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Biochemical Toxic Response of Phosphine on Human Health Estimated From Enzymatic Variance in Trogoderma granarium

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Abstract

The primary purpose of the current study was to study the possible pernicious effects of phosphine gas on enzyme activity alterations in *Trogoderma granarium* to determine its harmfulness to human beings after its prolonged exposure and intake. The saline extract of the adult Khapra beetle was biochemically analyzed at different doses, that is, from 10ppm to 30ppm, to accurately evaluate the effects of various phosphine concentrations (LC_{30} and LC_{50}) on 2 distinct strains of this insect pest gathered from different godowns of Pakistan as resistant (Chitral [Chi], Haroon Abad [Hbd], and Lahore [Lhr]) and susceptible (Faqeer wali [Fqw], Khanewal [Khw], and Rawalpindi [Rwp]) populations. Our experimental results suggest that the enzyme levels (AcP, AkP, ALAT, ASAT, LDH, and ICDH) seemed to be elevated with increasing dosage of phosphine from 10ppm to 30ppm in the resistant populace of the susceptible ones. It also illustrates that phosphine and its residues can inhibit the workability of certain enzymes that are vital for respiration and neuro reactions in hexapods and mammals. It has detrimental effects of phosphine on human health profile to consume stored food products containing such tenacious enemies.

Keywords

alkaline phosphatase, acid phosphatase, alanine aminotransferase, aspartate aminotransferase, phosphine, Trogoderma granarium

Introduction

Khapra beetle, *Trogoderma granarium* Everts, is an ancient, treacherous insect pest of stored grains worldwide.[1] Warehouses (a kind of storehouse for manufactured goods), godowns, and tower silos or metallic containers[2] originated in South Asia, including several countries with huge populations, for example, Pakistan, India, Bangladesh, Afghanistan, and Nepal. It mostly prefers dry, hot, and less humid environments and is thus found from 35° North to 35° South latitude but mainly near the equator. It usually loves to attack starch and protein-rich commodities, including cereals, whole grains, spices, dried seeds, fruits, and gums (made by some plants). The larvae of this beetle are proficient in penetrating through the pericarp (an outer covering or layer of the fruit or seed) and consume all the edible portions of those stored products, causing a substantial dropping in weight and quality of crop reservoirs on a large scale.[3] Furthermore, *T. granarium* has minimal spread ability, and it utterly depends on humanitarian aid and support as it is incapable of flying. Therefore, poor handling negligence in storing and transporting protocols may result in the proliferation of these pests.

For centuries, man has brawled to preserve his crops from various vicious contenders such as insect pests, microbial pathogenic agents, and rodents (eg, rats).[4] For this purpose, he

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has always relied on many pesticides. Metallic phosphides such as zinc, aluminum, calcium, and magnesium phosphide salts are the most common and renowned among those chemicals. These are widely used due to their ease of application (as they come in pellets and powder) and low-cost availability to protect stored grains from insects and rodents and a microbial attack.[4] Moreover, the exposure of these robust and effective phosphides with moisture or water produces a highly toxic gaseous product[5] with a solid garlicky aroma of the phosphine gas. Phosphine has been one of the most impactful candidates to combat insect pests worldwide. Phosphine PH_3 was first discovered in the late 17th century and has been employed as a vital and powerful fumigant. Pesticides having high vapor pressure or volatility rate and thus can be readily invaded through the minute crevices to avoid stored crop pests since the 1930s in the whole world.[3,6] It is being generated naturally (as un marshes) but tends to be degraded into less harmful constituents in the environment.[7] Still, their amount or dose is neither calculated nor inspected in a proper way[1] and that is the reason why PH3 has become a sluggish but savage poison[2] that is eating its consumers steadily for a long time as it serves as a part of their diet, so it should never be overlooked. Phosphine is a powerful nucleophilic reducing agent that can interact and halt the enzymes involved in metabolic processes in different body cells, thus causing severe multi-organ injury and malfunctioning.

