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Fumigation as a quarantine disinfestation treatment of imported germplasm: A case study of cereals

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ABSTRACT

During two decades (1996 to 2015), over 2.3 million germplasm samples and breeding trials of cereals comprising, *Avena sativa* L., *Hordeum vulgare* L., *Oryza sativa* L., *Triticum aestivum* L. and *Zea mays* seeds were imported into India. These were imported through Consultative Group on International Agricultural Research (CGIAR) Centers and from about 60 countries. In addition, 10,772 samples of transgenic planting material of *O. sativa*, *T. aestivum* and *Z. mays* with different genes were also imported. These were processed for quarantine clearance at ICAR-National Bureau of Plant Genetic Resources, New Delhi, a nodal organization to issue the Import Permit for germplasm including transgenics meant for research purposes. The samples were processed for the detection of pests and over 9,000 samples of germplasm were found infested with insect-pests mainly coleopterans [*Sitophilus granarius* L., *S. oryzae* L., *S. zeamais* (Motschulky), *Trogoderma variabile* (Ballian), *Cryptolestes ferrugineus* (Stephens), *Rhizopertha dominica* (Fabricius), *Tribolium castaneum* (Herbst), etc.] and lepidopterans (*Plodia interpunctella* (Hubner), *Sitotrog cerealella* (Fabricius)). Of the 10,772 samples of transgenics, 1,568 samples of *O. sativa* were found infested with different pests. As pest tolerance in quarantine is zero, all the infested samples were salvage using suitable phytosanitary techniques/ treatments. The infested samples were fumigated with a mixture of ethylene dichloride-carbon tetrachloride (EDCT) in the ratio of 3:1 by volume @ 320 mgL⁻¹ for 48 h or 640 mgL⁻¹ for 24 h at 30°C in an airtight fumigation chamber at normal air pressure. Prophylactic fumigation was also given to > 11 lakh samples with EDCT mixture as above. The regulations applicable to the use of fumigants were diligently followed to be in compliance with international agreements. Fumigation used for disinfestation purposes resulted in quarantine security and has effectively prevented the entry and spread of insect-pests of quarantine significance into clean areas.

Key words: Barley, Cereals, Disinfestation, EDCT mixture, Fumigation, Germplasm, Maize, Oat, Quarantine, Rice, Transgenics, Wheat

The basic tenet of plant quarantine is to regulate the movement of plant material to mitigate the associated pest risk to facilitate its pest-free exchange. Plant quarantine is a government endeavor enforced through legislative measures to regulate the introduction of plant material, plant products, soil, living organisms etc. to prevent inadvertent introduction of pests, pathogens and weeds harmful to the agriculture of a country/ state/ region and if introduced, to prevent their establishment and further spread. The pests introduced in an area can be much more devastating than they have

been in their native places. There are several glaring examples of such devastations worldwide (Khetarpal, 2004; Bhalla et al., 2014). Various international and national quarantine regulations are in place to check such spread of pests across geographical boundaries.

The globalization and liberalized trade with the advent of World Trade Organization (WTO) has enhanced the risk of inadvertent movement of dangerous pests along with commodities, which may harm the agriculture and the environment of clean areas. WTO provides for principles to frame and implement the regulations for trade among different countries. WTO-Agreement on Applications of

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Sanitary and Phytosanitary Measures (WTO-SPS) relates to phytosanitation in trade to protect animal or plant life or health within a territory of the member state from the risks arising from the entry, establishment or spread of pests. The International Plant Protection Convention (IPPC), recognized by the WTO under the SPS agreement, frames International Standards for Phytosanitary Measures (ISPMs) (<http://www.ippc.int/IPPC/En/standards.htm>), which provides the guidelines for harmonization of national quarantine regulations for effective implementation of the SPS agreement.

At present, India imports agricultural commodities, germplasm including transgenics as per the provisions of Plant Quarantine (Regulation of Import into India) Order 2003 issued under Destructive Insects and Pests (DIP) Act 1914 (herein after referred to as PQ Order 2003) promulgated by Ministry of Agriculture and Farmers' Welfare (MoA&FW), Government of India (<http://plantquarantineindia.nic.in/PQISMain/Default.aspx>). This is to fulfill India's legal obligation under various International Agreements. The Directorate of Plant Protection, Quarantine and Storage (DPPQS) of MoA & FW is the apex body for implementation of plant quarantine regulations. It has a national network of 57 plant quarantine stations at different airports, seaports and land frontiers. In all, two categories of material are being imported under the PQ Order, 2003: (a) bulk consignments for consumption and sowing/ planting, and (b) samples of germplasm in small quantities for research purposes. The DPPQS undertakes quarantine processing and clearance of bulk consignments for consumption or sowing purposes (<http://plantquarantineindia.nic.in/PQISMain/Default.aspx>). The various schedules of PQ Order, 2003 specify the details of fumigation treatments as a mandatory procedure to be followed during quarantine processing.

Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources (ICAR-NBPGR) is the nodal agency to undertake the quarantine of plant genetic resources and transgenic planting material imported into the country for research purposes. Every year about 80,000 germplasm accessions are imported and processed for quarantine clearance, which involves inspection of material for detection of pests therein and salvaging of the infested material using various treatments/ techniques.

Quarantine treatments demand a very high level of security as the pest tolerance in quarantine is extremely low. Disinfestation is an important quarantine strategy to facilitate movement of plant material in a pest-free state. This involves exclusion of pests using various techniques/ treatments. The kind of disinfestation method to be employed depends upon

the type of material to be disinfested and the nature of disinfestation. Of the various treatments, fumigation is able to provide 100% quarantine security against the seed pests of cereals. It is a unique treatment for disinfestation of space and commodities especially during pre-shipment because a fumigant acts as a gas, penetrates the commodity during fumigation and diffuses out without disturbing the commodity in any way. Fumigation results in complete mortality relatively quickly, leaving no or minimum residue and is thus the most befitting of various quarantine treatments. A fumigant may be a gas (methyl bromide), liquid (ethylene dichloride-carbon tetrachloride mixture), solid (aluminium phosphide) or a crystalline powder (naphthalene) at room temperature, but to be effective it should be highly volatile and with good penetrability. A fumigation schedule generally comprises dosage, concentration of fumigant (C), exposure period/ time (T), temperature and pressure. The specific quarantine fumigation schedules are generally documented for each country. The Clause 3(16) and 3(17) of the PQ Order deal with fumigation, disinfestation/disinfection of consignments imported into the country as specified in the various schedules of the legislation (<http://plantquarantineindia.nic.in/PQISMain/Default.aspx>).

MATERIALS AND METHODS

During the two decades (1996–2015), about 23 Lakh germplasm samples and breeding trials of cereals comprising, *Avena sativa* L., *Hordeum vulgare* L., *Oryza sativa* L., *Triticum aestivum* L. and *Zea mays* L. seeds from different countries/ sources were imported into India (Table 1). In addition, 10,772 samples of transgenic *O. sativa*, *T. aestivum* and *Z. mays* were also imported for research purposes (Table 2). These samples were imported through Consultative Group on International Agricultural Research (CGIAR) Centres viz., International Rice Research Institute (IRRI), the Philippines; International Centre for Maize and Wheat Improvement (CIMMYT), Mexico; International Centre for Agricultural Research in Dry Areas (ICARDA), Syria, and from about 60 countries. All the samples were processed for quarantine clearance and examined visually by naked eye or with magnifying glass/ illuminated magnifier/ stereoscopic binocular for the detection of external symptoms of damage/ insects and stages thereof. As *O. sativa* and *Z. mays* may carry hidden infestation, the suspected samples were subjected to X-ray radiography (Bhalla et al., 2003). The infested samples were disinfested using fumigation with ethylene dichloride-carbon tetrachloride (EDCT) mixture in the ratio of 3:1 by volume @ 320 mgL⁻¹ for 48 h or 640 mgL⁻¹ for 24 h at

