J. Pest. Control & Environ. Sci. 23: 16-34 (2023)

Release dynamics and comparative toxicity of phosphine and formaldehyde against two stored-product insects

Ahmed F. El-Aswad[1]; Gehad Z. Mohamed[1] and Neama A. Gouda[2]

[1] Pesticide Chemistry and Technology Dept., Faculty of Agriculture, Alexandria University, Egypt

[2] Pesticide Chemistry Department, Faculty of Desert and Environmental Agriculture, Matrouh University, Egypt

ABSTRACT

The release kinetics of phosphine and formaldehyde were examined, revealing that phosphine evolved rapidly under acidic conditions, whereas formaldehyde generation depended on optimizing the reactant ratios to ensure stable gas release. The optimized mixture ratio of 1:1:2 ($KMnO_4$: formalin: sand) produced the highest steady-state formaldehyde concentration while minimizing heat generation and maintaining reaction stability. The efficacy of both fumigants was evaluated against two major stored-product pests, *Sitophilus oryzae* and *Tribolium confusum*, across life stages. Phosphine exhibited strong, time- and concentration-dependent toxicity, with LC_{50} values markedly lower than those of formaldehyde, indicating more than an order of magnitude higher potency. Larvae were generally more sensitive than adults to both fumigants. Formaldehyde required higher concentrations or longer exposure to achieve comparable mortality, reflecting its slower, cumulative action. These results underscore phosphine as a highly effective fumigant for stored-product pest control, though careful application is essential to prevent resistance development. Formaldehyde may serve as a secondary option in controlled scenarios. Optimizing fumigant release, exposure duration, and species-specific strategies is critical for safe, effective, and sustainable pest management.

Keywords: Fumigation; phosphine; formaldehyde; release kinetics; insecticidal activity; stored-product insects; *Sitophilus oryzae*; *Tribolium confusum.*

INTRODUCTION

Environmental contamination and the rise of insect pests resistant to synthetic pesticides have intensified concerns about the sustainability of current pest-management systems. These issues have driven global efforts to develop alternative control methods that offer high efficacy with improved environmental safety (Souto et al., 2021). Fumigation remains one of the most important strategies in agriculture, food protection, and structural pest management due to its ability to deliver gaseous pesticides that penetrate deeply into commodities and hidden insect habitats (Opit et al., 2012). Because fumigants act in the vapor phase, they show superior penetrability and typically leave minimal residue on treated products.

Despite its effectiveness, fumigation carries significant hazards, as fumigants are among the most toxic pesticides used in practice. The phase-out of methyl bromide due to ozone-depletion concerns created a major gap in soil fumigation (Duniway, 2002),

* Correspondence to Prof. Dr. **Ahmed F. El-Aswad**, Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Aflatoun St., El-Shatby 21545, Alexandria University; Alexandria, Egypt. Phone: 00201064010643. E-mail: ahmed.elasouad@alexu.edu.eg

and although alternatives such as chloropicrin, methyl iodide, metam sodium, and metam potassium have been evaluated, none match the broad reliability of methyl bromide (Cal et al., 2004). Consequently, reliance on other fumigants in stored-product protection has increased.

Phosphine (PH_3) is currently the most widely used fumigant for grains and stored products. It is a highly toxic, colorless gas with a characteristic odor (WHO, 1988), used globally since the 1930s as an effective, low-cost replacement for many halogenated fumigants (Carlson and Thompson, 1998). It is highly effective at very low concentrations and is approved for a wide range of commodities (Longobardi et al., 2008). Phosphine is commonly generated from aluminum phosphide (AlP), which releases phosphine gas upon exposure to moisture, a process widely documented in fumigation practice and toxicology reports (Leeseh et al., 1990). Factors such as temperature, humidity, and grain moisture strongly influence gas release and adsorption by commodities (Carlson and Thompson, 1998).

Regulatory agencies have set strict maximum residue limits, typically 0.1 mg/kg for cereals and 0.01 mg/kg for other foods (Longobardi et al., 2008; WHO, 1988) and residue levels usually remain well below these limits. Phosphine's mode of action involves inhibition of cellular respiration and oxidative stress, but it poses risks to humans and animals, causing acute respiratory effects and chronic organ damage (Nath et al., 2011). Occupational limits are low, with a recommended threshold limit of 0.3 ppm. Sensitivity varies among insect life stages, with diapausing larvae being more tolerant. Effects on plants are limited unless seed moisture is high (WHO, 1988), and its antimicrobial activity is inconsistent.

Due to phosphine's limitations and safety concerns, attention has also turned to other fumigants such as formaldehyde. Formaldehyde (CH_2O) is widely used industrially and is a significant indoor and outdoor pollutant (Salthammer et al., 2010). Background atmospheric levels reach up to 0.03 ppm but can be higher in polluted or enclosed environments. Major sources include combustion processes, industrial emissions, and off gassing from building materials and consumer products (Dar et al., 2016). In the atmosphere, formaldehyde contributes to HOx radical formation and plays a role in ozone-depleting reactions (Iraci and Tolbert, 1997).

Historically, formaldehyde has been used as a fumigant in storage and quarantine settings, typically generated by reacting formalin with potassium permanganate at specific ratios and humidity conditions (Mitchell and Olsen, 2000). Early studies showed that gas generation is reduced when formalin is diluted with water, whereas mixing $KMnO_4$ with sand improves release, with gas liberation completing within 30 minutes (Ackland et al., 1980).

Formaldehyde is highly toxic, with irritation occurring above 0.1 ppm (Dar et al., 2016), and long-term exposure linked to nasopharyngeal cancer and myeloid leukemia. These hazards have greatly restricted its modern fumigant use (Watnick, 1999). Nonetheless, formaldehyde exhibits insecticidal activity against stored-product pests

(Krogmann et al., 2010) and has demonstrated efficacy against wood borers such as *Anobium punctatum*. Its volatility and penetrability enable it to reach hidden life stages, similar to other fumigants (Salthammer and Gunschera, 2021). Although no longer widely used, formaldehyde may still have limited applications in controlled or quarantine environments or as part of integrated pest-management strategies, especially where resistant pests or fumigant restrictions create challenges (Syarifah and Yaakop, 2016).

The present study aims to evaluate and optimize the use of two important fumigants (phosphine and formaldehyde) for the control of major stored-product insect pests. The work focuses on developing reliable and efficient generation protocols for both fumigants, with particular attention to factors influencing their release kinetics under different conditions. In addition, the study investigates the insecticidal efficacy of phosphine and formaldehyde against two key storage pests, the rice weevil *Sitophilus oryzae* and the confused flour beetle *Tribolium confusum*. By combining fumigant generation optimization with detailed toxicity assessments, this research provides insights into improving fumigation performance, enhancing safety, and supporting sustainable stored-product protection.

