

Allyl isothiocyanate

– An alternative biofumigant to manage post-harvest losses

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Allyl isothiocyanate (AITC), a bio-fumigant obtained from Brassicaceae family, is a potential alternative to synthetic pesticides in controlling the fungi, bacteria and insect-pests during storage. LC50 values of AITC against various stored grain insect-pests i.e. Sitophilus oryzae, S. zeamais, Tribolium spp, Rhyzopertha dominica, etc. were reported to be around 0.5 to 5.5 µl/L and the Minimum Inhibitory and Bactericidal Concentration values against different fungi and bacteria were around 60 to 180 µg/mL, thus proving its antimicrobial property. Different innovative formulations like encapsulation and nano-emulsions of this compound have been emerging which makes it more effective and feasible for use. Chemical fumigants were discouraged due to their health impacts to human and environment, AITC can be a better alternative as an eco-friendly biofumigant.

Key words: Allyl isothiocyanate, Biofumigant, Food preservative, Pest management, Post-harvest, Storage

POST-HARVEST losses can be defined as the loss that occurs from harvest of the crop until its consumption. Besides physical or chemical changes in metabolite contents due to ageing, post-harvest losses also occur due to the incidence of insect-pests, mites, rodents, etc. during storage. The food material during storage may be contaminated with microbes and mycotoxins. In India, about 450 million tonnes of raw food materials of plant and animal origin are refined, stored and transformed into usable substances. Out of which, post-harvest losses occur 10% in durables (cereals, oilseeds and pulses), 20% in semi-perishables (vegetables and fruits) and 25% in other commercial products like milk, meat, fish and eggs. Fumigation is one of the universal method employed in reducing the problem of pests and diseases during storage. Fumigation is a process of releasing toxicants (pesticides) in the form of gas or vapour to kill the pests. Various

fumigants are used such as aluminium phosphide, magnesium phosphide, methyl bromide, sulphuryl chloride, carbon dioxide, etc. Among them methyl bromide was popularized worldwide for its broad spectrum efficacy, but it causes severe lung damage and respiratory problems in humans and animals. It is also cited to be an ozone depleting substance. It was banned for applications other than quarantine and pre-shipment purposes by Montreal protocol from 2005. This restriction arose a grave concern for environmentalists and scientists for its suitable alternative which is equally effective against pests as well environment friendly.

Fumigants like metam sodium, dazomet, 1,3-dichloropropene, chloropicrin, phosphine (magnesium phosphide and aluminium phosphide), allyl isothiocyanate, sulfuryl fluoride, carbonyl sulfide, ethyl formate, ozone, hydrogen cyanide and carbon disulphide are different alternatives. Combinations

were also used such as dazomet + chloropicrin, metam sodium + chloropicrin, 1,3-Dichloropropene + Chloropicrin, etc. Among the suggested fumigants, each of them has usage restrictions. Some of them did not have enough efficacy against pests and diseases whereas others were reported to cause health and environmental hazards. Among all the alternatives, Allyl isothiocyanate (AITC), a plant derivative, has proved to be safe and promising against storage pests.

Isothiocyanates are the volatile organosulfur compounds with pungent character produced by the plants belonging to Brassicaceae family (mustard, radish, horseradish, wasabi) having biocidal properties. Chemically, it is a glucosinolate compound and is produced by plants as defensive mechanism against herbivores. When the plant is chewed by the herbivore, the enzyme myrosinase acts on this glucosinolate compound named as sinigrin and releases AITC in presence of water.

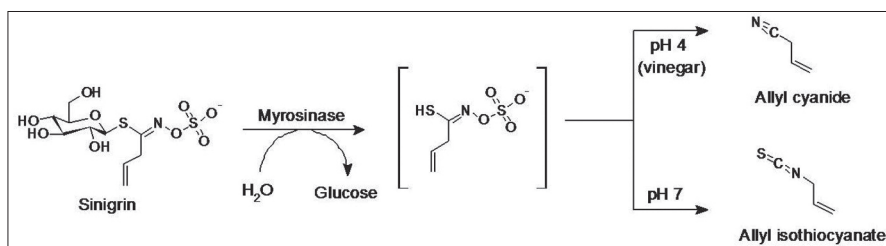
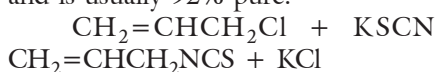


Fig 1. Artificial synthesis of AITC

AITC can also be extracted from dry distillation of mustard seeds. This is also called as volatile oil of mustard. Artificially, AITC is produced by the reaction of allyl chloride and potassium thiocyanate (Fig 1). This is also known as synthetic mustard oil and is usually 92% pure.



