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Phosphine resistance: Development, monitoring, strategies and alternatives for management in stored grain pests

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Abstract

The resistance of stored-product pests in general and coleopterans in particular to phosphine fumigation is becoming a global concern which has put the viability and sustainability of phosphine in jeopardy. The problem of phosphine resistance has been aggravated over the past two decades mostly due to the lack of suitable alternatives matching to the major attributes of phosphine, including its low price, ease of application, proven effectiveness against a broad pest spectrum, compatibility with most storage conditions, and international acceptance as a residue- free treatment. In this review, we compile a broad overview of phosphine resistance with special emphasis on the genetic basis of resistance development, countering the resistance development, key management strategies and alternative fumigants that need to be addressed.

Keywords: Dihydrolipoamide dehydrogenase, LC_{50}, phosphine resistance, *rph*1 and *rph*2, stored product insects

Introduction

The food grains are stored over long period of time in bulk storage units in most of the countries to maintain buffer stock, or to distribute to the masses under Public Distribution System etc. Major post-harvest losses are posed by biotic agents including insects, mites, rodents, birds and microbiota;on conservative estimate causing 15-20% loss in Indian context. Stored grain pests are a substantial danger to global food security and economic stability [1]. Beetles, weevils, moths, and mites can infest stored grains and cause significant harm [2]. They devour and contaminate grains, lowering their quality and making them unsuitable for human consumption or processing [3]. In addition to direct damage, stored grain pests can cause secondary difficulties such mould growth and mycotoxin contamination, jeopardising grain quality and safety [4]. Controlling stored grain pests is critical for reducing economic losses and maintaining food security [5]. Effective pest management measures help to preserve grain quality, reduce post-harvest losses, and protect food supplies [6]. Farmers and food producers can protect their stored grain products and preserve food resources for current and future use by implementing proper storage practices, monitoring for pest infestations, and employing appropriate control measures, such as fumigation or insecticides [7, 8]. Phosphine is a popular fumigant for the control of stored grain pests because to its efficiency and ease of administration [9]. It is a colourless, odourless gas made up of phosphorus and hydrogen that serves as an effective pesticide [10]. Phosphine is widely used to manage a variety of stored grain pests, such as beetles, weevils, moths, and mites, at various phases of their life cycle [11]. One of the primary benefits of phosphine is its capacity to penetrate deep into stored grain masses, reaching pests that are buried within the grains [12]. This makes it extremely effective for managing pest infestations, especially in big storage facilities. Furthermore, phosphine has a shorter fumigation period than other fumigants, making it a handy and cost-effective pest management solution [13]. Another essential feature of phosphine is that it does not leave harmful residues, making it suitable for use in food storage facilities. This is especially crucial in ensuring that stored grain products are safe for human consumption following fumigation [14]. Overall, phosphine's effectiveness, convenience of use, and safety profile have made it a popular choice for stored grain pest management around the world [15]. The over-reliance on this fumigant throughout the world has led to the development of resistance to major stored grain pests. Recent monitoring of phosphine resistance in the red flour beetle, *T. castaneum*, and the Khapra beetle, *Trogoderma granarium*, and the lesser grain borer *Rhyzopertha dominica* in India indicated strong resistance levels in North India [16].

The rise of phosphine resistance in stored grain pests presents a substantial challenge to global pest management efforts [17]. Phosphine has been a staple of stored grain pest control for decades due to its efficiency, low cost, and low residual issues [18]. However, the overreliance on phosphine as the major control strategy has put severe selection pressures on pest populations, resulting in the development of resistance [19]. Resistance to phosphine can develop through a variety of processes, including changes in the insect's respiratory enzymes, detoxification routes, and behavioural adaptations. These resistant pests can survive exposure to normally fatal phosphine concentrations, resulting in treatment failures and increasing economic losses for farmers and storage facilities [18]. The emergence of phosphine resistance emphasises the significance of employing integrated pest management (IPM) strategies that include several control approaches [20]. This method reduces selection pressure on pest populations, making resistance more difficult to develop [21]. Furthermore, continued research is required to better understand the mechanisms of resistance and create novel techniques for managing resistant pest populations [16, 22]. This paper seeks to give an in-depth analysis of phosphine resistance in stored grain pests, including genetic, biochemical, and behavioural factors. The plausible implications of consistent enhancement of phosphine resistance could only be minimised if a thorough knowledge on the biochemical and oxidative mechanisms underlying strong phosphine resistance. The advancement in the resistance monitoring techniques would play a greater role to check the further increase in the resistance allele proportion in the populations. The complete knowledge of high-throughput next generation sequencing techniques, molecular markers and genotyping techniques in the process helps the academicians, research scholars and law makers to arrive at a comprehensive solution to this emerging threat in post-harvest storage of grains. It emphasises the importance of alternative pest management tactics in light of resistance. Integrated pest management (IPM) tactics and the development of novel fumigants are important strategies for managing resistant populations and ensuring long-term pest control in stored grain facilities.