MATERIALS AND METHODS

Chemicals

Aluminum phosphide (Phostoxin, Tablets), Formalin 37.0%, Potassium permanganate 99.0%, Sodium sulfite, Silver nitrate >99%, Sulfuric acid 95–97%, HCl 37%, Sodium hydroxide >98%.

Tested insects

Two stored-product insect species were tested: the rice weevil (*Sitophilus oryzae*) and the confused flour beetle (*Tribolium confusum*). The insects were obtained from the laboratory colonies maintained in the Bioassay Laboratory, Pesticide Chemistry and Technology Department, Faculty of Agriculture, Alexandria University.

Experiments

Release kinetics of phosphine gas in different media

Calibration curve of phosphine in air

Known weights of aluminum phosphide pellets were placed in PH_3-free airtight bottles, and fumigation was allowed to proceed for 7 days at room temperature. Gas samples were collected from the headspace 3 hours after the fumigation reached steady state to construct a calibration curve for spectrophotometric analysis within the concentration range of 20–90 mg/L (Longobardi et al., 2008). The calibration curve yielded a k value of 0.0042 (mg phosphine/L of space) and showed a very good linear relationship, with $R^2 = 0.9887$ (Figure 1). The high R^2 value confirms that the analytical method used for quantifying phosphine was reliable and precise across the tested concentration range (Draper and Smith, 1998).

Determination of phosphine in air

Phosphine gas in air was determined using a colorimetric method. An aliquot (5 mL) of the headspace gas was transferred using a gas-tight syringe to a 125-mL screwcap vial with a rubber septum containing 10 mL of $AgNO_3$ solution (30 µg/mL). The reaction between the gas and $AgNO_3$ was allowed to proceed with mixing on a vortex mixer for 3 minutes. The resulting solution was then transferred to a cuvette, and the absorbance was measured at 415 nm against a blank ($AgNO_3$ solution). An egg-yellow to brown product was formed.

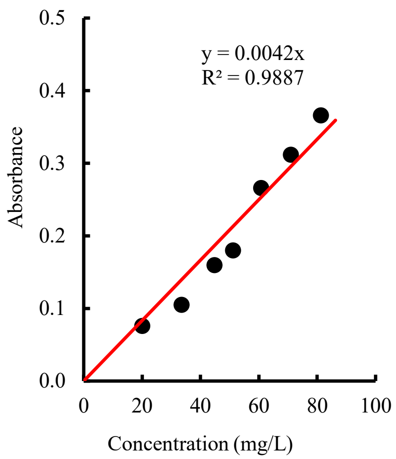

Figure 1. Calibration curve of phosphine in air.

Release kinetics of phosphine gas

The phosphine gas released from aluminum phosphide was monitored over 5 days at room temperature. The effects of moisture and acidic media on the release kinetics of phosphine were evaluated. Known weights of aluminum phosphide were mixed with either distilled water or 1 N HCl at a 1:5 ratio. In addition, aluminum phosphide was tested without any amendments. The release rate of phosphine was then monitored.

Optimization of formaldehyde generation

Formaldehyde gas was generated by adding formalin to potassium permanganate (Mitchell and Olsen, 2000). Different ratios of potassium permanganate to formalin (1:1, 1:2, 1:3, and 1:4 w:v) were tested. In addition, dilution of potassium permanganate with sand as an inert substance was evaluated. Various ratios of potassium permanganate:formalin:sand (1:1:1, 1:2:1, 1:1:2, and 1:2:2) were tested to determine the optimum combination for efficient gas generation.

Sampling and determination of formaldehyde

The method is based on the quantitative release of sodium hydroxide when formaldehyde reacts with sodium sulfite to form the formaldehyde-disulfite addition product:

$CH_2O + Na_2SO_3 + H_2O\ NaOH + CH_2(NaSO_3)OH$

The released NaOH is then titrated with sulfuric acid in the presence of 0.1% thymolphthalein indicator (Braymen and Songer, 1970).

Sampling of formaldehyde gas

A 5-mL aliquot of 0.1 M sodium sulfite (prepared by dissolving 12.6 g of the anhydrous salt in distilled water to make 1 L of solution) and three drops of 0.1% thymolphthalein indicator were placed in glass impinger air samplers. The outlet of each sampler was connected to a vacuum source, while the inlet was connected to a hose leading into the space to be sampled. Air samples were collected for 30 minutes.

Determination of the vapor concentration of formaldehyde

The impinging fluid was titrated slowly with 0.1 N H_2SO_4. The endpoint of the titration was carefully observed using the thymolphthalein indicator.

The formaldehyde content in each sample was calculated using the equation:

Formaldehyde (g/sample) = (mL of acid) × (Normality) × 0.03003, where 1 mL of 1 N H_2SO_4 is equivalent to 0.03003 g of formaldehyde

Fumigant activity of phosphine against the Rice weevil (Sitophilus oryzae) and the Confused flour beetle (Tribolium confusum)

Known weights of aluminum phosphide were placed in vials and suspended inside sealed bottles to generate the required phosphine concentration series. After confirming the phosphine concentration in each bottle, ten adults of *S. oryzae* and ten adults or larvae of *T. confusum* were exposed to the generated gas. Three replicates were used for each concentration. Mortality was recorded at 10, 20, 30, 40, 50, and 60 minutes after exposure. Percent mortality, LC_{50}, and LT_{50} values were calculated using Probit analysis according to Finney (1971).

Fumigant activity of formaldehyde against the Confused flour beetle (Tribolium confusum)

Different amounts of potassium permanganate, formalin, and sand (1:1:2 ratio) were mixed to generate a series of formaldehyde concentrations. Ten adults or larvae of *T. confusum* were placed in vials suspended inside the reaction flasks and exposed to the generated gas. Three replicates were used for each concentration. Mortality was recorded at 10, 20, 30, 60, and 90 minutes after exposure. Percent mortality, LC_{50}, and LT_{50} values were calculated using Probit analysis.

Statistical analysis

All bioassay data were subjected to Probit analysis to estimate median lethal concentrations (LC_{50}) and median lethal times (LT_{50}), along with their 95% confidence limits. Mortality percentages were corrected using Abbott’s formula when necessary. Probit analyses were performed using Probit software, and goodness-of-fit was evaluated through chi-square statistics. Descriptive statistics, including means and standard errors, were calculated for all measured parameters. Differences among treatments were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Release kinetics of phosphine gas in different media

The release kinetics of phosphine from aluminum phosphide were strongly influenced by the surrounding medium (Figure 2). Phosphine concentrations are expressed as the percentage of PH_3 released relative to the total phosphine content in AlP (100%). Release was highest in the acidic medium, followed by the aqueous medium, and lowest in the dry (blank) medium. In acidic conditions, gas evolution was rapid and reached a plateau within 3 hours, whereas in neutral aqueous medium the plateau occurred after 24 hours. In contrast, release in the dry medium was initially slow and plateaued only after 48 hours. These patterns highlight the strong influence of pH and moisture on AlP decomposition and phosphine generation.

