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Mode of Action of Phosphine and Resistance Mechanisms in Insect Pests

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Philippe Gengembre discovered phosphine (PH_3) in the late 1700s, and it has been utilized as a grain fumigant since the 1930s. Phosphine is a poisonous gas that is colourless, odourless, and explosive. When aluminium phosphide, calcium phosphide, or zinc phosphide (solid metal phosphides) pellets come into proximity to moisture in atmosphere, they emit PH_3. It is a fumigant, meaning it is volatile above -88°C and has a density 1.17 times that of air. Phosphine is extremely poisonous to aerobically respiring species but not to anaerobic or dormant organisms. As a result, it can be employed to destroy insect pests in grain while preserving crop viability. Phosphorus oxides, which are absorbed into normal cellular metabolism as phosphate, are the breakdown products of phosphine. PH_3 itself is the toxic form of the element, whereas the more oxidised oxyacids, hypophosphite, phosphite, and phosphate are not toxic. The ease of application, together with its effectiveness, lack of residues, and low cost of the chemical, has resulted in its use in internationally traded grain.

Application of Phosphine

- PH_3 fumigation is done once in 3 months
- Exposure period: 5-7 days or more at 25^0C
- Used for controlling stored grain pests and rodents as target pest species
- Applied usually with CO_2 to limit its flammability and potentiate its action
- Diphosphine (P_2H_4) is known to be spontaneously explosive as compared to phosphine
- Uptake of PH_3 and action in insects require O_2
- Trade name of phosphine is Profume and Ecofume (2% phosphine and 98% CO_2)

Features That Make Phosphine An Ideal Fumigant

- It is effective against all respiring pest species
- Do not leave toxic residues on treated commodities
- Generated in-situ from solid metal phosphide products
- Do not harm viability of seeds
- Rapidly diffused in air due to comparable densities

Electronic Configuration of Phosphine

The electronic configuration of phosphine is $1S^2\ 2S^2\ 2P^6\ 3S^2\ 3P^3$, with 5 valence electrons in the outer orbits. It has 2 lone s-electrons and thus shows nucleophilic properties and is less basic and electronegative atom owing to its size as compared to hydrides of NH_3 i.e., ammonia.

Chemical Chracteristics of Phosphine

A single phosphorus atom and three hydrogen atoms make up the phosphine molecule. As a result, the chemistry of the molecule is dominated by the chemistry of the element phosphorus, which is critical to the molecule's toxicity. Phosphorus is found in biological tissues in its completely oxidized state, phosphate, which is highly thermodynamically preferred over its significantly reduced equivalents, even at physiological pH. Each of the three elements' hydrides, NH3, PH3, and AsH3 (group 15 in the periodic table), are hazardous gases. Three chemical bonds can be actively contributed to by the three p orbital electrons. Phosphorus and arsenic s orbital electrons can also contribute in bond formation.

Symptoms of Phosphine Toxicity

Inhalation of phosphine vapours causes hyper-excitation and hyper- activity in insects. This is followed by lowered metabolic output and the organism becoming lethargic. There is increment in levels of oxidative stress in the cells and tissues of the insect, thus anesthetizing them. Loss of control and coordination, restlessness, paralysis, tachycardia, and whole-body convulsions are primary symptoms visible in insects and rodents.

Mode of Action of Phosphine

Phosphine belongs to IRAC Group 24A: Mitochondrial Complex IV Electron Transport Inhibitors.

- **Neural Mechanism:** Phosphine is known to increase the transmission of the neurotransmitter acetylcholine by inhibiting the action of enzyme acetylcholinesterase. Continuous firing of impulses dur to acetylcholine signaling causes hyper-excitation, paralysis, convulsions, and death.
- **Mitochondrial Action:** Phosphine inhibits the flow of electrons through the Electron transport chain present (ETC) on the inner membrane of mitochondria. Phosphine reduces the cytochrome a and not cytochrome a3 within the ETC.