Mode of action (MoA) of Phosphine in insect control

Phosphine, a commonly used fumigant for insect management, works by inhibiting cellular respiration, causing target insects to die [23]. The mode of action (MoA) of phosphine is its interaction with mitochondria, the cellular organelles that generate energy in the form of adenosine triphosphate (ATP) by oxidative phosphorylation [24]. Understanding phosphine's mode of action is critical for effective pest management tactics and preventing resistance development [25]. Phosphine (PH_3) enters the insect's body via the respiratory system and diffuses across the membranes before entering the cells [26]. Once entering the cell, phosphine interacts with a variety of cellular components, but its main target is the enzyme cytochrome c oxidase (COX), also known as complex IV of the mitochondrial electron transport chain. COX is in charge of transporting electrons from cytochrome c to molecular oxygen, the final step in the electron transport chain that produces ATP [27]. Phosphine binds to COX's heme group, reducing its function and interrupting the electron transport chain [28]. This inhibition prevents oxygen reduction to water, resulting in electron build-up and the production of reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) [29]. ROS are extremely reactive chemicals that can cause oxidative damage to biological components such as lipids, proteins, and DNA, eventually leading to cell death [30]. Phosphine disrupts mitochondrial activity, causing the membrane potential ($\Delta\Psi m$) to collapse and cytochrome c to be released into the cytosol [31]. Cytochrome c is an important component of the apoptotic pathway, and its release initiates a cascade of events that leads to programmed cell death, or apoptosis [32]. Apoptosis is important in the toxicity of phosphine to insects because it causes controlled and organised cell death, adding to the insect's total mortality [33]. In addition to affecting mitochondrial function, phosphine can disturb other cellular processes such as ATP generation, protein synthesis, and ion homeostasis [34]. The disruption of ATP synthesis depletes cellular energy reserves, which contributes to cell death [35]. Phosphine can also impede protein synthesis by attaching to ribosomes, the cellular organelles that synthesise proteins, disrupting normal cellular activity [36]. Mode of action of phosphine is illustrated in Figure 1.

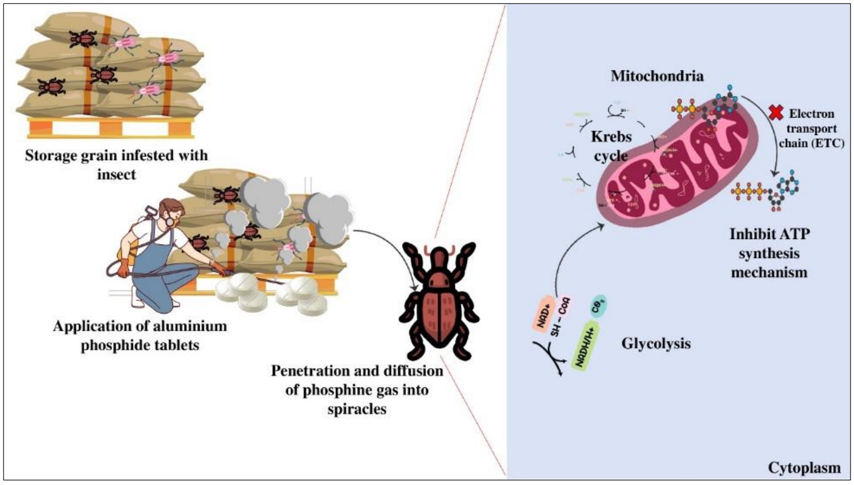

Fig 1: Infographic representing the method of application and mode of action of Phosphine fumigant

Factors contributing to phosphine resistance

Over-reliance on phosphine in the grain storage industry: One of the key causes of phosphine resistance in stored grain pests is an overreliance on phosphine in the grain storage business. Phosphine has long been the preferred fumigant for managing stored grain pests due to its efficacy, convenience of use, and low cost. However, extensive use has raised selection pressure on pest populations, favouring the survival and reproduction of individuals with natural resistance or resistance acquired through genetic changes. The scarcity of acceptable fumigant alternatives has compounded the overreliance on phosphine. With the phase-out of methyl bromide due to its ozone-depleting effects and a scarcity of alternative effective fumigants, phosphine has become the dominant choice for many grain storage facilities. This reliance on a single fumigant has generated conditions that promote the development and spread of resistance among stored grain pests.

