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Important Insect-Pests of Stored Seed, their Detection and Management

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INTRODUCTION

Seed health testing is a pre-requisite for quality assurance in seed production, supply and finally its success in the field as a healthy crop. Since seed produced in one season is utilised only in the succesive season(s), it passes through various conditions that prevail during the storage period which normally ranges from 3-7 months to more than a year in case of carry over stock and several years for buffer stocks. During storage, abiotic (temperature, humidity, oxygen) and biotic (birds, rodents, insects, microbes, type of seed and its moisture content) factors influence the health of seed. Among the biotic factors, insects play vital role in determining the quality of stored seed.

A study on revalidated seed samples revealed that out of 1.36 lakh samples of 60 crops from different seed testing laboratories in India, 3.43% samples were rejected on account of insect damage (ID) during 1989-90 to 1993-94. The rejection in wheat alone was 5.6% and maximum (18.3%) seed samples were rejected from U. P. [1]. The status of farmer's saved seed was more appalling. In Haryana only 13.2% samples were found free from insect infestation. Around 68% of samples were rejected, when evaluated as per the Indian Minimum Seed Certification Standard [2]. Further, it was found that insects that damage the seed embryo caused maximum loss to seed viability and vigour, as compared to those that damaged the endosperm.

Loss of seed viability based on extent of seed damage

	Normal	Abnormal	Dead	
Embryo	4.1	7.8	88.1	85.3
Endosperm	57.3	6	36.7	816.9
Undamaged	96.6	2.2	1.2	1384

The survey of health of Farmer Saved Seed from different regions conducted under NSP during 1997 to 2007 reveals varying degree of insect damage (ID) to seeds of cereal, oilseeds, pulses etc. [3].

FACTORS AFFECTING SEEDS IN STORE

The most important aspect of preserving seeds for long periods is the maintainance of seed viability and other seed quality attributes above prescribed Indian minimum seed certification standards. Therefore, it is important to understand various factors that directly or indirectly influence seed during storage.

A. ABIOTIC FACTORS

Temperature: This is one of the factors that influence metabolic activities of all biological material. At higher (>20°C) temperature, most of the microbes, mites and insects multiply and infest seed, affecting its health.

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Health status of Farmer saved seed conducted under NSP during 1997 to 2007

Regions	Crops	Storage method	Seed treatment	Moisture %	Germination %	Insect Damage %
Durgapura (Raj)	Wheat, Barley, Bajra	-	-	6-10	75-96	1-5
	Gram, Moth, Mung, Til	-	-	7-10	70-95	2-4
Bangalore (Karnataka)	Paddy, Finger Millet, Redgram, Fieldbean, Castor, Horse gram	-	-	6-7	92-97	-
Coimbatore (TN)	Paddy	Gunny bag lindane dust-few farmers	Malathion/	9-12	84-100	0.5-3
	Groundnut			9-12	80-86	0-1
Bhubaneswar (Orisa)	Paddy	Gunny bag Polythene bag Paddy Straw bin	No treatment	10-12	82-90	1-4
Akola (MS)	Cereals	-	-	8-10	70-89	0-8
	Pulses	-	-	8-10	75-90	0-7
	Cotton	-	-	9-10	75-82	0-1
Rahuri (MS)	Bajra	Gunny bag	Neem Leaves	10-12	67-94	0-3
	Gram	Gunny bag	-	9-12	81-99	0-22
	Wheat	Gunny bag Leaves	Neem	8-12	57-96	0-18
	Soyabean	Gunny bag	-	8-9	70-84	0-0.5
	Jowar	Gunny bag	Neem Leaves	11-12	79-83	6-9
	Mung bean	Gunny bag	-	10-11	85-97	5-12
Pondicherry (TN)	Paddy	Gunny bag	-	12-15	16-96	-
	Red gram	Gunny bag	-	8-12	85-97	-
Kanpur (UP)	Cereals	-	-	10-14	61-91	3-20
Mau (UP)	Cereals	-	-	-	74-88	2-8
	Pulses	-	-	-	78-92	4-80

(Source: Seed Entomology-Ten Years (1997-2007) of Research Work (under NSP)

Standard for Insect Damage (ID) as per Indian Minimum Seed Certification Standard

Class	Crop Seed	Max. visible ID (%) in seed
Foundation and Certified Seed	Maize & Legumes	1.00
	Other Crops	0.50

Thermal requirements of different groups of storage pests

Bio-agents	Temperature (°C)			Moisture content (%) minimum
	Minimum	Maximum	Optimum	
Insects	15-20	33-38	27-37	9.0-11
Mites	5-12	32-36	19-31	—
Fungi	2-5	34-40	19-31	15.4-16.8
Microbes	5-6	50-58	28-35	—

Humidity : Like temperature, humidity is also an important ecological factor that influences longevity of seed and pest activity. Most of the seed under tropical environment deteriorates faster due to high humidity coupled with temperature.