Bio-efficacy of AITC

AITC is very effective against gram positive and gram negative bacteria, fungi, yeast, molds, etc. in both liquid and vapour states (Table 1). AITC displayed bactericidal activity at all growth stages of the bacteria when used as a vapour. It disrupts the microbial cell membrane integrity provoking the leakage of cellular metabolites. Studies also demonstrated that AITC could alter protein structures at low concentration. Proteins, such as insulin, bovine, serum, albumin, ovalbumin, and lysozyme, were shown to be attacked by AITC at the disulfide bonds, and thiourea like derivatives were formed at lysine and arginine residues in these proteins. It inhibits the conidial germination and mycelia growth of various fungal pathogens. It also restricts the production of secondary metabolites (mycotoxins) of fungi.

AITC is also recommended against different storage pests (Table 2). Insecticidal mode of action of AITC is not clearly understood. But according to certain researchers, it inhibited the enzymatic activities of glutathione S-transferase (GST), catalase (CAT), cytochrome c oxidase, and acetylcholinesterase (AChE) in few stored grain pests. It is also reported that AITC induces mitochondrial alterations by rarefaction and vacuolization of the mitochondrial matrix, which may act

on the respiratory system. It is believed to reduce the cristae and density of the electron matrix in certain stored grain pests. There are also certain reports, which advocate increased CO₂ expiration from AITC exposed insects, which result in exhaustion of their energy and stored reserves finally leading to their death.

The practical application of AITC by farmers to reduce their crop losses by various pest, diseases and weeds

are given in Table 3.

Advantages of AITC

Natural preservatives are perceived to have lower toxicity, less negative environmental effects, better consumer acceptance. Allyl isothiocyanate is preferred over synthetic chemicals as it is safe for non-target organisms like predators, parasitoids, pollinators, etc. Based on the research conducted, AITC exhibits minimum residual effect in the applied places and environment. It is also advocated by few scientists that development of insect resistance to botanical insecticides is rather a very slow process.

Limitations of AITC

AITC is a highly volatile compound. It easily decomposes,

Table 1. Literature of the antimicrobial efficacy of AITC against various organisms

Organisms against which AITC acts	Result	Remarks
<i>Pseudomonas aeruginosa</i> , <i>P. fluorescens</i> , <i>P. putida</i> , <i>Escherichia coli</i>	MIC-12.5 µg/mL–25 µg/mL	Good bactericidal effect by inducing cell arrest
<i>Salmonella typhimurium</i> and <i>Lysteria monocytogenes</i>	MIC-60-100 µg/mL MBC-120-180 µg/mL at 37°C	At bactericidal concentrations, AITC damages bacterial cell membrane and can also cause inhibitory effects
<i>Botrytis cinerea</i>	MIC=10-40 µg/mL MBC =200-600 µg/mL at 21°C EC ₅₀ (µg/mL) = 1.35 for mycelium and 0.62 for conidia	Conidia is more susceptible to AITC than mycelium
<i>Aspergillus parasiticus</i>	Gaseous AITC ≥ 0.5 µL/L combined with RH=85% aflatoxins production	Completely inhibited the growth of fungus
<i>Aspergillus parasiticus</i> and <i>Fusarium poae</i>	Gaseous AITC at 10 µL/L	Completely inhibited production of Aflatoxins, Beauvericins and Enniatins

*MIC= Minimum inhibitory concentration and, MBC= Minimum bactericidal concentration

Table 2. Fumigant action of AITC over the stored grain pests

Organism against which AITC acts	Result	Remarks
<i>Sitophilus zeamais</i>	LC ₅₀ = 5.69 µg/ml LC ₅₀ = 3.55 to 4.22 µL/L LC ₅₀ = 1.5 µL/L LC ₅₀ = 0.74 µg/L	Transcriptomic alterations of <i>S. zeamais</i> were observed Induced various physiological impairments. Like low rate of emergence. Reduction up to 80.9% in the daily emergence Showed good fumigant toxicity, repellency and progeny inhibition. Inhibited the enzyme activities of cytochrome c oxidase, AChE and CAT
<i>Sitophilus oryzae</i> , <i>Plodia interpunctella</i> , <i>Tribolium confusum</i> (adult and larvae)	LC ₅₀ =2.0, 2.2, 3.7, 4.70 µL/L respectively.	Alterations in mitochondria by reducing the cristae and density of electron matrix.