Inadequate fumigation practices: Proper fumigation necessitates that storage facilities be well sealed to prevent the escape of phosphine gas, ensuring that a sufficient concentration is maintained to effectively kill pests. However, in many circumstances, storage facilities may contain breaches or gaps that allow phosphine gas to escape, limiting its effectiveness and increasing the possibility of resistance formation. Inadequate fumigation techniques can be caused by a variety of issues, including poor storage facility maintenance, incorrect fumigant application, and inadequate fumigation process monitoring. For example, failing to correctly seal storage bins or silos can result in the loss of phosphine gas, lowering its concentration and allowing pests to thrive. Similarly, improper application tactics, such as under dosing or overdoing, can build to resistance by exposing pests to sub lethal or ineffective amounts of phosphine.

Selection pressure: Repeated and unsuccessful fumigations have a substantial impact on the development of phosphine resistance in stored grain pests. When phosphine, a common fumigant for pest control, is administered repeatedly or ineffectively, it exerts substantial selection pressures on pest populations. This can result in the survival and expansion of people who are naturally resistant or who develop resistance through genetic alterations. Repeated fumigations with phosphine can result in a situation in which only the most resistant individuals survive as susceptible pests are eradicated. Over time, this may result in an increase in the proportion of resistant individuals in the population. Furthermore, unsuccessful fumigations, in which pests are exposed to sub lethal amounts of phosphine, can contribute to the development of resistance. Pests that survive such exposures may develop detoxification mechanisms or lessen their vulnerability to phosphine's effects, resulting in resistance. Sub lethal amounts of phosphine can cause these beetles to develop resistance. In one investigation, beetles were exposed to phosphine concentrations lower than the lethal dosage for multiple generations. The insects gradually developed resistance, with following generations demonstrating higher tolerance to phosphine. Similarly, inadequate fumigation can contribute to the development of resistance in stored grain pests. Inadequate storage facility sealing, for example, can cause phosphine gas to escape, reducing its concentration and effectiveness;pests that survive such exposures may have genetic traits that confer resistance, allowing them to survive and reproduce, resulting in the spread of resistance within the population. In addition to genetic resistance, pests can develop behavioural responses to phosphine exposure. For example, some pests may exhibit avoidance behaviour, migrating away from treated areas to avoid fumigant exposure. This behaviour can lower the efficacy of fumigation treatments while increasing the risk of resistance development in survivors. The newest reports of phosphine resistance to major stored grain pests across the world is compiled in Table 1.

Mechanism of phosphine resistance in stored grain beetles

Phosphine resistance in insects is a major concern in pest management, especially in stored grain facilities where this fumigant is widely utilised [42]. Understanding the mechanisms of phosphine resistance is critical for creating efficient pest management measures [16]. Several variables contribute to insect phosphine resistance, including genetic, metabolic, and behavioural pathways [12]. Resistance to phosphine is frequently related with mutations in the genes that encode the insect's respiratory enzymes. The genetic basis for resistance can be explained by the following phosphine toxicity mechanism proposed by Schlipalius *et al* [43], which is based on the fact that the DLD enzyme (*rph*2) produces reactive oxygen species (ROS) as a by-product of its normal role in aerobic respiration [44]. The FADS enzyme (*rph*1) produces desaturated fatty acids, which ROS targets. Exposure to phosphine increases ROS generation, causing oxidative damage to the fatty acids in cell membranes. Thus, the synergistic interaction between *rph*1 and *rph*2 stems from the normal function of FADS (*rph*1), which sensitises animals to ROS [45], and DLD's (*rph*2) propensity to generate high levels of ROS, which is enhanced by phosphine exposure. When insects are homozygous for *rph*1 resistance alleles, their cellular membranes become less sensitive to ROS. When insects are homozygous for the *rph*2 resistance alleles, they create less ROS. Individuals that are homozygous for both genes' resistance alleles create less ROS and are less sensitive to the ROS that is produced, resulting in extremely high phosphine resistance. The finding of genetic polymorphisms at the *rph*2 locus that modify the action of the dihydrolipoamide dehydrogenase (DLD) gene is attributed to point mutations in amino acid sequences and linked to phosphine resistance.

