-50 folds and ≥100 folds more concentrations of weak and strong resistance to phosphine, respectively (Afful et al., 2020; Afful et al., 2021). Rajendran, 1999 and Zeng,1998, found a high level of resistance in India to the insect, *S. oryzae*. Current studies proved that a high rate of phosphine resistance was noticed in the most destructive stored grain pests (*T. castaneum* and *S. oryzae*) in various regions throughout the world (Schlipalius et al., 2014; Kaur et al., 2015; Gautam et al., 2020). A lot of research has recently been conducted for the identification and characterization of resistance to phosphine in the stored pest, *T. castaneum*, and this pest was found to be resistant (Daglish, 2004; Opit et al., 2012; Konemann et al., 2017).

However, reports on phenotypic traits of stored grain pests, mainly weak and strong resistance to phosphine, are limited in Indian populations. It is necessary to get more information on the present status of resistance in observable traits of insects. Taking into consideration that management of these two pests is necessary to ensure food security by maintaining the nutritionally rich quality of food grains.

MATERIALS AND METHODS

The current research was carried out in the Grain Entomology Laboratory of the crop protection division, ICAR-National Rice Research Institute (20°45' N latitude, 85°93' E longitude and 36 m altitude), Cuttack, Odisha, India.

Table 1. Sampling locations for *T. castaneum* and *S. oryzae* populations from Odisha.

Sl. no.	District	Block	Location	Latitude	Longitude
1	Balasore	Remuna	Bampada (CWC)	21.5266° N	86.8697° E
2	Kendrapara	Derabish	Chhata (OSCSCL)	20.5396° N	86.3351° E
3	Cuttack	Cuttack Sadar	Nayabazar (CWC)	20.4551° N	85.9179° E
4	Jagatsinghpur	Erasama	RRC, Erasama (OSCSCL)	20.2019° N	86.4010° E
5	Khordha	Jatni	Jatni (OSCSC)	20.1624° N	85.7011° E
6	Jagatsinghpur	Balikuda	RRC, Ambasala (OSCSCL)	20.1412° N	86.2730° E
7	Puri	Nimapara	Nimapara (OSWC)	20.0537° N	86.0071° E
8	Dhenkanal	Kamakhyanagar	Godaribil (OSCSCL)	20.9229° N	85.5561° E
9	Bargarh	Bargarh	Kendupali (OSWC)	21.2550° N	83.5070° E
10	Nayagarh	Odagaon	Akhupadara (OSCSCL)	20.0159° N	84.9870° E
11	Sambalpur	Dhankauda	Durgapali (OSWC)	21.4681° N	84.0056° E
12	Balangir	Bolangir	Madhiapali (CWC)	20.5411° N	83.5310° E

CWC: Central Warehouse Corporation; OSWC: Odisha State Warehouse Corporation; OSCSCL: Odisha State Civil Supplies Cooperation Limited.

Collection of test insects and their maintenance in the grain entomology laboratory

Twelve insect strains of *T.castaneum* and *S. oryzae* were collected from various Central Warehouse Corporations (CWC), Odisha State Warehouse Corporations (OSWC) and Odisha state civil supplies cooperation limited (OSCSC) of Odisha for a period of 2019-2020 (Table 1). These test populations were collected from rice grains and damaged products stored in various storage structures such as unsealed, bulk, and bag storage. The collected samples were kept in a zip lock bag, labeled and brought to the laboratory. The adult insects were separated from the samples by sieving (Wakil et al., 2014).The collected insects were reared on rice grains and wheat flour for the rice weevil and the red rust flour beetle, respectively (FAO, 1975). These rearing food materials were sterilized for atleast 30-60 minutes at 121ºC temperature under 15 psi to kill immature and adult stages of existing insect pests. Individuals from each strain were reared separately in a plastic jar (15 x 10 cm) containing 500g of rice grains or wheat flour and kept in a bod Incubator at 25-35ºC and 70-80% relative humidity (RH) to ensure proper growth of the insect populations. Adults from each strain were removed every 5-6 days and released in a new plastic jar (15 x 10 cm). In this manner, successive jars were maintained separately for each strain to get a continuous supply of the same aged insect populations. The insect individuals in the jars were kept in darkness and allowed to grow without any disturbance until the newly adult insects emerged. Adults were separated and cultured in a similar manner for at least 6-7 generations in order to obtain a sufficient number of individuals of each strain for bioassay study (Opit et al., 2012). The laboratory strains of these two insect pests were found in the grain entomology laboratory of the crop protection division, ICAR-NRRI, Cuttack, Odisha, India. The insect population, which has been reared for more than ten years without being exposed to any chemical pesticides or phosphine, will be used as a laboratory strain.