Table 3 Insect-pests intercepted against which fumigation was given

Crop	Source/ Country	Interception
<i>Hordeum vulgare</i>	Morocco	<i>Rhizopertha dominica</i> (Fabricius), <i>Tribolium castaneum</i> (Herbst)
	Syrian Arab Republic	<i>R. dominica</i>
	ICARDA, Syria	<i>Lasioderna serricornis</i> (Fabricius)
<i>Oryza sativa</i>	Argentina	<i>R. dominica</i> , <i>Sitotroga cerealella</i> (Olivier)
	Bangladesh	<i>R. dominica</i> , <i>S. cerealella</i> , <i>Sitophilus oryzae</i> (L.), <i>T. castaneum</i>
	Malaysia	<i>R. dominica</i> , <i>S. oryzae</i>
	Nepal	<i>R. dominica</i> , <i>S. cerealella</i> , <i>S. oryzae</i> , <i>T. castaneum</i>
	Philippines	<i>Cryptolestes ferrugineus</i> (Stephens), <i>R. dominica</i> , <i>S. oryzae</i>
	Thailand	<i>L. serricornis</i> , <i>R. dominica</i>
	USA	<i>R. dominica</i> , <i>S. cerealella</i>
	Singapore	<i>C. ferrugineus</i> , <i>R. dominica</i> , <i>S. cerealella</i> , <i>S. oryzae</i> , <i>T. castaneum</i>
<i>Triticum aestivum</i>	Australia	<i>R. dominica</i> , <i>Trogoderma variabile</i> Ballion
	Azerbaijan	<i>R. dominica</i>
	CIMMYT, Mexico	<i>C. ferrugineus</i> , <i>R. dominica</i> , <i>S. oryzae</i> , <i>S. cerealella</i> , <i>T. castaneum</i> , <i>Trogoderma granarium</i> (Everts)
	ICARDA, Syria	<i>R. dominica</i>
	Nepal	<i>S. oryzae</i> , <i>S. zeamais</i> (Motschulsky), <i>T. castaneum</i>
	USA	<i>Sitophilus granarius</i> (L.)
	Serbia	<i>R. dominica</i>
	South Africa	<i>S. cerealella</i>
	Syrian Arab Republic	<i>R. dominica</i>
	USA	<i>S. granarius</i>
	Lebanon	<i>R. dominica</i>
<i>T. durum</i>	Lebanon	<i>R. dominica</i>
	Argentina, CIMMYT (Mexico), Mexico	<i>S. zeamais</i>
<i>Zea mays</i>	Bolivia	<i>S. cerealella</i>
	Colombia	Larval form
	Egypt	<i>S. oryzae</i>
	Indonesia	<i>S. oryzae</i> , <i>S. zeamais</i>
	Philippines	<i>R. dominica</i> , <i>S. zeamais</i> , <i>S. oryzae</i> , <i>S. cerealella</i> , <i>T. castaneum</i>
	Thailand	<i>R. dominica</i> , <i>S. oryzae</i> , <i>S. zeamais</i> , <i>S. cerealella</i> , <i>T. castaneum</i>
	U.S.A	<i>Carpophilus</i> sp., <i>Plodia interpunctella</i> (Hübner), <i>S. oryzae</i> , <i>S. zeamais</i> , <i>T. castaneum</i>

*Pest not yet reported from India; # pest regulated under Plant Quarantine (Regulation of Import into India) Order 2003.

of the most important quarantine treatments due to the penetrability of the fumigants. Methyl bromide (MB), aluminium phosphide and EDCT mixtures are registered fumigants in India to mitigate the pest problem in stored commodities, plants and plant products meant for import and export. MB has been designated as ozone depleting substance (ODS) under the Montreal protocol and has only restricted use in quarantine. EDCT, a liquid fumigant is a mixture of

two chemicals namely, ethylene dichloride-carbon tetrachloride in a ratio of 3:1 by volume. It is used for the disinfestation of cereals, pulses and seeds. However, it is not used for disinfestation of products rich in fat, living plants or vegetable. Earlier workers studied the efficacy of EDCT mixture against various stored product pests and its safety. It is very effective, cheap, non-inflammable, non-explosive, non-injurious to stored commodities and not dangerous to human

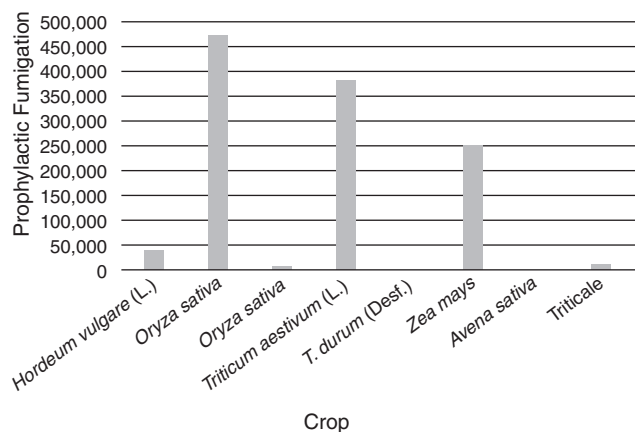


Fig. 2. Prophylactic fumigation given to different cereals against insect infestation

beings. Khalsa et al., 2014) tested EDCT mixture (3:1) against *T. castaneum* (Herbst), *T. granarium* (Everts) and *Latheticus oryzae* (Watrh) in various animal feed and found that animal feed like crushed barley, crushed gram and wheat bran can be effectively disinfested by fumigation with EDCT mixture. However, the order of susceptibility of the three test insects varied considerably. In all the cases, adults and pupae were found to be more susceptible than larvae.