New research on rat liver (after phosphine intake by the rat) has claimed that phosphine interferes with the oxygen uptake by the hepato-mitochondria by impeding the function of uncoupling proteins (specific transporter proteins in the inner mitochondrial membranes), hence deregulating the enzymatic levels such as pyruvate malate and succinate dehydrogenase.[8] Additionally, PH3 induces oxidative stress in insects (as in the Khapra beetle) as well as other animals by halting catalase and peroxidase activities (present in the liver to normalize the toxic effects of hydrogen peroxide), and chemically reacting with H2O2 to produce more harmful and reactive free radicals.[5,6] Further, novel histological findings suggest and prove the toxic effects of phosphine on hepatocytes[9] as it is rapidly absorbed throughout the GI tract after ingestion (may directly or along with some grains having its leftover residues); however, partly carried to the liver via the hepatic portal vein.[9] Further, the fat body is the primary site of metabolism in insects, including *T. granarium*, which performs all necessary metabolic tasks in insects as the mammalian liver does. Therefore, the fat body controls and processes essential enzyme levels and functions.

Moreover, the deleterious effects of phosphine have been observed in *T. granarium* species and found that the toxicity of phosphine (gas) changes in different developmental phases as the adult beetles are more prone to its effects as compared to the larval stages (ie, fourth and sixth instar larvae), this may happen because of the elevated enzyme levels in larval stages to run the catabolic as well as anabolic processes in these insects, as the larval stage requires a surplus amount of food and energy to grow and develop.[3] It can be estimated from the previous studies that an unrestrained and blind usage of phosphine (or phosphides) results in an abrupt demise of the Khapra beetle (irrespective of attaining the resistance to overdoses of phosphides) because due to obstruction of detoxifying enzymes by phosphine gas (PH3), the beetle (as *T. granarium*) would not be able to subsist anymore.[10] Hence, the grain products are also supposed to carry the harmful phosphine residues that would be supplied publicly in markets and shops and are more likely to be readily dissolved in the circulatory system of human beings worldwide.[7]

Our present research is entirely based on a new idea to scrutinize the effects of phosphine remnants in stored food products on human liver enzymes and health, assessed from the enzymatic alterations in *T. granarium* Everts.

Material and Methods

Six populations of an orthodox, notorious insect pest *T. granarium* Everts with the familiar name Khapra beetle were collected in sterilized plastic bags from 6 different godowns of Pakistan and used in our present study. The collected populations of *T. granarium* were raised from egg to fourth and sixth instar larvae, and developed in the biochemistry lab of the University of Punjab, Lahore. The crushed wheat grains were used as a helping medium to get the desired and expected consequences. Wheat was fumigated before application to kill the hidden insect pests if any. Furthermore, the whole body of the acclimatized larvae of Khapra beetles was used and homogenized with the help of centrifuging.[11]

Physical Conditions Provided

The fumigated wheat was thoroughly spread in fresh air for about 4 to 5 hours and placed in an oven overnight at 60°C. Moreover, the master cultures of Khapra beetles of both strains were provided with 34 or 36°C temperature and 60 or 70% humidity[3,12] for their better rearing and bred up to 22 generations to meet the experimental needs.

Chemical Fumigant Used

Phosphine gas (produced from Aluminum phosphide) was used in this experiment. Hydrogen Phosphide, phosphorus trihydride, and chemical formula PH_3 or H_3P are alternative names for this chemical.[3] This stored grain insecticide belongs to the inorganic phosphine family, with a 33.998 g/mol molecular weight. Further, its EPA chemical code, IUPAC ID, and PubChem CID are 066 500, Phosphane, and 24 404 [2].

Physical and Chemical Properties

Phosphine is a colorless, flammable, extremely poisonous gas with a pungent odor of rotten egg. It is sparingly soluble in

water (solubility in water, 31.2 mg/100 mL) but utterly soluble in organic solvents like benzene and carbon tetrachloride. It possesses 132.8°C melting point and 87.7°C boiling point along with 1.37 g/L density and 1.1×10^{-5} Pa.s.[2]