Phosphine gas generation

Using the procedure of the Food and Agricultural Organization of the United Nations (FAO) for generating phosphine gas, FAO (Anonymous, 1975) Method No. 16. For phosphine gas generation, a beaker of 5L capacity, an inverted funnel, a collection tube (cylinder), muslin cloth, and an aluminium phosphide tablet (QUICKPHOS) were used. Phosphine gas was produced in a gas generation chamber using commercially available aluminium phosphide tablets. The aluminium phosphide tablet (QUICKPHOS) weighing 1g was tightly warped in muslin cloth and placed in the lower container of the gas generation chamber. A glass funnel was placed invertedly over the tablet, trapping and channeling the gas across the neck and passing into the center vessel of the upper container. The lower container was poured with about 2 liters of water containing a 5% solution of sulphuric (H_2SO_4) acid. A metal ring was placed on the collar of the lower container, and the upper container was placed on top so that the center of the vessel was exactly over the funnel neck. The inner vessel was sealed with an airtight silicon rubber septum. The collection tube was devoid of air, and the pure phosphine gas was noticed as gas bubbles. It is critical to calculate the exact volume of phosphine gas to be injected into the desiccators in order to measure the volume of the desiccators. The desiccators were completely poured with water, and the weight of the water was comparatively equal to the volume of the desiccators, allowing us to obtain an accurate volume of the desiccators. The volume of phosphine gas required for each desiccator was calculated using the formula given by the FAO (Anonymous, 1975) Method No. 16.

$$d_1\,(\mu l) = \frac{298 \times X_1\,(mg/l) \times V_1\,(l) \times 22.41 \times 1000 \times 1000 \times 100}{273 \times 1000 \times 33.9977\,(\text{GMW phosphine}) \times 1000}$$

Where, X_1 (mg/l) = Required dose of phosphine in desiccator

V_1 = Volume of the desiccators

Bioassays technique

For detecting phosphine resistance levels in collected strains of *T. castaneum* and *S. oryzae* assayed by using the FAO method, No. 16. To detect the rate of resistance to phosphine, the same aged adults (one to two weeks old) of the rust red flour beetle and the rice weevil were fumigated with seven discriminating doses of phosphine over a 24 hours exposure period (Table 2). This bioassay was conducted at a room temperature of 30±2 ºC with a relative humidityof 60±5%. Each bioassay experiment was replicated three times for each strain of two populations, along with their control treatment, and ten adults of each strain were kept in each small tube with their food material (rice grains or wheat flour) of 2 g each and placed inside each gas-tight desiccator. Discriminating doses of phosphine gas were injected into gas-tight desiccators with a micro-syringe through a rubber septum fitted to a socket in the lid of the desiccator, which contained test insects in a small plastic box having holes for respiration. After 24 hrs of exposure, all boxes were removed from desiccators and examined under a stereozoom microscope (Nikon SMZ745T) to record insect mortality (insects with movement in their legs and antennae were considered alive, otherwise dead). The test insects were fed for a week and kept in a room at a temperature of 25 ºC with 70% relative humidity for recovery. Adult insects from each box were observed, counted, and classified as dead or alive after 7 days post-exposure period (Jagadeesan et al., 2012). The lower dose of phosphine of each insect population was exposed to the laboratory population because they are greatly susceptible to chemical fumigants, *i.e*., phosphine.

Table 2. Discriminating doses of phosphine exposed against *S. oryzae* and *T. castaneum* populations of Odisha.

Sl. no.	*S. oryzae*	*T. castaneum*
1	0.001 mg/l	0.25 mg/l
2	0.005 mg/l	0.50 mg/l
3	0.010 mg/l	0.75 mg/l
4	0.025 mg/l	1.00 mg/l
5	0.050 mg/l	1.25 mg/l
6	0.075 mg/l	1.50 mg/l
7	0.100 mg/l	1.75 mg/l
8	Control	Control

Statistical analysis

The mortality data of tested populations was analyzed using EPA PROBIT analysis program, software version 1.5. lethal concentrations of LC_{50} and LC_{99} and fiducial limits were found for each tested population. Resistance ratios (RRs) were determined by dividing the LC_{50} of the tested population by the LC_{50} of the susceptible population.