Seed Moisture : Moisture content in seed is a function of RH in the atmosphere, if seeds are stored in permeable containers. And it is the most detrimental factor that directly affects seed deterioration and pest multiplication. The safe moisture content however depends upon storage length, type of storage structure, kind/variety of seed, type of packing material used. For cereals, in ordinary storage conditions for 12-18 months, seed drying up to 10% moisture content appears quite satisfactory. However, for storage in sealed containers, drying up to 5-8 % moisture content depending upon the species, may be necessary.

The moisture content requirement of different important storage insects for their breeding and multiplication

Storage insects	Moisture content (%)	
	Minimum	Maximum
Rice weevil, *S. oryzae*	9.5-11.0	14.0-14.7
Lesser grain borer, *R. dominica*	9.0-11.0	11.0-14.0
Khapra beetle, *T. granarium*	0.0-1.9	11.5
Red rust flour beetle, *T. castaneum*	10.0	11.5-16.0
Rice moth, *C. cephalonica*	9.0	15.0-20.0

Oxygen availability: The role of oxygen in respiration is well known. In an airtight container, the oxygen content of the air is consumed in respiration both by seeds and by the insects, if any. It is estimated that oxygen consumption by insects is 130,000 times higher than that of seeds. Hence, oxygen content depletes faster in insects infested lots than in insect-free lots. Each insect species requires a particular minimum concentration of oxygen for its survival. The minimum concentration of oxygen required by different stages of two species of storage pests has been determined at ICAR-IARI (Table 6). This indicates the role of oxygen in survival and multiplication of storage insects. Seeds remain completely free from insect infestation under vacuum or modified atmosphere where oxygen is <3%.

Minimum concentration of oxygen required by different stages of two species of storage pests

Insect species	Stage	Oxygen content (%)	Reference
Khapra beetle, *Trogoderma granarium*	Egg	16.77	3, Girish, GK 1964 Ph.D. Thesis of IARI, New Delhi
	First instar larvae	5.35	
	Full grown larvae	1.08	
	Adults	3.39	
Red rust flour beetle, *Tribolium castaneum*	Full grown larvae	6.37	4, Sinha, BP, 1965 M.Sc. Thesis of IARI
	Adults	7.24	

B. BIOTIC FACTORS

A large number of biotic agents affect quality of seeds during storage. Most of our knowledge on seed damaging insects is based on those that infest stored grain. Only a dozen insect species cause serious damage in the seeds.

IMPORTANT INSECT-PESTS OF STORED SEEDS

Several insect pests feed on seeds during storage. Seed attracts wide variety of insects that infest seed during the process of its development and maturation on the plants, in threshing yards, during transit and in the store. Storage insect pests also move along with germplasm from one area to another and even to different countries. Adult insects are strong fliers and may spread from one place to another. They may destroy the seed and contaminate the rest. The majority of insect pests belong to the orders Coleoptera (beetles & weevils) and Lepidoptera (moths). These insects can be categorized as major or minor pests, according to the damage to seeds/ grains caused by them and also on the basis of their feeding behaviour.

(a) Primary feeders: These insects mostly lay eggs inside or on the seed and spent a part or entire larval and pupal life inside the seed. They cause significant loss of germination which is not detectable from outside e.g. rice weevil, lesser grain borer, pulse beetle, Angoumois moth etc.

(b) Secondary feeders: This group of insects feed on germ and endosperm from outside. They may attack whole seed and damage the embryo or feed on the seeds directly, if its moisture is high, or on seeds that have already been damaged /infested by other insects. These insects and their different stages are generally visible among the seeds e.g., red rust flour beetle, saw toothed beetle, Indian meal moth, etc.

Damaged seeds result in loss of germination, serious contamination like webbing and ball formation and also inconspicuous deterioration. Other changes such as increased fungal activity, hot spot and moisture migration can also occur. Some insects like pulse beetle infest crop at ripening stage and infestation continues to damage seed during storage.