Insects resist phosphine through a complex interplay of biochemical systems that allow them to detoxify or tolerate the fumigant's effects [46]. These systems can be roughly classified as detoxification pathways, repair mechanisms, and sequestration processes [47]. Understanding these biochemical pathways is critical for creating efficient phosphine-resistant insect management techniques [48]. Detoxification mechanisms contribute significantly to phosphine resistance by lowering fumigant concentrations in insect tissues [49]. One of the primary detoxification processes involves the enzyme glutathione S-transferase (GST), which catalyses the conjugation of glutathione (GSH) to phosphine, resulting in a less toxic and more easily excreted molecule [50]. Increased GST expression or activity can improve the insect's ability to detoxify phosphine, lowering its potency as a fumigant [12]. For example, in the rice weevil (*Sitophilus oryzae*), phosphine resistance has been linked to enhanced GST activity. According to studies, resistant rice weevil strains express more GST than susceptible strains, allowing them to metabolise phosphine more efficiently [51]. Another detoxification process involves the enzyme cytochrome P450 monooxygenases, which can convert phosphine to phosphine oxide [52]. The insect can metabolise and excrete this oxidised version of phosphine, which is less hazardous than the original chemical [53]. Increased cytochrome P450 expression or activity can help insects resist phosphine by lowering the fumigant levels in their tissues. In addition to detoxification routes, repair mechanisms contribute to phosphine resistance by reducing fumigant damage [54]. Furthermore, reactive oxygen species (ROS) play an important part in the molecular pathways underlying insect phosphine tolerance [55]. ROS are very reactive chemicals that include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen (O_2^-) [56]. These compounds are created as by-products of regular cellular metabolism, but their levels can rise dramatically in response to stress, such as phosphine exposure [57]. ROS are known to induce oxidative damage to biological components such as DNA, proteins, and lipids, resulting in cell death [58]. However, insects have evolved ways to combat the effects of ROS, allowing them to survive and even thrive in the presence of phosphine [59]. One of the primary strategies by which insects protect themselves from ROS-induced damage is the action of antioxidant enzymes [60]. These enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), serve to neutralise ROS and prevent oxidative damage [61]. Phosphine exposure has been found in studies to promote the development of antioxidant enzymes in insects, providing them with improved protection against ROS [62]. For example, in the red flour beetle (*Tribolium castaneum*), phosphine treatment has been found to upregulate SOD and CAT expression, resulting in enhanced enzyme activity. This increase in antioxidant enzymes protects beetles from ROS-induced damage and improves their ability to survive phosphine exposure [63]. In addition to antioxidant enzymes, insects use non-enzymatic antioxidants such glutathione (GSH), vitamin C, and vitamin E to protect themselves against ROS. These antioxidants can directly scavenge ROS or replenish other antioxidants, such GSH, which is essential for maintaining cellular redox equilibrium [64].

Insects' phosphine resistance develops and spreads mostly through behavioural factors. These mechanisms entail changes in insect behaviour that allow them to avoid or minimise exposure to phosphine, lowering its efficacy as a fumigant [41]. Understanding these behavioural factors is critical for creating measures to control phosphine-resistant insect populations. One of the key behavioural mechanisms of phosphine resistance is avoidance behaviour, in which insects actively migrate away from phosphine-treated areas or avoid contact with phosphine-treated surfaces [65]. This behaviour can limit the insect's exposure to the fumigant while increasing its chances of survival. Avoidance behaviour can be natural or learnt, and it varies among species and communities [66]. For example, in the rice weevil (*S. oryzae*), phosphine-resistant strains have been found to migrate and disperse more than susceptible strains [67]. This increased movement allows resistant insects to avoid phosphine-treated areas, minimising their exposure to the fumigant and boosting their chances of survival [68]. Temperature, humidity, and the presence of food sources can all have an impact on avoidance behaviour [40]. Insects may avoid phosphine-treated areas because they believe them to be unsuitable for eating or reproduction. Understanding the environmental cues that drive avoidance behaviour can help to build more effective phosphine-resistant insect management tactics. The different mechanisms of PH_3 resistance are represented in Figure 2.