RESULTS AND DISCUSSION

Relative toxicity and resistance ratio of *S. oryzae* populations against phosphine

About 12 Odisha populations of *S.oryzae* were assessed for their toxicity and resistance ratio against phosphine. We observed that except for Jagatsinghpur (RRC, Erasama), all locations of tested populations were shown to be resistant and exhibited various levels of resistance (either strong or weak) to phosphine gas as a fumigant for a 24 hrs exposure period. The susceptible/laboratory population was found to have an LC_{50} value of 0.004 mg/l, whose LC_{50} value was similar to that of the tested population of region RRC, Erasama (Jagatsinghpur). Among all the tested populations of various regions of Odisha, only the population of one location *i.e*., Chhata (Kendrapara) was found to have strong resistance to phosphine with an LC_{50} value of 0.038 mg/l, *i.e*., 9.50 fold more resistant in comparison with the laboratory population. However, populations in all other regions were shown to be weakly resistant to phosphine with a LC_{50} of (0.018 mg/l), *i.e.*, 4.50 times in Bampada (Balasore) to an LC_{50} of (0.005 mg/l), *i.e.*, 1.25 times in Akhupadara (Nayagarh) populations (Table 3).

Similar kind of results were obtained by Sonai Rajan et al. (2017), who studied the dispersal of *S. oryzae* populations in various geographical regions of Tamil Nadu to regulate the degrees of resistance to phosphine and it was noticed that levels of resistance varied from 21.21 to 93.38%. Kumar et al. (2021) collected twenty-five *S. oryzae* populations from four southern districts of India and noticed that twelve populations (13.33 to 48.89%) revealed moderate resistance, whereas the other thirteen populations (50.00 - 80.90%) were observed to have strong resistance. The previous and present findings may also give an idea for developing location-specific resistance management approaches in the near future by fumigating phosphine at recommended doses and waiting periods to reduce the resistant ability of insect pests.

Relative toxicity and resistance ratio of *T. castaneum* populations against phosphine

The current research confirmed that, after 24 hrs exposure period of phosphine fumigation, except for Balangir (Madhiapali), all assessed populations of *T. castaneum* from different regions of Odisha were found resistant to phosphine gas in comparison with the laboratory population. Moreover, resistance to phosphine varied across populations tested. According to Probit analysis, the LC_{50} value of the location Madhiapali (Balangir) was 0.010 mg/l, which was similar to the susceptible/laboratory population.The phosphine resistance levels differed from the LC_{50} values of 0.130 mg/l (Kendupali, Barghar) to 0.011 mg/l (Durgapali, Sambalpur), *i.e.*, they were 13.00 and 1.10 times more resistant than the laboratory-susceptible population, which had the highest and lowest resistance levels among tested populations, respectively. Strong resistance degrees were observed in regions of Odisha

Table 3. Relative toxicity and resistance ratio of phosphine against *S. oryzae*.

Sl. no.	District	LC_{50} (mg/l) 95% FL	LC_{95} (mg/l) 95% FL	LC_{99} (mg/l) 95% FL	Slope ± SE	Chi-square	RRs
1	Balasore	0.018 (0.015-0.023)	0.077 (0.057-0.117)	0.138 (0.094-0.245)	2.663 ± 0.305	9.440	4.50
2	Kendrapara	0.038 (0.030-0.045)	0.071 (0.059-0.095)	0.091 (0.073-0.141)	6.183 ± 1.231	9.141	9.50
3	Cuttack	0.007 (0.005-0.009)	0.041 (0.029-0.069)	0.085 (0.054-0.175)	2.151 ± 0.260	2.357	1.75
4	Jagatsinghpur (E) (sus)	0.004 (0.002-0.005)	0.030 (0.020-0.055)	0.072 (0.041-0.171)	1.790 ± 0.229	9.235	1.00
5	Khordha	0.006 (0.004-0.007)	0.036 (0.025-0.061)	0.077 (0.047-0.162)	2.054 ± 0.251	6.984	1.50
6	Jagatsinghpur(B)	0.008 (0.005-0.011)	0.050 (0.034-0.091)	0.108 (0.064-0.260)	2.032 ± 0.306	6.270	2.00
7	Puri	0.010 (0.007-0.013)	0.076 (0.051-0.135)	0.180 (0.106-0.400)	1.828 ± 0.212	8.036	2.50
8	Dhenkanal	0.013 (0.010-0.016)	0.051 (0.038-0.078)	0.089 (0.061-0.160)	2.783 ± 0.335	3.931	3.25
9	Bargarh	0.012 (0.009-0.015)	0.063 (0.045-0.103)	0.127 (0.082-0.248)	2.260-0.264	4.323	3.00
10	Nayagarh	0.005 (0.003-0.006)	0.015 (0.011-0.036)	0.024 (0.015 -0.096)	3.310 ± 0.894	5.965	1.25
11	Sambalpur	0.012 (0.009-0.015)	0.067 (0.047-0.111)	0.139 (0.088-0.280)	2.148 ± 0.250	2.938	3.00
12	Balangir	0.010 (0.007-0.013)	0.072 (0.049-0.124)	0.162 (0.098-0.347)	1.921 ± 0.223	10.998	2.50
13	Laboratory	0.004					