Some important storage insects are described below:

A. Primary (Internal) feeder

1. Rice weevil (*Sitophilus oryzae*) (*Coleoptera: Curculionidae*)

This is the serious pest of cereal and millet seeds such as paddy, wheat, maize, sorghum etc. and causes both qualitative and quantitative loss during storage. Both larval and adult stages attack seeds and feed voraciously. It can also infest cereal crops at maturing stage in the field. It is distributed all over the world. The adult is a tiny weevil about 2.5 mm long and dark brown or reddish brown in colour. Female lays around 300-400 eggs. Generally only one egg is laid on one seed but two or more eggs can also be seen. Female weevil makes a slit like opening to lay an egg in the hole and plugs it. Under optimum conditions, eggs hatch in about four to five days and the tiny grub bores into seed kernel. As soon as it emerges from egg, it starts feeding on the starchy material of the seeds till it becomes fully-grown and leaves behind only an intact shell filled with frass. The grub stage lasts for 19-34 days and then it pupates. The pupal period lasts for one week under optimum conditions. The adult emerges out after cutting a hole and is ready for breeding. Seed with emergence hole becomes quite hollow and can float in water. At 70% RH and $28^0 \pm 1^0$C, the life cycle is completed within a month.

2. Lesser grain borer (*Rhyzopertha dominica*) (*Coleoptera: Bostrichidae*)

Lesser grain borer is second most important storage insect that spoils seed. This insect is mostly found in warmer region of the world and damages wheat, barley, maize, paddy, sorghum and their products. Both adults and larvae cause serious damage. Heavily attacked seeds are hollowed out and only thin shell remains. Up to four beetles can be present in bigger seeds like maize. It is also a good flier. Adults are smaller than rice weevil and without snout but resemble in colour. It is nearly 2-2.5 mm. long with small triangular head which is deflexed under the thorax. It prefers dark and dingy places. Female lays 300-500 eggs in its lifetime. Eggs are laid either on the seed surface near the embryo end which is soft or easy for young larva to penetrate or interstices of the grain or in other parts of the stores like on cracks and crevices, bags, walls etc. Eggs are white but later on change to pinkish opaque. The newly hatched larva bores straight into the seed. Its incubation period is 4-7 days. It is active and undergoes five moults. Full grown larvae are dirty white with light brown head and curved abdomen covered with tiny hairs. Larval period lasts for 35-40 days. Profuse powdery substance is the characteristic of its damage. Pupal period is for 7-8 days and it completes life cycle in 6-8 weeks. The most destructive stages are larva and adult.

3. Pulse beetle or bruchid (*Callosobruchus maculatus, C. chinensis*) (*Coleoptera: Bruchidae*)

This is an important pest of legume seed both in storage and in the field. It is distributed throughout India. It attacks peas, Bengal gram, pigeonpea, black gram, chickpea, cowpea etc. Larva grows inside the seed, eats its endosperm, and renders it totally damaged. Adults do not feed. Cross-infestation is very common because adults are good fliers. Adults are black, dark brown, 5-6 mm in size. Infestation starts from maturing pods. Females lay about 80-100 eggs and attach them to seed by means of transparent glue like substance. Freshly laid eggs are creamy but become white later on. Incubation period is for 3-7 days. Leg-less larva is cylindrical, fleshy and with wrinkled body. It is white or creamy yellow in colour and remains inside the seed in curved position. Newly hatched larva enters in the seed through the seed coat. After hatching, larval stages develop inside the whole pulse seed. Seed germination is spoiled even during developing stages of beetle. Adults come out of the seed after pushing out a circular lid prepared by prepupal stage of larva. Generally one hole is seen in one seed but it depends on the size of seed also.

C. chinensis is smaller than *C. maculatus* and its colour is less brownish. Habit and nature of damage matches with those of *C. maculatus* but this species can develop in smaller seeds also and more than one emergence holes are present on a damaged seed. Duration of life cycle is also comparatively shorter.