The various pests intercepted are presented in Table 3. Of these, *T. variabile* (Matsush) intercepted in wheat from Australia is most common on cereals and cereal products, such as rice, wheat, maize, barley, oatmeal, pasta and breakfast cereals, but has been found on pulses, fruit and nuts. It is known from Europe, Asia, Australia, Central America, Mexico, Canada and USA where it favours drier areas (Partida and Strong, 1975). Although it has been recorded from Central, South and West Asia, Saudi Arabia, China, Mongolia and the USSR (erstwhile), it is yet not reported from India and is therefore of high quarantine significance. The pest develops at temperatures of 17°–38°C, but does not have the tolerance of hot, dry conditions that is a characteristic of *T. granarium*. Another quarantine pest *Sitophilus granarius* intercepted in wheat from USA is a species reported from India more than a century ago based on records of NHM, London, 1905 with no reports thereafter and is, thus, very important from quarantine viewpoint. It is distributed throughout the temperate regions of the world and in tropical countries it is rare and limited to cool upland areas (CAB International, 2007). The FAO global survey of insecticide susceptibility recorded it from more than 18 countries spanning five continents. All the samples infested with these quarantine pests were salvaged using fumigation (Gupta et al., 2013).

Some of the pests such as *C. ferrugineus*

intercepted in both transgenic and non-transgenic material, are regulated pests under the PQ Order in imports of maize from several countries (Table 3). Likewise, *S. zeamais* intercepted in wheat and maize is a regulated pest under PQ Order. Also, many of the pests intercepted despite being reported from India, pose a quarantine risk during import due to their high economic significance and the possibility of presence of new area specific strains/ biotypes. New strains or biotypes are also included in the category of pests according to the definition of ‘pest’ by IPPC (<http://www.ippc.int/IPP/En/standards.htm>). New strains having greater physiological adaptability pose a higher degree of risk during import.

A quarantine treatment for the elimination of pests associated with plant material is conducted in accordance with phytosanitary regulations of the importing country so as to prevent the dispersal of the pests to the clean areas. The major considerations for any phytosanitary treatment include:

Pest tolerance - theoretically, quarantine pest exclusion implies pest tolerance after a quarantine treatment as ‘Zero’, i.e. the treatment must result in 100% kill of the pest, but practically it is not achievable. Hence, ‘Probit 9’ is the level of kill acceptable in quarantine. The concept of ‘Probit 9’ is not valid for pests which can develop from a single individual. The concept of pest tolerance varies from country to country. Commodity tolerance- as there is very fine margin between the lethal dose for a pest and that causing phytotoxicity in the plant material infested. A quarantine treatment is expected to cause no or minimum acceptable changes in the commodity. Residue tolerance- A quarantine treatment should have no or minimum residue in commodity so that it does not harm human beings, animals or the environment. The residue tolerance for a quarantine treatment is established by maximum residue limits (MRL) prescribed by the Codex Alimentarius Commission, which was recognized by the WTO to develop international standards. Also, a quarantine treatment must not be very time consuming so that it causes only a minimum delay in quarantine clearance of the material (Kapur, 1995; Anonymous, 1998).

There are certain factors that need to be considered while undertaking fumigation. A fumigation schedule generally comprises dosage, concentration of fumigant (C), exposure period/ time (t), temperature and pressure. Concentration Time Product (C·T) is usually constant for a Schedule. After a minimum concentration builds up, exposure period can be adjusted or *vice versa*. Dose as per the schedule is calculated on the basis of volume of the space containing the commodity and not on the

volume of commodity. However, sorption/ uptake of gas by solids in the system, results in a loss of fumigant and necessitates monitoring of concentration and also if required, topping up of the fumigant as and when required during fumigation. Penetrability (capacity of a gas to diffuse through air and commodity) can be improved by fans or blowers, or by vacuum i.e. reducing the air pressure; temperature affects physical absorption of fumigant as well as the insect respiration (Couey and Chew, 1986).