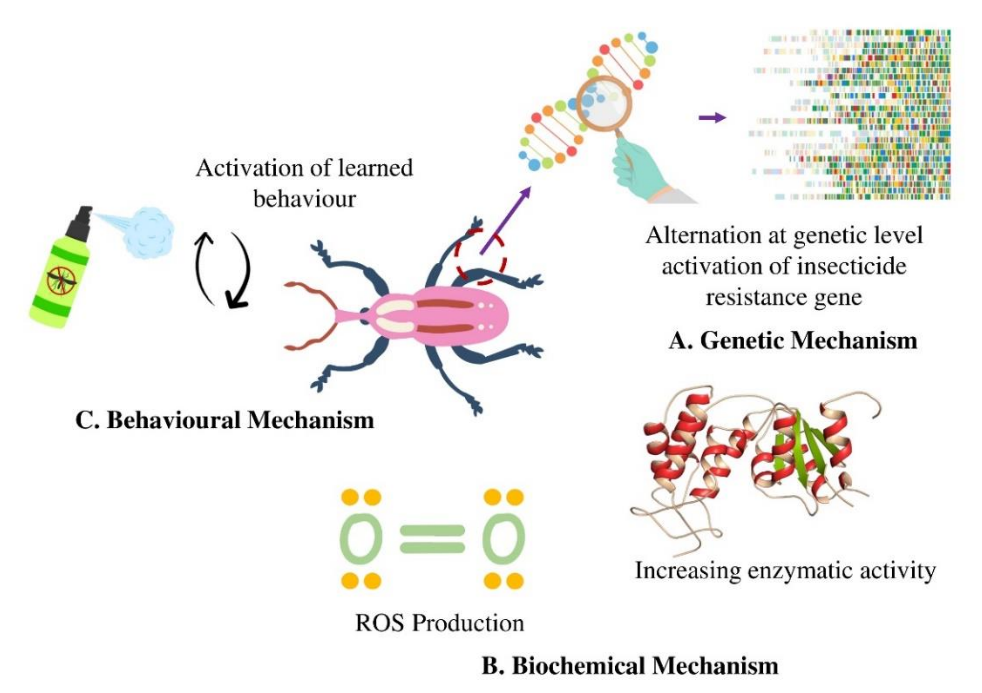

Fig 2: Various mechanism involved in the phosphine resistance in any stored grain pest

Table 1: Recent reports of phosphine resistance to stored grain pests across the world