LC: lethal concentration; FL: fiducial limits; RRs : resistance ratios; RRs = LC_{50} - resistant population/LC_{50} - laboratory-susceptible population; Total no. of insects = 30.

populations such as Kendupali (Barghar) of LC_{50} (0.130 mg/l) *i.e.*, 13.00 times subsequently Nayabazar (Cuttack) of LC_{50} (0.110 mg/l) *i.e.*, 11.00 times, RRC, Ambasala (Jagatsinghpur) of LC_{50} (0.099mg/l) *i.e.*, 9.90 times, Godaribil (Dhenkanal) of LC_{50} (0.088mg/l) *i.e.*, 8.80 times, RRC, Erasama (Jagatsinghpur) of LC_{50} (0.088 mg/l) *i.e.*, 8.80 times, Nimapara (Puri) of LC_{50} (0.076 mg/l) *i.e.*, 7.60 times and Chhata (Kendrapara) of LC_{50} (0.070 mg/l) *i.e.*, 7.00 times more resistant than the laboratory-susceptible population. When compared to the laboratory population, all other assessed populations exhibited weak resistance to phosphine (Table 4).

Our results corroborate the results of (Ali et al., 2013) where in they obtained *T. castaneum* populations from backward regions of Pakistan and shown to be resistant to phosphine by following a bioassay study. Similarly, Opit et al. (2012) tested 11 populations of *T. Castaneum* among them four were found strongly resistant to phosphine in California and Australia, with a resistance rate varying from 42 to 100%. In another study of the 25 populations in the USA and Canada, twelve populations were confirmed to be resistant to phosphine (Cato et al., 2017).

Our present study indicated varying degrees, intensity and frequencies of phosphine resistance. Of the twelve assessed populations, seven and only one strong resistant population were found in *T. castaneum* and *S. oryzae*, respectively from different locations in Odisha. According to the current findings, *Tribolium* populations were more resistant than *Sitophilus* populations (Ahmad and Ali, 2013). This could be due to the appearance of more phosphine resistant genes and high selection pressure triggered by phosphine fumigation being used frequently over time (Schlipalius et al., 2014; Nguyen et al., 2015; Afful et al., 2018; Nayak et al., 2021). The storage structures (gunny bags and bulk storage) in godowns and warehouses were not properly sealed prior to fumigation to maintain fumigant intensity, which increased the susceptibility

Table 4. Relative toxicity and resistance ratio of phosphine against *T. castaneum*.

Sl. no.	District	LC_{50} (mg/l) 95% FL	LC_{95} (mg/l) 95% FL	LC_{99} (mg/l) 95% FL	Slope ± SE	Chi-square	RRs
1	Balasore	0.063 (0.040-0.088)	0.446 (0.312-0.736)	1.002 (0.626-2.056)	1.939 ± 0.245	1.777	6.30
2	Kendrapara	0.070 (0.051-0.092)	0.446 (0.318-0.708)	0.958 (0.618-1.809)	2.052 ± 0.223	14.571	7.00
3	Cuttack	0.110 (0.067-0.161)	2.239 (1.389-4.471)	7.795 (3.992-21.665)	1.258 ± 0.147	10.359	11.00
4	Jagatsinghpur (E)	0.088 (0.063-0.116)	0.684 (0.488-1.077)	1.602 (1.026-3.005)	1.844 ± 0.186	8.300	8.80
5	Khordha	0.022 (0.017-0.027)	0.113 (0.080-0.185)	0.222 (0.142-0.438)	2.317 ± 0.271	4.236	2.20
6	Jagatsinghpur (B)	0.099 (0.073-0.129)	0.668 (0.484-1.030)	1.473 (0.966-2.672)	1.986 ± 0.201	5.363	9.90
7	Puri	0.076 (0.049-0.107)	1.317 (0.854-2.397)	4.304 (2.370-10.252)	1.325 ± 0.140	13.487	7.60
8	Dhenkanal	0.088 (0.055-0.127)	2.145 (1.310-4.347)	8.053 (4.032-22.653)	1.186 ± 0.130	9.939	8.80
9	Bargarh	0.130 (0.103-0.160)	0.496 (0.376-0.732)	0.864 (0.606-1.460)	2.826 ± 0.318	1.726	13.00
10	Nayagarh	0.012 (0.009-0.015)	0.064 (0.045-0.104)	0.128 (0.082-0.251)	2.245 ± 0.262	8.518	1.20
11	Sambalpur	0.011 (0.008-0.014)	0.105 (0.068-0.201)	0.270 (0.150-0.667)	1.659 ± 0.195	7.763	1.10
12	Balangir (sus)	0.010 (0.007-0.013)	0.077 (0.052-0.137)	0.182 (0.107-0.407)	1.822 ± 0.211	8.341	1.00
13	Laboratory	0.010					