Procedure Protocols

The phosphine (gas) generation for sub-lethal LC_{30} and lethal LC_{50} determination was the first step according to the FAO method's techniques.[13] PH3 was generated from commercially available Aluminum phosphide tablets collected over the acidified water. The incubated wheat was shifted into sterilized jars for culture rearing. These jars were one-fourth filled with wheat, and >50 beetles (from different localities) were added inside them. After that, jars were finely covered with a muslin cloth to prevent entry and escape of stray insects and Khapra beetles, respectively. The beetles were then transferred to the following jars after 48 hours for the sound maintenance of the larval age. Further, wheat-containing eggs were placed back in the same earlier vessel, in which the adults were obtained after 5±1 days of hatching used in this study. The glass vials holding prominent clusters of *T. granarium* were placed in the desiccators. Phosphine was then injected with the help of a microsyringe through a rubber septum, fitted on the lid of those desiccators, and were kept at 30±1°C and 65±5% for 20 hours in the laboratory,[1,3] after which the mortality rate was supposed to be examined. The quantitative analysis of the killed beetles was corrected by applying Abbott's formula.

Results

The biochemical analysis of a saline extract of adult Khapra beetle showed the effects of phosphine on the enzymatic activity of the beetle at different doses from 10ppm to 30ppm, observed in various strains collected from discrete areas including Chitral (Chi), Hyderabad (Hbd), Lahore (Lhr), Faqeer wali (Fqw), Khanewal (Khw), and Rawalpindi (Rwp).

Figure 1 shows an increase in AcP activity with 19% at 10ppm and 7% at 20ppm doses that decreases at 30ppm dosage of phosphine with 6% in the resistant population of Chi. Hbd and Lhr populations have shown an upraised AcP enzyme enterprise with prolonged phosphine concentrations from 16 to 38% and 14 to 38% at 10ppm to 30 ppm doses, respectively. Further, AcP level was decreased with 10ppm phosphine treatment but raised from 13 to 24% at 20ppm to 30ppm concentrations in Fqw populace. Khw and Rwp susceptible population masses have also shown an elevated AcP activity at various doses of phosphine (say 10ppm to 20ppm) but reduced at 30ppm in Rwp up to 40%.

An abnormal rate of change in AkP activity is shown in graph Figure 2. The increased activity of AkP in Chi was 6%, 15%, and 2%; in Hbd, it was 17%, 7%, and 23%, while in Lhr resistant population, it was 3%, 1%, and 6% at 10, 20, and 30ppm doses of phosphine compound, respectively. Moreover, Fqw masses of adult susceptible beetles have shown an increased level of AkP with a nice percentage of 6 to 20%. Khw populations showed reduced AkP activity with the least percentage of 3% and 6% at 10ppm and 20 ppm, respectively, while it rose to 13% at the 30ppm dose. However, Rwp has shown up to 6% increase in enzyme activity at 10ppm, 7% elevated at 20ppm, and only 2% enhanced at 30ppm dosage.

Figure 3 represents an increase or decrease in ALAT levels or activity in different batches of Khapra beetles from different places. ALAT activity seems to be augmented in Chi populations up to 8% at 10ppm, which is then supposed to be lowered to 2% at 20ppm, while it again rose to 4% at an enhanced concentration of phosphine 30ppm. Hbd community also showed an elevated level of ALAT from 3 to 13% at 10ppm to 30ppm doses. Lhr population displays a little increase (as 2%) in ALAT activity at 10ppm dose, that improved to 5% at 20ppm and again came to the same level or 2% at

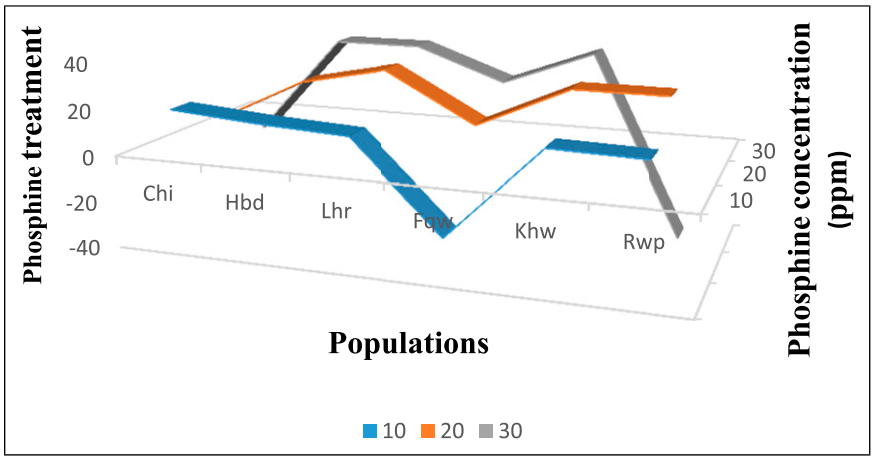

Figure 1. Percent increase or decrease in AcP activity in three adult phosphine resistant and three susceptible populations of T. granarium at 10, 20, and 30ppm dose concentrations is shown in the graph.