Sl no	Storage pest	Dose of phosphine	Findings	Reference; Country
1	*Tribolium castaneum*	0.038 to 1.277 mg/lit	Red flour beetle has acquired 2.11 to 70.94-fold resistance to phosphine compared to susceptible check. The magnitude of catalase, peroxidase and superoxide dismutase were found to be more in resistance as compared to susceptible population	[12]; India
2	*Sitophilus granaries*, *S. oryzae* and *S. zeamais*	Two different dose were used (i) As per FAO protocol (30 ppm for 20 h) and (ii) the dose–response protocol (50–1000 ppm for 3 d)	*S. oryzae* G1 showed 100% active individuals after 20 h or even 7 d post-exposure, while low survival was noted for all populations of *S. granarius* and no survival for *S. zeamais*; no active individuals were recorded after exposure to 700 ppm for any of the populations tested, with *S. oryzae* G1 showing 89% survival after 3 d at 50 ppm and 1.1% at 700 ppm, and no survival for all concentrations and populations of *S. granarius* and *S. zeamais*.	[37]; Greece
3	*Liposcelis bostrychophila*	The full assay comprised eight concentrations ranging from 0.003 to 1.000 g/m^3. The most resistant strain was subjected to extended exposure periods of 72 and 144 hours.	Two out of eleven strains were still susceptible to phosphine fumigation, while nine showed varying degrees of resistance: 2 strains with very low resistance, 3 strains with moderate resistance, 1 strain with high resistance, and 3 strains with very high resistance. The most susceptible strain was collected from La Union (lr1lug strain) with LC_{50} and LC_{99} values of 0.004 and 0.024 g/m−3 respectively, while the highest resistance level was recorded in Tarlac (lr3tr strain) with LC_{50} and LC_{99} values of 0.917 and 2.081 g/m^3 respectively.	[13]; Philippines
4	*Rhyzopertha dominica*, *S. granarius*, *T. castaneum*,*Trogoderma granarium*	1, 2, 3, 6, 9, 12 and 15 ppm	LC_{50} values for laboratory-susceptible populations of four stored grain pests in Pakistan: *R. dominica* (2.85 ppm), *S. granarius* (1.90 ppm), *T. castaneum* (2.54 ppm), and *T. granarium* (2.01 ppm). Resistant populations from various locations showed significantly higher LC_{50} values, indicating high resistance levels compared to the laboratory population	[14]; Pakistan
5	*S. oryzae*, *Oryzaephilus surinamensis* and *R. dominica*	3000 ppm	57.1% of tested field populations in the Czech Republic were classified as phosphine-susceptible, with significant variations among species. *R. dominica* had the highest percentage of resistant populations (71.4%), followed by *S. oryzae* (57.1%) and *O. surinamensis* (9.5%). Intra-population variability in response to phosphine was observed, suggesting a need for an action plan to mitigate resistance.	[15]; Czech Republic
6	*T. castaneum* and *S. oryzae*	-	Phosphine gas bioassays on *S. oryzae* showed LC_{50} values ranging from 0.004 mg/l to 0.038 mg/l, with the Chhata (Kendrapara) population exhibiting strong resistance (9.50-fold) compared to the laboratory population. For *T. castaneum*, LC_{50} values ranged from 0.011 mg/l to 0.130 mg/l, indicating 1.10 to 13.00 times more resistance than the laboratory-susceptible population across different locations.	[38]; India
7	*S. oryzae*	0.014 to 0.76 mg/l	Phosphine resistance in *Sitophilus oryzae* populations from Şanlıurfa, Adana, and Kahramanmaraş provinces in Turkey was investigated, showing resistance levels up to 57.5 times higher than the susceptible population. Survival rates at discriminating concentrations ranged from 0-99%, 0-90%, and 0-89%, indicating high resistance levels in these areas, posing challenges to phosphine use for pest management.	[39]; Turkey
8	*R. dominica*	0.01, 0.03, 0.06, 0.1, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L	Field populations of Rhyzopertha dominica showed median lethal concentration values ranging from 0.024 mg/L to 1.991 mg/L, indicating 1.63 to 82.96-fold resistance compared to laboratory susceptible checks. Antioxidant enzyme activities varied significantly, with peroxidase activity ranging from 1.28 to 336.8 nmol H_2O_2 reduced/min/mg protein, superoxide dismutase inhibition rate from 81.29% to 99.66%, and catalase activity from 6.28 to 320.13 nmol H_2O_2 reduced/min/mg protein.	[16]; India
9	*S. oryzae*, *O. surinamensis* and *T. castaneum*	3000 ppm of phosphine	Susceptible insect populations were quickly immobilized even with short phosphine exposure, while resistant populations remained active even after prolonged exposure (up to 300 min). Higher phosphine concentrations (500-3000 ppm) showed a "sweet spot" effect, with decreased mortality at higher concentrations, particularly notable at 1000 and 2000 ppm for 5 h, irrespective of resistance levels, indicating non-linear recovery patterns.	[40]; Greece
10	*S. oryzae*, *O. surinamensis* and *T. castaneum*	30 ppm	Trials conducted in nitrogen chambers with 1.0% O_2 at 28 and 40 °C for 2.5, 3, and 9 days showed complete parental mortality for *O. surinamensis* and *S. oryzae*, and partial survival for *T. castaneum* at 28 °C and 3 days. Progeny production was completely suppressed for all species and populations, indicating the effectiveness of low oxygen regardless of phosphine resistance, suggesting it as a potential alternative for resistance management.	[41]; Greece

Conclusion

The review emphasizes on the growing worldwide problem of the development of phosphine resistance. This resistance challenges the sustainability of the phosphine as the cheapest and most versatile fumigant for the disinfestation of stored food grain products. Major breakthrough has been made with the inheritance and biochemistry of resistance. Two major genes are responsible for the resistance, with resistance expressed as two major phenotypes (i.e., weak and strong). Historically, the FAO diagnostic test and its variations have underpinned resistance surveys, but the development of same day knockdown tests offer the possibility of faster testing, and molecular diagnostics allow for rapid and accurate screening for resistance genes. Quantification of the effects of the phosphine concentration, exposure period, and other variables such as temperature is providing a basis for the development of effective fumigation protocols for resistant populations. Over the past decade, we have gleaned the new insights into the ecological implications of phosphine resistance from the field studies on dispersal, gene flow, and polyandry. There are ongoing attempts in many countries to manage strong levels of resistance in major pests that seriously compromising the effectiveness of currently registered rates of phosphine. Management options include the early detection of strong level of resistance through monitoring, characterization of resistance, development of improved fumigation procedures, and the use of alternative treatments. However, several areas need attention from ongoing and future research that will help in extending the usefulness of this unique fumigant into the foreseeable future

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