LC: lethal concentration; FL: fiducial limits; RRs: resistance ratios; RRs = LC_{50}-resistant population/LC_{50}-laboratory- susceptible population; Total no. of insects = 30.

of stored insects (Lorini et al., 2007; Pimentel et al., 2007; Gautam et al., 2020). Because adult stored insect pests can fly due to their well-developed wing mechanism, these stored pests are shifted to various areas via transportation of stored products with a few horizontal and vertical resistant genes as the primary reason for gene flow, continuing to increase the intensities of phosphine resistance (Hernandez Nopsa et al., 2015). Furthermore, the climatic conditions of the storage godowns/warehouses are congenial for the multiplication of stored grain pests, and the exclusion of any control measures may be the probable basis for the occurrence of potent resistant populations (Collins et al., 2005; Lorini et al., 2007). In resistant populations, the phosphine resistance pathway is activated by taking less or an overdose of phosphine gas than susceptible individuals (Opit et al., 2012).

According to current findings, tested populations exhibited either more resistance, less resistance, or more susceptible to phosphine fumigation. This could be due to more resistant populations of insect pests acquiring less phosphine gas than less resistant or susceptible populations, which may be due to more resistant populations having a lower cellular respiration rate than less resistant or susceptible populations (Pimentel et al., 2007; Pimentel et al., 2008). We also noticed that different degrees of resistance to phosphine were found among assessed populations of the same species, which could be attributed to different inherited traits within a species and diverse geographic locations (Schlipalius et al., 2012; Nguyen et al., 2016; Cato et al., 2017). The increasing rate of phosphine fumigation in a particular region in retaining less inheritable traits of species diversification of stored pests than regions with less use of phosphine may be due to the expression of polymorphism in an individual insect species being less (McCulloch et al., 2019; Thangaraj et al., 2019).

Taking into consideration the above concern, an awareness training programme should be conducted in the locality of Odisha where resistance populations were found on how stored pests become resistant to phosphine and how it is harmful to human beings by consuming fumigated stored products. So the time has come to encourage people/farmers to replace synthetic insecticides or chemical fumigants with varying modes of action after a certain period of time in order to delay the emergence of insect resistance (Hernandez Nopsa et al., 2015; Afful et al., 2018). Other management approaches include increasing the waiting period for phosphine; reviewing, analyzing, and increasing the recommended doses of phosphine, especially in comparison to laboratory and field experiments;using advanced phosphine equipment; properly closing the fumigation storage rooms; using an alternative fumigant (sulfuryl floride); detecting pest populations in storage structures (godowns, warehouses, and farmers houses/ homes) by checking, testing,and cleaning and using of grain protectants should be adopted to suppress the resistance activity of phosphine (Ahmedani et al., 2007; Nayak et al., 2010; Emery et al., 2011).

CONFLICTS OF INTEREST

Individual authors declare no conflict of interest with regards to this publication.

CONCLUSION

We observed varying levels of phosphine resistance in the populations studied. It is possible that this is because storage structures are not tightly sealed after fumigation, and a lack of knowledge about fumigation procedures raises the rate of incidence of resistant individuals in a tested population. As a result, either alternative fumigants or alternate management approaches should be used to delay the development of insect resistance. We need to upgrade to advanced storage systems and structures, as well as conduct training programs to manage resistant insect populations in stored grains.

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