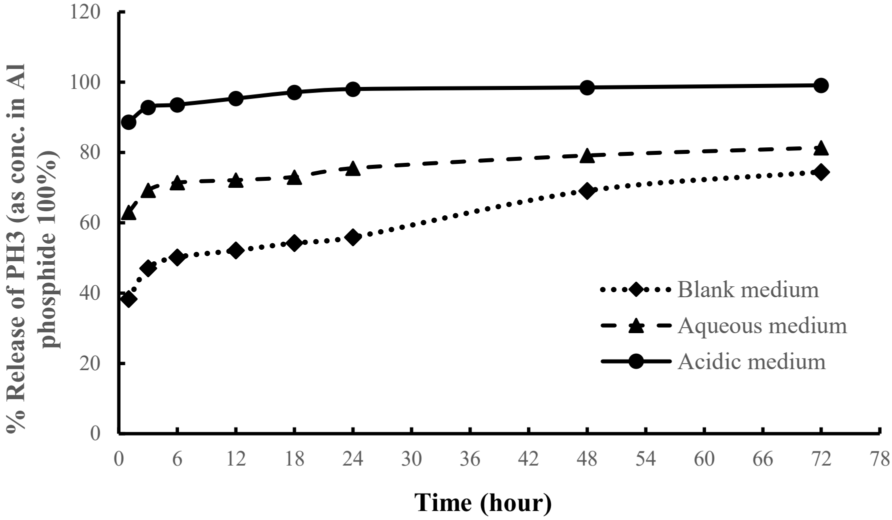

Figure 2. Release kinetics of phosphine gas from aluminum phosphide in different media.

The rapid and nearly complete release of phosphine in the acidic medium, reaching ~82% within the first hour, eflects the accelerated decomposition of AlP in the presence of protons. Acid-driven hydrolysis ($AlP + 3H^+ \rightarrow Al^{3+} + PH_3$) proceeds much faster than hydrolysis by water alone ($AlP + 3H_2O \rightarrow Al(OH)_3 + PH_3$) (Ozoemena et al., 2021). Under typical fumigation conditions, complete decomposition of AlP tablets generally takes 48–72 hours (Caliboso and Tiongson, 1998), indicating that the experimental acidic setup (1 N HCl, 1:1 w:v) created conditions far more favorable for rapid gas evolution than those encountered in field applications.

The observation that PH_3 concentrations on the second day were highest in the acidic medium, intermediate in the water-amended medium, and lowest in the unamended medium aligns with prior studies on residual AlP. Rajashekar et al. (2006) reported that 3–3.6% of AlP often remains unreacted in the residue and continues to release PH_3 slowly, particularly when exposed to acid, which can further react with remaining AlP particles more effectively than water alone.

By the third day, PH_3 levels in all media converged, suggesting that most reactive AlP, both in the bulk tablet and in the residue had decomposed. This plateau is consistent with reports that gas evolution slows markedly after the main decomposition phase (Prabhakaran et al., 2001). The convergence may also reflect equilibrium between phosphine generation, diffusion, and gas–liquid partitioning across the different media.

Optimization of reaction ratios for maximum formaldehyde generation

The generation of formaldehyde gas varied noticeably with the ratio of potassium permanganate ($KMnO_4$), formalin, and sand (Figure 3). Increasing $KMnO_4$ relative to formalin accelerated gas release due to more vigorous oxidation of formalin; however, excessively high $KMnO_4$ levels caused abrupt and difficult-to-control reactions.

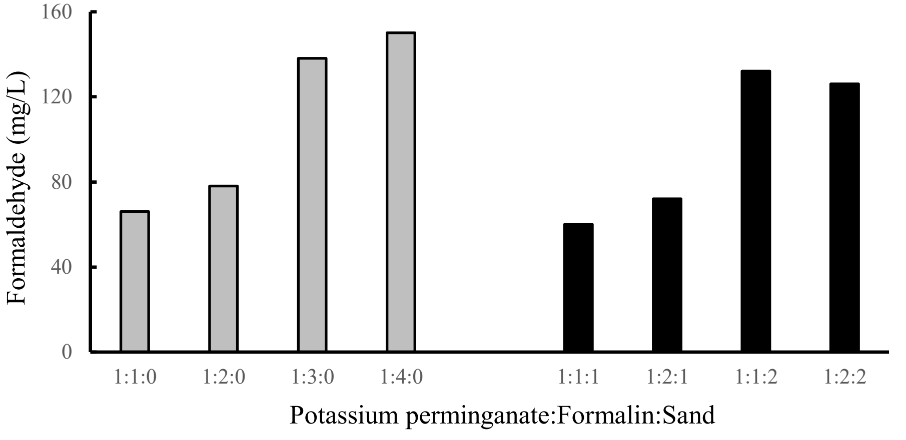

Figure 3. Optimization of the $KMnO_4$:Formalin:Sand ratio for formaldehyde generation.

The inclusion of sand moderated the reaction and improved gas-release stability. Mixtures containing sand produced smoother and more sustained formaldehyde evolution compared with those without sand, which tended to release gas rapidly and unevenly. The optimized ratio (1:1:2, $KMnO_4$:formalin:sand) achieved the highest steady-state formaldehyde concentration while minimizing heat generation and maintaining reaction stability.

Although higher formalin ratios (e.g., 1:3 or 1:4) produced slightly higher formaldehyde concentrations, the 1:1:2 ratio is preferred because it efficiently generates gas using lower total amounts of chemicals. Other ratios, such as 1:1 and 1:2 ($KMnO_4$:formalin) or 1:1:1 and 1:2:1 ($KMnO_4$:formalin:sand), produced similar concentrations but with less optimal stability or overall efficiency.

Formaldehyde generation using the $KMnO_4$–formalin method relies on controlled oxidation of formalin by $KMnO_4$, a strong oxidizing agent that reacts rapidly with aldehydes under suitable conditions (Felix et al., 2018). Because the reaction is highly exothermic, uncontrolled conditions can lead to rapid gas release and thermal spikes, posing safety risks (Australia, 2022). The presence of an inert diluent such as sand mitigates these risks by absorbing heat, moderating the reaction rate, and smoothing gas release (Maznoy et al., 2016).

In our system, sand likely acted as a physical buffer, dispersing heat and ensuring a stable gas-release profile.

The 1:1:2 ratio strikes a balance between vigorous oxidation for efficient formaldehyde generation and thermal control to prevent hazards such as splashing, overheating, or sudden surges in gas output. These findings have practical significance for safe and controlled fumigation, particularly in confined environments, where precise control of formaldehyde generation is essential for maintaining effective concentrations without compromising safety.