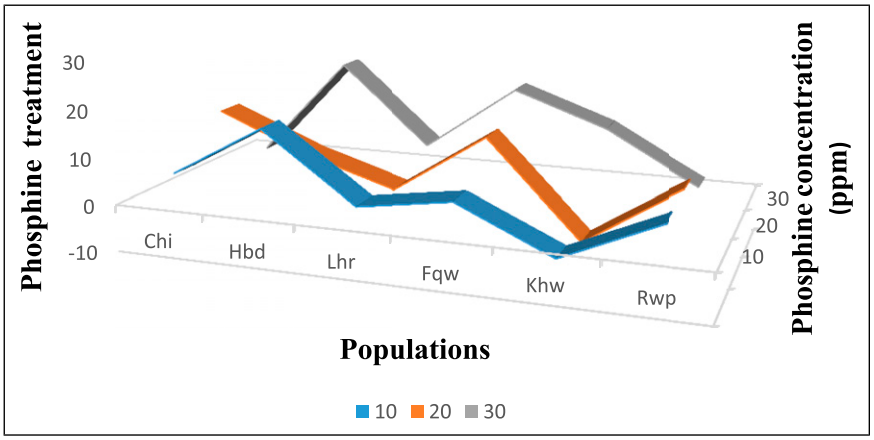

Figure 2. An overall increase or decrease in the percentage of AkP levels in phosphine tolerant and susceptible populations of adult Khapra beetles at 10, 20, and 30ppm is shown.

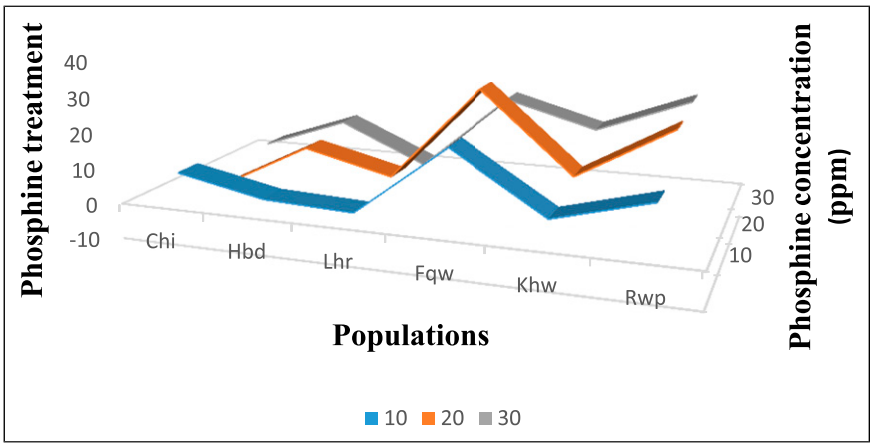

Figure 3. Percent increase or decrease in ALAT activity in phosphine resistant and susceptible strains of adult beetles T. granarium at different concentrations obtained from six discrete areas.