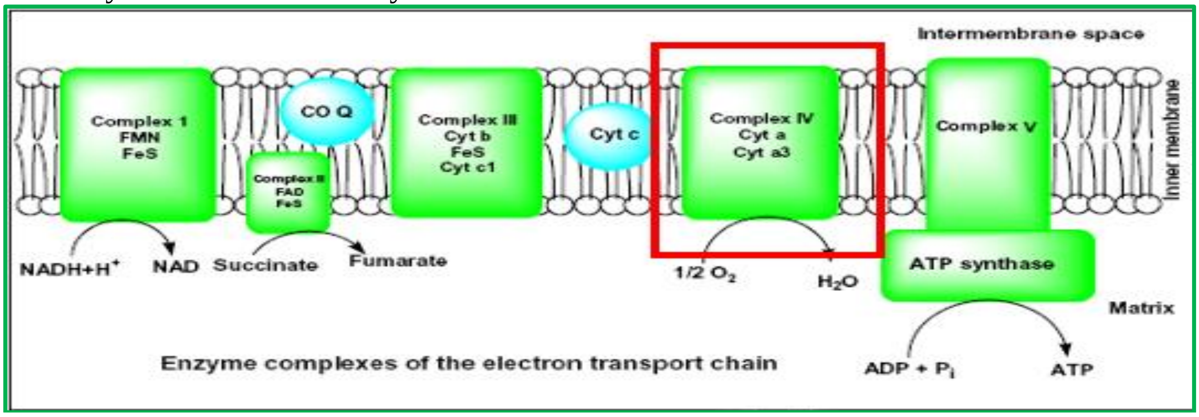

Fig.1: Site of phosphine action in mitochondrial ETC

- **Phosphine Induced ROS Generation:** The inappropriate flow of electrons through ETC generates significant amount of reactive oxygen species (ROS) at complex I and III. Phosphine induced ROS generation causes damage to DNA and macromolecules of cell causing cell death and apoptosis, ultimately leading to mortality of organism due to energy insufficiency.

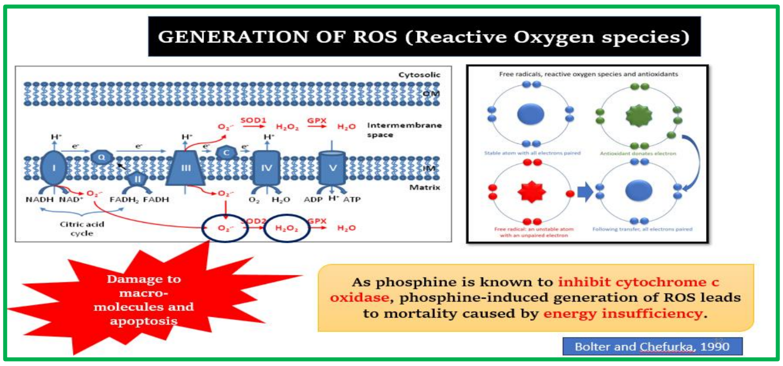

Fig. 2: Phosphine induced generation of ROS by interrupted flow of electrons in ETC

- **Enzyme Inhibition by Phosphine Action:** Phosphine interferes with the active site of the enzymes and inhibits enzyme action. For instance, phosphine inhibits the activity of enzyme Glycerophosphate dehydrogenase, Pyruvate dehydrogenase, Keto glutarate dehydrogenase, Catalase, and peroxidase. This further leads to formation of superoxide and oxidative stress.