Phosphine toxicity against stored-product insects

Phosphine toxicity showed a clear time- and concentration-dependent pattern in all tested species. LC_{50} values consistently declined with increasing exposure duration, reflecting enhanced cumulative mortality as the fumigant penetrated insect tissues more effectively. Similarly, LT_{50} values decreased with increasing phosphine concentration, demonstrating accelerated mortality under stronger toxic stress. Slope values for both LC_{50} and LT_{50} were generally steep, indicating relatively homogeneous population responses, though some variability occurred at high doses or prolonged exposures. Elevated χ^2 values in these conditions likely reflect biological variability, microhabitat effects, and physiological stress influencing mortality dynamics (Islam et al., 2021).

These patterns agree with the known mode of action of phosphine, which disrupts mitochondrial oxidative phosphorylation by inhibiting cytochrome c oxidase, collapsing membrane potential, impairing ATP production, and generating reactive oxygen species (Nath et al., 2011; Sciuto et al., 2016; Valmas et al., 2008; Aulicky et al., 2022). Prolonged exposure enhances these cumulative effects, whereas excessively high concentrations may induce narcotic-like metabolic suppression that temporarily reduces lethal efficiency, contributing to deviations from optimal probit-model fits (Nakakita, 1987; Nath et al., 2011). These findings emphasize that achieving effective fumigation requires careful optimization of both exposure duration and phosphine concentration to avoid survival of tolerant individuals and reduce the risk of resistance development (Bond and Monro, 1984; Sciuto et al., 2016; Wakil et al., 2021).

Data for *S. oryzae* (Table 1) revealed a sharp decline in LC_{50} values from 0.373 mg/L at 10 minutes to 0.083 mg/L at 60 minutes, demonstrating steadily increasing susceptibility with extended exposure. Confidence intervals also narrowed with time, indicating improved precision. Slope values ranged from 1.757 to 4.212, suggesting relatively steep concentration–mortality relationships. Goodness-of-fit (χ^2) values were acceptable overall, though slightly elevated at 40–60 minutes, indicating minor deviations from the probit model at longer exposures. LT_{50} values also decreased consistently with increasing concentrations, from 60.63 minutes at 0.085 mg/L to 23.39 minutes at 0.205 mg/L. Slope values (2.346–2.863) indicated uniform responses across the population, while higher χ^2 values at the strongest doses reflected increased variability in mortality time under intense toxic stress.

These results align with FAO-based bioassay methods and previous findings showing increased efficacy during prolonged exposures, especially in tolerant or resistant strains (Harahap et al., 2023). Mechanistically, the observed patterns reflect phosphine's slow-acting inhibition of mitochondrial respiratory enzymes, particularly cytochrome c oxidase (Aulicky et al., 2022; Valmas et al., 2008), making adequate exposure duration essential for complete control.

Table 1. Toxicity indices of phosphine gas against adult Rice weevils (*Sitophilus oryzae*)

Exposure time (min)	LC_{50} (mg/L space) (Upper-Lower)	Slope	P	χ^2
10	0.373 (0.515-0.270)	2.993 ± 0.265	0.971	0.528
20	0.300 (0.407-0.221)	2.107 ± 0.150	0.597	2.771
30	0.233 (0.303-0.180)	1.757 ± 0.129	0.713	2.127
40	0.157 (0.182-0.136)	1.840 ± 0.122	0.020	11.669
50	0.101 (0.115-0.089)	2.246 ± 0.128	0.471	3.543
60	0.083 (0.091-0.076)	4.212 ± 0.210	0.046	9.674
Concentration (mg/L space)	**LT_{50} (min.) (Upper-Lower)**	**Slope**	**P**	**χ^2**
0.085	60.633 (69.866-52.650)	2.792 ± 0.104	0.987	0.342
0.095	50.682 (56.740-45.289)	2.824 ± 0.087	0.867	1.266
0.105	43.912 (48.427-39.827)	2.863 ± 0.752	0.490	3.420
0.145	37.667 (41.678-34.047)	2.530 ± 0.060	0.041	9.940
0.195	30.369 (33.739-27.333)	2.346 ± 0.0518	0.001	25.061
0.205	23.394 (25.881-21.139)	2.737 ± 0.054	0.001	20.900

A strong time-dependent toxicity trend was also observed for *T. confusum* adults (Table 2). LC_{50} values decreased from 0.681 mg/L at 10 minutes to 0.300 mg/L at 30 minutes and further to 0.044 mg/L at 60 minutes—a more than 15-fold increase in toxicity. Confidence limits narrowed at longer exposures, and slope values (0.989–1.963) indicated moderately steep dose–response curves. Elevated χ^2 values at 60 minutes suggest increased variability in susceptibility during long exposures. LT_{50} values fell from 60.34 minutes at 0.04 mg/L to 26.35 minutes at 0.21 mg/L, with slope values between 2.511 and 3.009 indicating relatively uniform population responses. Higher χ^2 values at stronger doses likely reflect heterogeneous tolerance or microenvironmental differences under severe toxic pressure.

These findings confirm the cumulative and metabolic-disruptive nature of phosphine toxicity, driven largely by its inhibition of oxidative phosphorylation (Nath et al., 2011; Alzahrani and Ebert, 2023). The patterns agree with earlier studies showing that both exposure duration and concentration strongly influence phosphine efficacy in stored-product beetles (Winks, 1982).

For *T. confusum* larvae (Table 3), LC_{50} values decreased from 0.273 mg/L at 10 minutes to 0.021 mg/L at 40 minutes, representing an over tenfold increase in toxicity with prolonged exposure. Confidence intervals narrowed correspondingly. Slope values (0.699–3.056) varied but generally indicated moderately steep responses. χ^2 values were acceptable except at 40 minutes (10.883), suggesting slightly greater variability at this exposure duration. LT_{50} values declined with concentration, from 30.77 minutes at 0.04 mg/L to 14.54 minutes at 0.21 mg/L. Slope values (2.914–3.820) indicated uniform responses, particularly at lower doses, while elevated χ^2 at the highest concentration (21.410) suggested increased variability in mortality time under strong toxic pressure.