Table 3. Practical application of AITC

Crop	Location	Result
Commercial tomato	China	AITC used as a soil fumigant and it effectively controlled major bacterial and fungal pathogens, root knot nematode, weeds and improved plant vigor, yield and farmers' income in tomato cultivation under protected agriculture.
<i>Allium tuberosum</i>	China	Proved toxic to all the developmental stages of <i>Bradysia odoriphaga</i> which feed on the underground roots, bulbs, and immature stems of Chinese chive. Adult was comparatively more sensitive to AITC.
Strawberry	USA	Vapours of 0.08 mg/L of Brassica meal derived AITC were applied to strawberry significantly controlled the <i>Botrytis cinerea</i> infection and also extended the shelf life of fruit.
Strawberry	USA	The bio-fumigant proved to be more effective in reducing the population of <i>Macrophomina phaseolina</i> .

owing to direct exposure to heat, humidity, light, or oxygen. This fast decomposition aids in reduction of its efficacy. It is colourless to pale yellow liquid with a very irritating pungent odour. This makes it unacceptable when used at high concentrations in food. When exposed to higher doses it is reported to cause harm to mucous membrane affecting respiratory system and irritant to eyes. Thus to overcome such limitations development of efficient formulations can be a problem-solving step.

Though AITC is a bio-fumigant, there occurs certain short term and long term health hazards if exposed directly to the vapours of this compound:

- Exposure to the vapours causes severe irritation to eyes, throat and nose. It results in runny nose, watery eyes, sneezing, coughing, breathing problems, etc.
- On contact with AITC to the skin for a long time results in blisters

and burns.

- It can also cause certain reproductive system problems by hampering the growth of fetus.

Precautions in application of AITC

Certain precautions should be taken at the workplace:

- Use of good ventilation to reduce the skin and eye exposures.
- Exhaust ventilation should be used.
- Wearing of gloves to avoid skin contact.
- Goggles with a face shield should be worn while working with fumigant.
- AITC should be stored in a cool and good ventilated area (refrigerated) away from heat and moisture.

Novel formulations for better delivery

New formulations of AITC are coming up and this will help in overcoming the limitations:

Microencapsulation of AITC: This capsule formulation helps to

overcome the pungent odour, control volatilization, avoid accidental health hazards and also aid in slow release of the substance as when required in suitable conditions. This makes the ingredients to be available at desired site and time with specific rate. Such sustained release agents significantly helps in improving the efficacy, safety and even reduce environmental pollution.

Nano-emulsions of AITC: It reduced AITC degradation and volatility and were able to extend AITC shelf-life compared with free AITC. Nano-emulsions are fine oil-in-water dispersions, non-equilibrium systems which have a tendency of spontaneous separation into their constituent phases. Nano-emulsions have relatively high kinetic stability due to its small size and steric stabilization between the droplets. The size of the droplet ranges from 10-500 nm.

SUMMARY

AITC, a plant based derivative has high potential as a natural fumigant for pest management and food preservative. This molecule is considerably safe to human and environment. However, the duration of its effect may fall short when compared to chemical pesticides. This may result in higher application rates and higher frequency of usage. But this can be overcome by developing sound formulations which are technically superior and economically feasible.

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Cropping Season and Fertilizer Requirement

To ensure adequate availability of fertilizers, Department of Agriculture and Cooperation (DAC) assesses requirement of major fertilizers before each cropping seasons. The assessment is made based on requirements projected by State Governments/UTs, past consumption, weather conditions, targeted area, cropping pattern, area under irrigation etc.

Most ideally, fertilizer requirements should be assessed on the basis of nutrient needs of the crops and cropping systems in a particular area (block, district, and state) commensurable with existing nutrient deficiencies. This will ensure balanced fertilization and also ensure availability of needed fertilizers, in particular, secondary and micronutrients which very often farmers do not get at right time and right place. IFFCO has made soil testing service robust and performing to ensure adoption of site specific nutrient management. The Government has rightly initiated the scheme of soil health cards which will help farmers to know about soil health.

— Editor