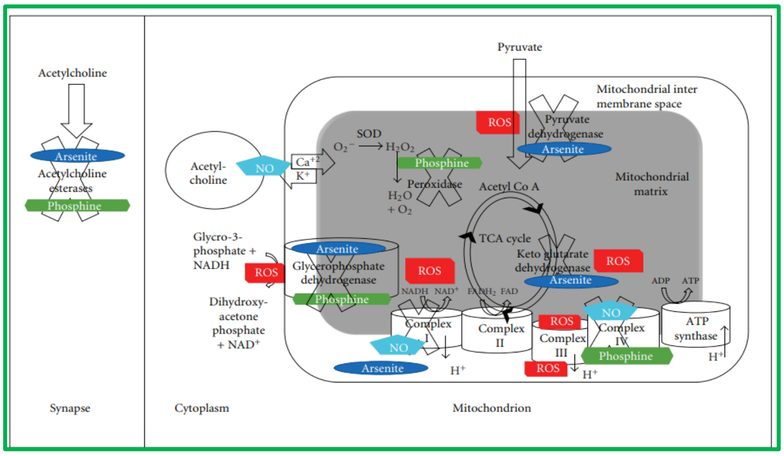

Fig. 3: Summary of phosphine action via neural, metabolic, and mitochondrial action

Glutathione and melatonin are known to act as antioxidants against ROS generated by phosphine.

Resistance to Phosphine in Insects

Over 10% of insect populations sampled showed resistance to phosphine. Repeated under dosing, inadequate exposure periods, no proper gastightness in storage godowns, improper contents and concentrations of fumigants being used, overdose or frequent applications, etc. are the primary causes of phosphine resistance in insects. Resistance to phosphine was first reported by Winks (1969) in *Tribolium castaneum*.

Phosphine Resistance Mechanism in Insects

The mechanisms adopted by insects to suppress phosphine induced mortality are stated below:

- Physiological adaptation
- Suppressed metabolic demands and aerobic respiration
- Decrease in O_2 consumption and respiratory rates- reduced intake
- Generation of strongest protective antioxidant
- Evolution of resistant genes with high frequency (Multigenic)
- Decreased absorption (seven times less gas/gram of insect)
- Active excretion of phosphine by R individuals
- Target site insensitivity (Point mutation and sterility)
- Additional detoxification mechanisms

Genetic Basis of Resistance to Phosphine

Resistance to phosphine is mediated by two genes rph1 and rph2. The gene rph1 is known to produce fatty acid desaturase (FADS) that further acts as a substrate for the ROS generated by inhalation of phosphine gas. The rph2 (Dihydrolipoamide dehydrogenase) produces ROS. If a insect possess homozygous resistant alleles of either of these genes, it is said to have weak resistance, whereas if both the genes are in

Table 1. Species of stored product insects reported to be resistant to phosphine

rice weevil	*Sitophilus oryzae*
granary weevil	*Sitophilus granarius*
lesser grain borer	*Rhyzopertha dominica*
rust-red flour beetle	*Tribolium castaneum*
confused flour beetle	*Tribolium confusum*
Saw-toothed grain beetle	*Oryzaephilus surinamensis*
Khapra beetle	*Trogoderma granarium*
flat grain beetle	*Cryptolestes ferrigineus*
almond moth	*Cadra cautella*
Indian meal moth	*Plodia interpunctella*
cigarette beetle	*Lasioderma serricorne*

Fig. 4: List of insect pests resistant to phosphine action

homozygous state, the insect is said to have strong resistance. The cell membranes of insect become less susceptible to ROS generated, if rph1 is in homozygous state; whereas less of the ROS is generated if the rph2 is in homozygous state; and if both the genes are homozygous, not only less ROS is generated but also the generated ROS is less damaging on the cellular membranes.

Enhancing Phosphine Toxicity to Insects

- Use of mitochondrial uncouplers
- Increased temperature to increase metabolic activity and respiratory uptake
- Extended exposure period (14 days instead of 5-7 days)
- Dry grain fumigation and storage in polyethylene sheet enclosure
- Multiple applications at appropriate time intervals are suggested
- Increasing CO_2 to 14% enhance toxicity through respiratory stimulation
- Use of other potent fumigants as Carbonyl sulfide, Sulfuryl fluoride, Methyl Phosphine in combination with phosphine.

Concluding Remarks

Phosphine is an effective grain fumigant used extensively after ban of methyl bromide in Montreal protocol. However, resistance to phosphine is witnessed in more than 10 species of insects worldwide. To combat phosphine resistance in insects a variety of approaches must be adopted in an integrated manner.

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