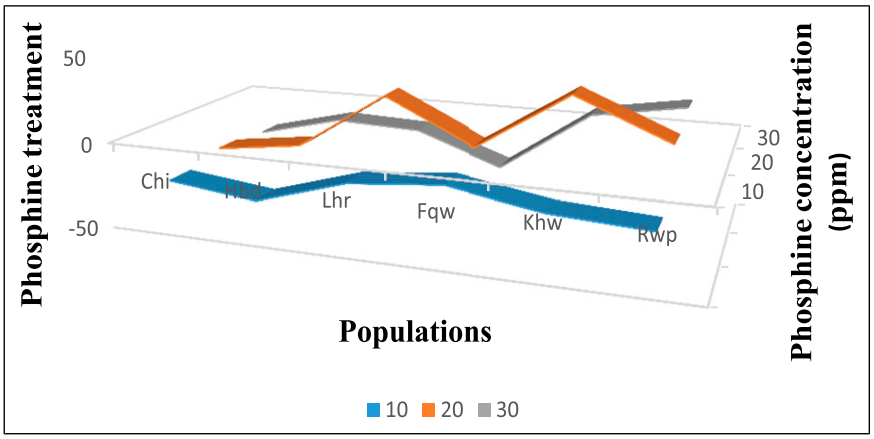

Figure 4. Percent increase or decrease in ASAT activity in phosphine tolerant as well as susceptible adult beetles T. granarium at 10 to 30ppm concentrations collected from different localities.

30ppm concentration. Furthermore, Fqw susceptible populace shows a peak line of enzyme activity with 22 to 32% at 10ppm and 20ppm which again reduced to 24% as the phosphine concentration was elevated (as 30ppm). Khw and Rwp populations exhibit a remarkable increase from 6 to 17% and 13 to 27% at 10ppm to 30ppm concentrations, respectively.

Figure 4 illustrates the ASAT enzyme activities in resistant and susceptible populations of Khapra beetles. Resistant beetle cluster of Chi showed a decreased enzyme activity at 10ppm to 30ppm doses of phosphine treatment. Hbd population has also encapsulated a 30% reduced level of ASAT at 10ppm, 12% at 20ppm, and 9% at 30ppm dose. In Lhr, ASAT performance initially decreased at 10ppm but upraised at 20ppm, followed by a reduction in its activity up to 11% at 30ppm phosphine dosage. Additionally, the susceptible populations of Fqw showed a decreased ASAT level with 9% at 10ppm, 3% at 20ppm, and 30% at 30ppm doses. However, Khw and Rwp susceptible mob of *T. granarium* have shown minimized enzymatic execution at commencing exposure to phosphine with 10ppm. After that, it starts to elevate from 20ppm to 30ppm treatment of phosphine.

Figure 5 encapsulates the dramatic changes in LDH enzyme levels in beetle mass of different regions. LDH level or activity has dropped in Chi populace on treating with 10ppm phosphine which raised at 20ppm and then again lessened at 30ppm up to 1%. Hbd resistant population showed a variable increase in a collapsing manner from 18 to 6% at 10ppm to 30ppm phosphine doses. Lhr beetle mob collection has displayed an enhanced enzymatic activity from 5 to 6% at 10ppm to 20ppm but decreased at 30ppm with 1% only. Further, Fqw populations have shown an uphill LDH activity with 2% at 10ppm, 11% at 20ppm, and then 4% at 30ppm phosphine concentrations. Khw and Rwp populations show a reduction in LDH level and performance with initial dosage of

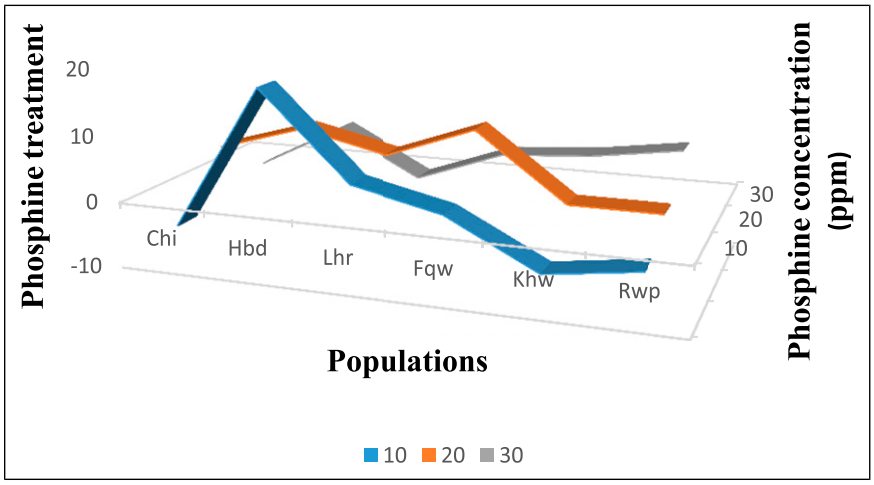

Figure 5. Percent increase or decrease in LDH activity in phosphine resistant and susceptible adult T. granarium strains at different doses gathered from other regions of Pakistan.