Table 2. Toxicity indices of phosphine gas against adult Confused flour beetle (*Tribolium confusum*)

Exposure time (min)	LC_{50} (mg/L space) (Upper-Lower)	Slope	P	χ^2
10	0.681 (1.544-0.303)	1.629 ± 0.167	0.972	0.058
20	0.468 (0.823-0.268)	1.637 ± 0.121	0.789	0.475
30	0.300 (0.526-0.172)	0.989 ± 0.062	0.785	0.483
40	0.147 (0.201-0.108)	1.008 ± 0.056	0.436	1.662
50	0.063 (0.082-0.048)	1.412 ± 0.056	0.585	1.073
60	0.044 (0.057-0.034)	1.963 ± 0.065	0.001	16.358
Concentration (mg/L space)	**LT_{50} (min.) (Upper-Lower)**	**Slope**	**P**	**χ^2**
0.04	60.340 (68.907-52.865)	3.009 ± 0.119	0.525	3.199
0.11	43.409 (48.464-38.894)	2.511 ± 0.066	0.036	10.266
0.16	34.278 (37.840-31.054)	2.527 ± 0.057	0.001	13.416
0.21	26.345 (28.870-24.037)	2.906 ± 0.058	0.001	37.028

Table 3. Toxicity indices of phosphine gas against larval Confused flour beetle (*Tribolium confusum*)

Exposure time (min)	LC_{50} (mg/L space) (Upper-Lower)	Slope	P	χ^2
10	0.273 (0.343-0.217)	3.056 ± 0.289	0.347	2.114
20	0.228 (0.431-0.122)	0.699 ±0.055	0.949	0.105
30	0.063 (0.082-0.048)	1.412 ± 0.056	0.585	1.073
40	0.021 (0.036-0.013)	1.612 ± 0.077	0.004	10.883
Concentration (mg/L space)	**LT_{50} (min.) (Upper-Lower)**	**Slope**	**P**	**χ^2**
0.04	30.770 (33.685-28.112)	3.820 ± 0.173	0.055	5.802
0.11	23.921 (26.204-21.836)	3.435 ± 0.120	0.690	0.741
0.16	19.562 (21.560-17.745)	3.329 ± 0.108	0.085	4.924
0.21	14.542 (16.561-12.759)	2.914 ± 0.101	0.001	21.410

Overall, the larval bioassay results confirm phosphine's characteristic slow-acting toxicity and its requirement for sustained exposure to achieve lethal effects, consistent with previous reports (Bond and Monro, 1984; Nath et al., 2011; Sciuto et al., 2016; Valmas et al., 2008). Very high doses may induce narcotic-like responses that suppress metabolism and limit immediate toxicity, contributing to deviations from probit-model expectations (Nakakita, 1987).

Comparative susceptibility of *Sitophilus oryzae* and *Tribolium confusum* to phosphine

A clear interspecific difference in phosphine susceptibility was observed at 30 minutes. *S. oryzae* adults exhibited a lower LC_{50} value (0.233 mg/L), indicating higher sensitivity to phosphine, whereas *T. confusum* adults showed a higher LC_{50} (0.300 mg/L), reflecting lower susceptibility under identical conditions (Figure 4).

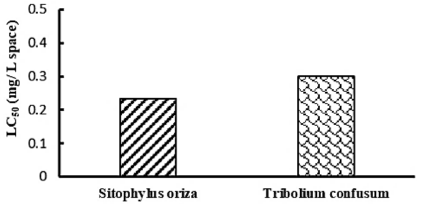

Figure 4. Comparison of LC_{50} values at 30-minute exposure to phosphine between adults of *Sitophilus oryzae* and *Tribolium confusum*.

Such differences align with previous findings demonstrating species-specific tolerance profiles. *S. oryzae* is well known for its ability to develop resistance to phosphine, often associated with mutations in dihydrolipoamide dehydrogenase (N505T), with resistant strains typically showing LC_{50} values of ~0.20–0.36 mg/L after 48-hour exposure (Nguyen et al., 2016). Metabolic and proteomic studies confirm altered oxidative-stress responses and detoxification pathways in resistant individuals.

These differences have practical implications for fumigation strategies. Because *S. oryzae* can develop resistance more readily, even modest changes in susceptibility may influence treatment outcomes. This emphasizes the need for resistance-management practices such as routine monitoring, gas concentration verification, and adherence to recommended exposure durations to ensure effective control (Nayak et al., 2015).

Although phosphine is broadly effective against both species, *T. confusum* may require slightly higher concentrations or longer exposures to achieve comparable mortality. Steep probit slopes for both beetles suggest generally uniform population responses, while deviations at high concentrations likely reflect physiological variability or microenvironmental effects (Wakil et al., 2021). Optimizing concentration and exposure time is especially critical in mixed-species infestations to avoid treatment failures and delay resistance development.

Formaldehyde toxicity against stored-product insects

Table 4 presents the LC_{50} and LT_{50} values describing the fumigant toxicity of formaldehyde gas against adult *T. confusum*. Formaldehyde showed a clear time-dependent toxicity pattern. No LC_{50} could be calculated at 10 min due to insufficient mortality, but from 20 min onward LC_{50} values declined steadily from 7.633 mg/L at 20 min to 2.104 mg/L at 60 min, indicating increased susceptibility with prolonged exposure. Slope values (1.937–6.928) reflected moderate to steep concentration–response relationships.

A strong concentration-dependent decrease was also observed in LT_{50} values. At the lowest concentration (1.2 mg/L), LT_{50} was 104.97 min, dropping sharply to 6.23 min at 9.6 mg/L. The slope values (2.13–2.96) indicated relatively uniform adult susceptibility across concentrations, and χ^2 values were low and within acceptable ranges.

These findings demonstrate that formaldehyde has clear fumigant toxicity against adult *T. confusum*, but effectiveness depends strongly on both concentration and exposure time. The lack of an LC_{50} at 10 min suggests that very short exposures are inadequate because formaldehyde requires sustained contact to penetrate tissues and interact with molecular targets. Over longer exposures, the progressive decline in LC_{50} reflects a cumulative mode of action. Nevertheless, LC_{50} values even at 60 min remained much higher than typical phosphine LC_{50} values, indicating substantially lower potency. This is consistent with the chemical's mechanism, as formaldehyde induces protein and DNA cross-linking such as DNA–protein crosslinks, which requires time to accumulate (Weickert and Stingele, 2022).

Table 4. Toxicity indices of formaldehyde gas against adult Confused flour beetle (*Tribolium confusum*)

Exposure time (min)	LC_{50} (mg/L space) (Upper-Lower)	Slope	P	χ^2
10	---	---	---	---
20	7.633	6.873 ± 0.737	0	6
30	6.519	6.928 ± 0.520	0	7.8
40	4.572	3.144 ±0.229	0	11.1
50	2.844 (3.832-1.418)	2.266 ±0.186	0	11.100
60	2.104	1.937 ±0.201	0.001	21.760
Concentration (mg/L space)	**LT_{50} (min.) (Upper-Lower)**	**Slope**	**P**	**χ^2**
1.2	104.968 (153.336-87.464)	2.774 ± 0.576	0.149	2.082
2.4	85.491 (118.072-72.681)	2.347 ± 0.533	0.289	1.124
4.0	67.014 (77.054-60.185)	2.963 ± 0.384	0.066	5.452
4.8	53.02 (59.89-47.726)	2.619 ±0.279	0.33	3.428
7.2	36.261 (39.833-32.763)	2.921 ±0.287	0.557	2.073
8.0	13.41 (17.094-7.094)	2.130 ±0.225	0.023	9.5
9.6	6.229 (8.289-3.765)	2.324 ±0.362	0.545	1.215

Formaldehyde also exhibited strong time-dependent toxicity toward *T. confusum* larvae (Table 5). LC_{50} values decreased from 6.990 mg/L at 10 min to 1.766 mg/L at 60 min, representing roughly a fourfold increase in toxicity over the exposure period. Slope values (1.995–5.060) indicated moderately steep to steep dose–response relationships, especially at shorter exposures.