On the basis of pressure at which the fumigation is conducted, it is termed as atmospheric or vacuum fumigation. Atmospheric fumigation is conducted at normal air pressure (NAP) in any airtight enclosure, which can retain a fumigant during the exposure period without appreciable loss through leakage. This can take care of most insects, mites and stages thereof that are external or surface feeders. In the process of vacuum fumigation most of the air in the chamber is removed, requiring a specially designed/ constructed chamber capable of withstanding external pressure up to one atmosphere. This hastens penetration of fumigant through the tightly packed material or the internal infestation. The operation generally takes 2-5 h as compared to atmospheric fumigation, which takes a minimum of about 24 h. Gas leak detectors, viz. Riken gas indicators or thermal conductivity meter, concentration monitoring units, first aid, a self-contained breathing apparatus (SCBA) and anti-dotes should always be kept readily available (Bhalla et al., 2014). The specific quarantine fumigation schedules are generally documented for each country viz., the Plant Quarantine Treatment Manual, USDA (1998) and NSPM-11 (<http://plantquarantineindia.nic.in/PQISMain/Default.aspx>). Any deviation from the prescribed schedule may be hazardous or result in fumigation failure.

REGULATIONS GOVERNING FUMIGATION TREATMENTS

The International Standard for Phytosanitary Measure- 28 (2007) provides for harmonizing the phytosanitary treatments to enhance mutual recognition of treatment efficacy by the National/ Regional Plant Protection Organizations (N/RPPOs) so as to facilitate trade.

As per Clause 3 (17) of the Plant Quarantine (Regulation of Import into India) Order 2003 issued under the Destructive Insects & Pest Act, 1914, all the fumigation, disinfestation or disinfection of the consignment shall be carried out through an agency approved by the Plant Protection Adviser (PPA) to the Government of India under the supervision of an officer

duly authorized by the PPA on his behalf. The various schedules of the PQ Order 2003 specify the details of fumigation treatments as a mandatory procedure to be followed during quarantine processing. The national standard (NSPM-11, 12) promulgated by the DPPQS gives the details on MB fumigation and accreditation of fumigation agencies, responsibility of these agencies like client transport etc. It also specifies the offshore (at the port of shipment) and onshore (at the port of entry) treatments required under the Schedule V and VI of the PQ Order (<http://plantquarantineindia.nic.in/PQISMain/Default.aspx>). The development of national standards is a vital breakthrough for compliance with international standards and facilitates trade (Khetarpal and Gupta, 2006).

The other relevant regulations applicable to the use of fumigants include, (i) The Insecticide Rules, 1971 issued under the Insecticides Act, 1968 as amended in 1999 pertaining to licensing for sale, stock, distribution and use of insecticides including fumigants; manner of labeling, packing, storage and transport; medical examinations, first aid measures, protective clothing, respiratory devices, training of workers in observing safety precautions and handling safety equipments; disposal of used packages; (ii) Gas Cylinder Rules, 1981 issued under the Indian Explosives Act, 1884 applying to specification of cylinders and valves for storing the gases such as MB, storage and transport of cylinders etc; (iii) Rules of the Prevention of Food Adulteration Rules, 1955 issued under the Prevention of Food Adulteration Act, 1954 about the prescribed tolerance limits of residues of insecticides including fumigants; and the relevant Bureau of Indian Standards for code of safety and fumigation practices for aluminum phosphide, MB; welded and seamless steel gas cylinders for MB filling; methyl bromide retention valve; specifications of fumigation covers; gas masks; and package requirements.

CONCLUSION

Disinfestation treatments are instrumental in facilitating the movement of plant material especially under the present liberalized WTO regime, which provides for free trade subject to countries meeting international phytosanitary standards. India can boost its trade prospects by developing disinfestation protocols in line with the international standards. Presently, the most commonly used fumigant in quarantine—MB—is being phased out due to its being designated as an ODS in the Montreal Protocol under WMO/ UNEP, and is to be eventually completely phased out. However, some exceptions such as Quarantine Pre-shipment (QPS) and emergency use of

MB are not regulated under the Montreal Protocol. The use of another important fumigant phosphine is also threatened due to pest resistance concerns. Therefore, efforts are being made the world over to develop alternatives (Kapur et al., 2004). However, use of chemical fumigants cannot be replaced because of their inherent advantages over other treatments. Fumigants are the most convenient, relatively residue-free and quick method of disinfestation which can be applied in case of bulk material and are also economically feasible. But at the same time being non-specific and toxic to all life forms, it is imperative to have trained manpower to handle the chemicals and the equipment taking all safety precautions to overcome their limitations in order to harness the full benefits of this technology. However, search for new/ alternate fumigants is also likely to continue because fumigation has been the most convenient, quick and economically feasible method of disinfestation especially in case of bulk material. There is also a renewed interest in developing safer eco-friendly fumigants (Kapur et al., 2004), which need to be standardized for use as quarantine fumigants.

Fumigation used for disinfestation purposes made available pest-free material to the researchers, is a success story, which has effectively prevented the entry and spread of insect pests of quarantine significance over the past two decades.

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