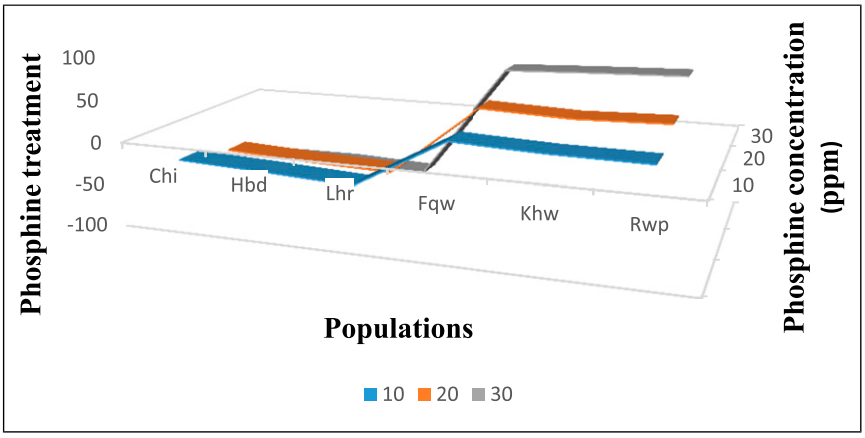

Figure 6. Percent increase or decrease in ICDH activity in adult phosphine tolerant and susceptible beetles a 10, 20, and 30ppm concentrations collected from different areas.

phosphine of 10ppm, which after that increases with an increased phosphine exposure from 20ppm to 30ppm.

Figure 6 presents crystal clear variations in ICDH enzyme activities with an elevated or reduced amount of phosphine compound. ICDH activity seems to be dropped in the resistant batch of *Trogoderma granarium* of Chi, Hbd, and Lhr (as negative percentage values or lower peaks in the above graph betray the %age decrease in enzyme functionality). In addition, Fqw, Khw, and Rwp have manifested an upraised activity of ICDH from 27 to 65%, 23 to 69%, and 22 to 72% with 10ppm to 30ppm phosphine administration, respectively.

Discussion

Metal phosphides (producing phosphine gas) are thought to be the efficient claimants against stored grain insect nemeses causing destabilization of the enzymatic levels in *T. granarium* (Khapra beetle). This notion is quite unique from the preceding work and has not yet been studied in this respect. The primary concern of the current research is to inspect enzymatic activity modulations under the action of phosphine on resistant and susceptible strains of *T. granarium* and raise awareness about the harmful effects of phosphine residues impregnated in the stored goods human health. Further, it may also be helpful to pave new ways to overcome the detrimental impacts of PH_3 remnants in preserved grains in future. Our study results illustrate enzyme alterations in 6 populations of Khapra beetle from different godowns due to phosphine exposure for a specific time interval, that is, from hours to days. Miscellaneous doses or concentrations of Phosphine fumigant were applied in 2 distinct populations of *T. granarium* and a variety of enzyme-level changes were examined.

Resistant Populations

Figures 1 to 6 show increased levels of AcP, AkP, ALAT, ASAT, LDH, and ICDH enzyme activities in the resistant mass of Khapra beetles on average. Gong et al 2013 also support our results that AchE activity enhanced in the tolerant strains compared to the susceptible ones. An increased, unchecked, and continuous utilization of insecticides like phosphine cause an unwanted alteration in the gene pool of an insect food pest thus making it resistant. Moreover, the resistant populace shows a very minute uptake of phosphine than that of the susceptible populations, which is why fatal doses for susceptible beetles do not cause mortality in resistant beetles.[3] Both phosphatases (AcP and AkP) are engaged in the dephosphorylation of other protein molecules and energy transfer. High acid phosphatase activity is vital for the pupal development and reproduction of T. granarium from the larval stages.[14] The decreased activity of these insect enzymes shows their retardation and depletion due to repeated insecticidal treatment. As a result, energy demands are reduced, ultimately aiding insect pest survival in phosphine's rapid and sudden toxic stress.