LT_{50} values declined sharply with increasing concentration: from 138.91 min at 1.2 mg/L to only 0.662 min at 9.6 mg/L. Slope values (0.923–2.216) suggested fairly uniform susceptibility at low concentrations, though greater variability occurred at the highest dose, consistent with intensified physiological stress. χ^2 values were acceptable and demonstrated a good model fit.

These results show that *T. confusum* larvae are sensitive to formaldehyde, with both exposure duration and concentration playing key roles in toxicity. The continuous decline in LC_{50} values suggests that formaldehyde's cumulative mechanism, mediated by cross-linking of proteins and nucleic acids, requires sustained exposure to cause lethal cellular disruption (Li et al., 2023).

Table 5. Toxicity indices of formaldehyde gas against larval Confused flour beetle (*Tribolium confusum*)

Exposure time (min)	**LC_{50} (mg/L space) (Upper-Lower)**	**Slope**	**P**	**χ^2**
10	6.990 (9.056-5.682)	5.060 ± 0.468	0.005	12.834
20	5.775	4.357 ± 0.338	0	9.5
30	4.100 (5.828-2.571)	2.636 ± 0.202	0	11.1
40	3.203 (4.195-2.078)	2.430 ± 0.190	0	11.1
50	2.547 (3.167-1.771)	2.447 ± 0.191	0.003	18.076
60	1.766 (2.241-0.977)	1.995 ± 0.205	0.032	10.568
Concentration (mg/L space)	**LT_{50} (min.) (Upper-Lower)**	**Slope**	**P**	**χ^2**
1.2	138.912 (343.142-98.076)	1.594 ± 0.387	0.722	0.65
2.4	61.969 (73.542-54.369)	2.216 ± 0.276	0.282	3.819
4.0	44.142 (51.681-38.429)	1.780 ± 0.188	0.461	3.615
4.8	30.381 (26.323-34.814)	1.798 ± 0.184	0.987	3.345
7.2	18.367 (22.096-14.342)	1.469 ± 0.179	0.43	3.849
8.0	5.366 (8.169-2.535)	1.328 ± 0.207	0.111	7.521
9.6	0.662	0.923 ± 0.495	0.117	2.461

Larvae were slightly more sensitive than adults, likely due to their thinner cuticle, faster metabolism, and less-developed detoxification pathways, characteristics also reported in other beetle larvae (Pedersen et al., 2020). Although LT_{50} values decreased sharply at higher concentrations, very low doses produced prolonged LT_{50} values, indicating that low-concentration formaldehyde acts slowly unless maintained over extended periods.

Previous studies support these trends. Formalin vapors achieved complete larval mortality in *Anobium punctatum* after several days of exposure (Wojcik, 2006), and formaldehyde-based treatments similarly produced increased larval mortality in the red palm weevil (*Rhynchophorus ferrugineus*) with longer exposure times. These findings underscore the cumulative nature of formaldehyde toxicity.

Comparative toxicity of phosphine and formaldehyde in two life stages of *T. confusum*

At 30 minutes of exposure, phosphine exhibited substantially greater toxicity than formaldehyde toward both adults and larvae of *T. confusum*. For adults, the LC_{50} of phosphine (0.300 mg/L) was far lower than that of formaldehyde (6.519

mg/L), and a similar trend was observed in larvae, where the LC_{50} values were 0.063 mg/L and 4.100 mg/L, respectively (Figure 5). Thus, phosphine was more than an order of magnitude, more potent than formaldehyde across both life stages, with larvae consistently showing higher sensitivity than adults.

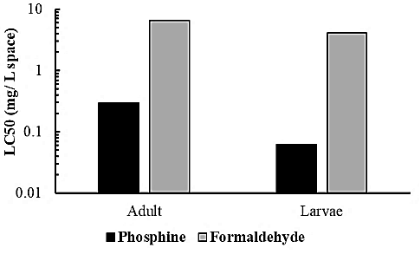

Figure 5. Comparison of the toxicity of phosphine and formaldehyde at 30-minute exposure against adults and larvae of *Tribolium confusum*.

This marked difference underscores phosphine's superior fumigant efficacy and explains its established role in stored-product pest management. Its effectiveness across all life stages, relatively low cost, and lack of residues have supported its widespread use, although resistance remains an increasing concern (Nayak et al., 2020). By contrast, formaldehyde exhibits a cumulative and slower mode of action, requiring higher concentrations or extended exposure periods to achieve comparable control levels. While formaldehyde can be effective under prolonged, high-concentration conditions (Eduardo et al., 2017), it is far less practical as a rapid, standalone fumigant for stored-product insects.

These results demonstrate that although formaldehyde is active against both larvae and adults, its lower potency and slower action limit its utility to controlled, long-duration fumigation scenarios where sustained concentrations can be safely maintained.

It can be stated that phosphine is a highly effective fumigant against both *S. oryzae* and *T. confusum* across life stages, provided that adequate exposure time is maintained. Its rapid release under acidic conditions (Figure 2) further supports its efficiency in environments that facilitate fast gas buildup. Formaldehyde, while capable of producing significant mortality, requires substantially higher concentrations or longer fumigation periods (Tables 4 and 5) and careful control of its generation system (Figure 3), which limits its practicality as a fast-acting fumigant.

From an applied standpoint, phosphine remains the preferred choice for most storage scenarios. However, formaldehyde may still serve as a secondary option where phosphine use is restricted, assuming release systems (such as the $KMnO_4$–formalin–sand mixture) are well-optimized. Finally, the growing evidence of phosphine resistance, including documented genetic mechanisms in *S. oryzae* highlights the need for proper fumigation design, avoidance of sublethal exposures, and continuous resistance monitoring to maintain long-term efficacy.