Further, Figure 2 shows AkP activity with a significant increment at 30ppm for more energy production for proficient respiration and overcoming abrupt stress.[14] Figures 3 and 4 show an induced level of transaminases (ALAT and ASAT) indicating an urgent need of energy supply for the larval stage (as fourth instar larvae) for feeding and development that would be possible by the breakdown of proteins into their building blocks, thus facilitating them to enter in the Kreb's cycle that will be obliging for the insect to get sufficient amount of energy. A notable decrease in LDH activity has been shown in Figure 5 that was found in fourth and sixth instar larvae of Khapra beetle at 30ppm phosphine concentration in Chi, Hbd, and Lhr populace. It is representing the reduced respiration by the blockage of lactate to pyruvate interconversion and hence the insect physiology disturbs; similar results were found by Byrne et al, 2003. It may also happen due to excessive diffusion of phosphine in the hemolymph of resistant beetles. ICDH activity is observed in fourth and sixth instar larvae of tolerant strains of Chi, Hbd, and Lhr shown in Figure 6 at 20ppm and 30ppm stipulating the deactivation of TCA (tricarboxylic acid) cycle due to phosphine.

Susceptible Populations

Figures 1 to 6 represent an exhausting level of enzyme performance in *T. granarium*. Figure 1 shows an induced level of phosphatases (the detoxifying enzymes) indicating an elevated respiratory process, energy production, and consumption by the breakage of phosphate bonds (high energy bonds) in Khapra beetle larval stages of Fqw, Khw, and Rwp susceptible collections to protect them from phosphine toxicity but to a certain limit as they are not tolerant to phosphine gas. Further, Figure 1 shows Fqw population's depleted AcP enzyme activity at 10ppm and Rwp at 30ppm because high concentrations of Phosphine cause a decrease of beetle enzymes and mortality at last, as they are impotent to resist the change like the resistant ones. Figure 2 shows a reduced level of AkP in Khw group at 10ppm and 20ppm. However, Figures 3 and 4 suggest the antagonist activities of transaminases observed in sixth instar larvae that show a time-dependent energy generation during the active period, not in the dormant phase but after that, that is, when the adult beetles need energy for metabolism. It also explained[15] the opposing effects of ALAT and ASAT under the employment of insecticides. Khw shows minimum ALAT performance at all concentrations in Figure 3.

In addition, the enzymatic alterations are entirely dependent on external cues such as physical conditions like temperature, pH, humidity, and even altitude of different localities.

Conceivable Effects of Phosphine on Human Health

Phosphine acts as a silent killer in the human body and has been consumed via food (such as grains and cereals) and the environment for an extended period along with its neglected impacts on human physiology, as the illiterate merchants

highly disregard and deny its death-dealing properties. Phosphine inhalation studies are rarely studied in specific animal models albeit field workers are more prone to its lethal toxicity and thus dragged the attention of researchers nowadays to elucidate the hazardous chemical effects of phosphine. Pulmonary edema, shortness of breath, atelectasis (breathing complications due to collapsing of lung or its part), dizziness, liver enzyme changes, and emphysema are reported in them.[16-18] Other adverse effects include hyperreflexia (overactive reflexes), poly-neuropathy (the damaged peripheral nerves), apprehensive temper, weak attention, and psycho-motor stimulations.[19,20] Additionally, the previously published research provides evidence that phosphine or its metabolites possesses inhibiting effects of certain enzymes that are necessary for respiration as well as neuro-reactions, for example,, cytochrome c oxidase and catalase enzymes which play a key role in oxygen uptake in arthropods and mammals including humans.[21]

Conclusion

It can be apprehended that phosphine disrupts the enzymatic functionality of both strains of Khapra beetle and is likely to devastate human biochemistry and hepatic enzyme activities. It is suggested that future studies must be expanded to explore the harmful effects of many other insecticides used to preserve the stored grains. Additionally, this novel data may also help to understand the mechanism of development of phosphine tolerance in Khapra beetles over time for effective control of these stored grain pests.

Declaration of Conflicting Interests

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