Conclusion

The present study demonstrates that phosphine is a highly potent fumigant against adults and larvae of *Sitophilus oryzae* and *Tribolium confusum*, with toxicity increasing markedly as concentration and exposure time rise. In contrast, formaldehyde exhibited measurable fumigant activity, especially at higher concentrations but remained substantially less effective than phosphine, requiring longer exposure periods to achieve comparable mortality. The results also underscore the importance of optimizing fumigant-generation methods, including adjustment of $KMnO_4$–formalin–sand ratios and consideration of pH-dependent release kinetics, to ensure consistent gas production, operational safety, and improved insecticidal performance.

Furthermore, the findings reaffirm the need for robust resistance-management practices. Given the widespread reliance on phosphine in stored-product protection, improper application or repeated sublethal exposure can accelerate resistance development and diminish long-term efficacy. Understanding fumigant dynamics, species-specific susceptibility, and appropriate management strategies is therefore essential for achieving effective and sustainable control of stored-product pests.

REFERENCES

Ackland, N. R., Hinton, M. R. and Denmeade, K. R. (1980). Controlled formaldehyde fumigation system. Applied and Environmental Microbiology, 39(3), 480-487.

Alzahrani, S. M. and Ebert, P. R. (2023). Pesticidal toxicity of phosphine and its interaction with other pest control treatments. Current Issues in Molecular Biology, 45(3), 2461-2473.

Aulicky, R., Stejskal, V., Frydova, B. and Athanassiou, C. (2022). Evaluation of phosphine resistance in populations of *Sitophilus oryzae*, Oryzaephilus surinamensis and Rhyzopertha dominica in the Czech Republic. Insects, 13(12), 1162.

Australia, W. (2022). Managing Risks of Hazardous Chemicals in the Workplace: Code of Practice. Department of Mines, Industry Regulation and Safety.

Bond, E. J. and Monro, H. A. U. (1984). Manual of fumigation for insect control (Vol. 54, p. 432). Rome, Italy: FAO.

Braymen, D. T. and Songer, J. R. (1970). Method for quantitating formaldehyde gas in air. Applied Microbiology, 19(6), 1021-1022.

Cal, A. D., Martinez-Treceño, A., Lopez-Aranda, J. M. and Melgarejo, P. (2004). Chemical alternatives to methyl bromide in Spanish strawberry nurseries. Plant disease, 88(2), 210-214.

Caliboso, F. M. and Tiongson, R. L. (1998). Pesticide Use and Residues in Stored Grains in the Philippines. Seeking Agricultural Produce Free of Pesticide Residues.

Dar, A., Shafique, U., Anwar, J. and Naseer, A. (2016). A simple spot test quantification method to determine formaldehyde in aqueous samples. Journal of Saudi chemical society, 20, S352-S356.

Draper, N. R. and Smith, H. (1998). Applied regression analysis (Vol. 326). John Wiley & Sons.

Duniway, J. M. (2002). Status of chemical alternatives to methyl bromide for pre-plant fumigation of soil. Phytopathology, 92(12), 1337-1343.

Eduardo, W. I., De Moraes, R. F. O., Almeida, L. F. V., Dos Santos, J. A., Dos Santos, R. F. and De Bortoli, S. A. (2017). Alternative to formaldehyde in artificial diet for sugarcane borer and its effects on the parasitoid. Comunicata Scientiae, 8(2), 194-201.

Felix, L. D., Solomon, O., Adesoji, A. and Adegoke, A. (2018). Kinetics of Acidic Potassium Permanganate Oxidation of Formaldehyde in Aqueous and 5% Ethanol-Water Solvents. Caribbean Journal of Sciences and Technology, 6(1), 09-17.

Finney, D. J. (1971). Probit Analysis (3rd ed.). Cambridge University Press.

Harahap, N. A., Witjaksono, W., Martono, E. and Trisyono, Y. A. (2023). Bioassay Method Development to Test *Sitophilus oryzae* Sensitivity against Phosphine. Jurnal Perlindungan Tanaman Indonesia, 27(1), 18-25.

Hendriks, A. J., Awkerman, J. A., de Zwart, D. and Huijbregts, M. A. (2013). Sensitivity of species to chemicals: Dose–response characteristics for various test types (LC_{50}, LR_{50} and LD_{50}) and modes of action. Ecotoxicology and environmental safety, 97, 10-16.

Iraci, L. T. and Tolbert, M. A. (1997). Heterogeneous interaction of formaldehyde with cold sulfuric acid: Implications for the upper troposphere and lower stratosphere. Journal of Geophysical Research: Atmospheres, 102(D13), 16099-16107.

Islam, M. A., Amin, S. N., Brown, C. L., Juraimi, A. S., Uddin, M. K. and Arshad, A. (2021). Determination of median lethal concentration (LC_{50}) for endosulfan, heptachlor and dieldrin pesticides to african catfish, Clarias gariepinus and their impact on its behavioral patterns and histopathological responses. Toxics, 9(12), 340.

Krogmann, L., Holstein, J., Eymann, J., Degreef, J., Häuser, C., Monje, J. C., Samyn Y. and Vanden Spiegel, D. (2010). Preserving and specimen handling: insects and other invertebrates. Manual on field recording techniques and protocols for all taxa biodiversity inventories, 2, 463-481.

Leeseh, J. G., Arthur, F. H. and Davis, R. (1990). Three methods of aluminum phosphide application for the in-transit fumigation of grain aboard deep-draft bulk cargo ships. Journal of economic entomology, 83(4), 1459-1467.

Li, Y., Ou, J., Huang, C., Liu, F., Ou, S. and Zheng, J. (2023). Chemistry of formation and elimination of formaldehyde in foods. Trends in Food Science and Technology, 139, 104134.

Longobardi, F., Pascale, M., Silvestri, M. and Visconti, A. (2008). Rapid method for determination of phosphine residues in wheat. Food Analytical Methods, 1(3), 220-225.

Maznoy, A. S., Kirdyashkin, A. I. and Gabbasov, R. M. (2016). Combustion synthesis of porous oxynitride materials under conditions of forced filtration of reacting gas. International Journal of Heat and Mass Transfer, 95, 264-271.

Mitchell, C. E. and Olsen, D. B. (2000). Formaldehyde formation in large bore natural gas engines part 1: formation mechanisms. Journal of engineering for gas turbines and power, 122(4), 603-610.

Nakakita, H. (1987). The Mode of Action of Phosphine. Journal of Pesticide Science, 12(2), 299-309.

Nath, N. S., Bhattacharya, I., Tuck, A. G., Schlipalius, D. I. and Ebert, P. R. (2011). Mechanisms of phosphine toxicity. Journal of toxicology, 2011(1), 494168.

Nayak, M. K., Daglish, G. J. and Phillips, T. W. (2015). Managing resistance to chemical treatments in stored products pests. Stewart Postharvest Rev, 11(1), 1-6.

Nayak, M. K., Daglish, G. J., Phillips, T. W. and Ebert, P. R. (2020). Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annual Review of Entomology, 65(1), 333-350.

Nguyen, T. T., Collins, P. J., Duong, T. M., Schlipalius, D. I. and Ebert, P. R. (2016). Genetic conservation of phosphine resistance in the rice weevil *Sitophilus oryzae* (L.). Journal of Heredity, 107(3), 228-237.

Opit, G. P., Phillips, T. W., Aikins, M. J. and Hasan, M. M. (2012). Phosphine resistance in Tribolium castaneum and Rhyzopertha dominica from stored wheat in Oklahoma. Journal of Economic Entomology, 105(4), 1107-1114.

Ozoemena, C. C., Bartimaeus, E. S., Igwe, F. U. and Nwachuku, E. O. (2021). Assessment of Chronic Toxicological Effects of 2, 2-Dichlorovinyl Dimethyl Phosphate (Sniper) on the Kidneys of New Zealand White Rabbits. International Journal of Advances in Nephrology Research, 4(1), 92-103.

Pedersen, K. E., Pedersen, N. N., Meyling, N. V., Fredensborg, B. L. and Cedergreen, N. (2020). Differences in life stage sensitivity of the beetle Tenebrio molitor towards a pyrethroid insecticide explained by stage-specific variations in uptake, elimination and activity of detoxifying enzymes. Pesticide Biochemistry and Physiology, 162, 113-121.

Prabhakaran, S., Ray, S., Schneider, B.M., Williams, R.E., Flinn, P., Phillips, T., Hagstrum, D., Arthur, F., Throne, J., Emery, R.N. and Tassone, R.A., 2001. Integrated commodity management methods with ca and/or fumigation. Phytoparasitica, 29, pp.S51-S57.

Rajashekar, Y., Reddy, P. V., Begum, K., Leelaja, B. C. and Rajendran, S. (2006). Studies on aluminium phosphide tablet formulation. Pestology, 30(4), 41-45.

Salthammer, T. and Gunschera, J. (2021). Release of formaldehyde and other organic compounds from nitrogen fertilizers. Chemosphere, 263, 127913.

Salthammer, T., Mentese, S. and Marutzky, R. (2010). Formaldehyde in the indoor environment. Chemical reviews, 110(4), 2536-2572.

Sciuto, A. M., Wong, B. J., Martens, M. E., Hoard-Fruchey, H. and Perkins, M. W. (2016). Phosphine toxicity: a story of disrupted mitochondrial metabolism. Annals of the New York Academy of Sciences, 1374(1), 41-51.

Souto, A. L., Sylvestre, M., Tölke, E. D., Tavares, J. F., Barbosa-Filho, J. M. and Cebrián-Torrejón, G. (2021). Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: Prospects, applications and challenges. Molecules, 26(16), 4835.

Syarifah, Z. S. A. and Yaakop, S. (2016). Mimicry of formaldehyde as aldehyde: A potential and novel chemical compound to control storage pests. Serangga, 21(2), 43–55.

Valmas, N., Zuryn, S. and Ebert, P. R. (2008). Mitochondrial uncouplers act synergistically with the fumigant phosphine to disrupt mitochondrial membrane potential and cause cell death. Toxicology, 252(1-3), 33-39.

Wakil, W., Kavallieratos, N. G., Usman, M., Gulzar, S. and El-Shafie, H. A. (2021). Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan. Insects, 12(4), 288.

Watnick, V. (1999). Risk Assessment: Obfuscation of Policy Decisions in Pesticide Regulation and the EPA's Dismantling of the Food Quality Protection Act's Safeguards for Children. Ariz St. LJ, 31, 1315.

Weickert, P. and Stingele, J. (2022). DNA–protein crosslinks and their resolution. Annual Review of Biochemistry, 91(1), 157-181.

WHO (1988). Phosphine and selected metal phosphides. In Phosphine and selected metal phosphides. International Programme on Chemical Safety, & World Health Organization.

Winks, R. G. (1982). The toxicity of phosphine to adults of Tribolium castaneum (Herbst): time as a response factor. Journal of Stored Products Research, 18(4), 159-169.

Wojcik, A. (2006). Formalin vapours as a fumigant for larvae of furniture beetle. Annals of Warsaw Agricultural University. Forestry and Wood Technology, 59.

J. Pest. Control & Environ. Sci. 23: 16-34 (2023)

حركية اطلاق غاز الفوسفين والفورمالدهيد وتقييم سميتهما ضد نوعين من حشرات المنتجات المخزنة

أحمد الأسود[1]؛ جهاد محمد[1]؛ نعمة جودة[2]

[1] قسم كيمياء وتكنولوجيا المبيدات، كلية الزراعة، جامعة الإسكندرية، مصر

[2] قسم كيمياء المبيدات، كلية الزراعة الصحراوية والبيئية، جامعة مطروح، مصر

تمت دراسة حركية إطلاق غازي الفوسفين والفورمالدهيد، وأظهرت النتائج أن الفوسفين يتصاعد بمعدل سريع تحت الظروف الحامضية، بينما يعتمد توليد الفورمالدهيد على النسب بين المواد المستخدمة لإطلاق الغاز بصزرة مستقرة، فقد اتضح أن النسبة المثلى هى (1:1:2، KMnO4: الفورمالين: الرمل) وقد حققت أعلى تركيز للفورمالدهيد بحالة ثابتة مع تقليل توليد الحرارة والحفاظ على استقرار التفاعل. وقد تم تقييم فعالية كلا الغازين ضد اثنين من الآفات الرئيسية للمنتجات المخزونة، حشرة سوسة الأرز (*Sitophilus oryzae*) وخنفساء الدقيق (*Tribolium confusum*). أظهر الفوسفين سمية عالية تعتمد على الزمن والتركيز، مع قيم LC_{50} أقل بكثير من تلك الخاصة بالفورمالدهيد، مما يشير إلى فعالية أعلى بمقدار أكثر من الضعف. كانت يرقات خنفساء الدقيق أكثر حساسية بشكل عام من الحشرات الكاملة تجاه كلا الغازين. تطلب الفورمالدهيد تركيزات أعلى وكذلك زمن تعرّض أطول لتحقيق معدل موت مماثل، مما يعكس تأثيره الأبطئ والمتراكم. تؤكد هذه النتائج على أن الفوسفين يعتبر مبيد فعال للغاية لمكافحة آفات المنتجات المخزونة، مع ضرورة تطبيقه بحذر لتجنب خطورته ومنع تطور صفة المقاومة لدى الحشرات. ومع ذلك قد يكون الفورمالدهيد خيارًا ثانويًا في سيناريوهات مكافحة خاضعة للتحكم. ومما لاشك فيه أن تحسين انتاج الغاز، وزمن التعرض، والاستراتيجيات الخاصة بكل نوع من الآفات يعد أمرًا حيويًا لضمان إدارة آمنة وفعالة ومستدامة.