4. Angumois grain moth. (*Sitotroga cereallela*) (*Lepidoptera: Gelechidae*): It is the most destructive pest of stored seed. The initial infestation takes place when the seed is in milk stage in the field and usually a small percentage of seed kernels is infested. By the time seeds are threshed and stored, infestation increases rapidly. In storage the infestation is restricted to upper surface. Early infestation is difficult to detect because hole made by young one is so small that it cannot be seen. Larva enters and eats its way in the seed, then turns about and spins a silken web over the opening by which it enters, thus it is difficult to locate it. The appearance of moths in the stores and round holes on the seed provide the first indication of infestation. Infested seeds are hollow and filled with excreta or webs of larva. Circular opening is for moth's escape. It is found

throughout the world mostly in warm temperate climate. It is serious pest of whole seeds of wheat, paddy, sorghum, pearl millet etc. Germination is seriously affected after infestation. Of all the lepidopteran storage pests, it is the most destructive insect. Adult moth measures 8-10 mm and is a good flier. Its longevity is for 1-2 weeks. Female lays around 120-350 eggs singly or in small clusters on the surface of the seed. Eggs look white at early stage but changes to bright red later on. Full-grown larvae are nearly 5 mm long, white in colour with yellowish head. Larva spins a silken cocoon inside the grain and changes into reddish brown pupa. Before pupation, larvae cut a small circular opening on the husk, for emerging, which is covered by silken cover. Pupal stage is for 7 days. Destructive stage is larva and damaged seed is very light in weight.

B. Secondary (External) feeder

5. Rice moth. (*Corcyra cephalonica*) (Lepidoptera: Pyralidae): It is widely distributed in all rice growing areas of the world and is a serious pest of rice and sorghum. It flourishes well in humid climates and also attacks wheat, maize, barley, millets, soybean and oilseeds. Larva is mainly responsible for damage. Young larva feeds on the broken or damaged seeds bywebbing together the seeds into large lumps. Presence of such webbing leads to loss of consumer preference. Adults are 10-12 mm in length, easily distinguished by closed straight beak pointed forward. Female is larger than male and the adult life is short (7-10 days). Head has tufts of hairs with grayish forewings and creamy hind-wings. Females lay about 200 eggs which are small, oval, white and are laid anywhere on bags, walls, or on grains, Young larva is creamy white with prominent light brownish yellow head. Larva webs silken shelter which soon gets densely covered with broken grain and frass. Larval life is for 4-5 weeks after that it pupates inside the silken cocoon for 8-10 days.

6. Red rust flour beetle. (*Tribolium castaneum*) (Coleoptera: Tenebrionidae): Cosmopolitan in distribution, it is the worst pest of flourmills. It feeds on broken grain flour, starchy material, fruit, nuts, millets and prepared cereal foods. Infested material emits sour and pungent smell, which is due to some secretions of beetles. Destructive stages are adult and larva. Adults are flat brown, 5-6 mm in length and reddish brown in colour. Head, thorax, and abdomen are distinct and antennae are well developed, of which the last segment is abruptly much larger than preceding ones. Neither adult nor larvae can usually damage sound grains. Individual female lays 400-450 eggs in its whole life. Eggs are sticky and are laid on the grain/ debris. They are small, cylindrical and of wheatish colour. Incubation period is 5-10 days. Larvae are very active and pupate after 3-4 weeks. Pupation takes place on the grain surface, which lasts for 6-8 days. Adult has 4-5 months longevity and feeds throughout the life.

SOURCES OF INFESTATION

- **Field**: Some of the insects like bruchids, *Sitophilus oryzae, Sitotroga cerealella* infest seed crops at reproductive stage in the field. They come along with the harvested produce and multiply during pre-storage or storage period. The infestation is normally detected at the time of emergence of adults.

- **Godowns**: Insects or its stage(s) hiding in the cracks and crevices, electrical fittings etc. are the major source of infestation in the godowns.

- **Old gunny bags/receptacles/containers:** Insects or its stage(s) hide in the weavings, seam or corners; infest the seed when stored in such contaminated bags/containers.

- **Vehicle:** Vehicles are another source of infestation when in regular use. Now a days, containers are used for transporting large quantity of bagged seed for exports. Such containers need thorough cleaning and treatment to kill insects in it.

MONITORING AND DETECTION OF INSECT INFESTATION

Detection of insect infestation in food grains helps in quality control and compliance of Prevention of Food Adulteration (PFA) Act (4). Many of these techniques are equally relevant in detection of insect infestation in seed to ensure supply of high quality and healthy seed to the farmers. It also serves as an early warning for taking appropriate control measures. Delay in detection may result in pest outbreaks, causing severe contamination of seed materials and quantitative loss. It also helps in assessment of effectiveness of fumigation and other pesticide treatments.

A. Monitoring of Insect Infestation in Seed Godowns

1. Visual Inspection: It includes inspection of the godown for live, flying or crawling insects during spring, summer or rainy seasons. Detection of live insects or its castings in sweeps and presence of flour deposits on bags caused by lesser grain borer are the indication of insect infestation. Presence of web in an undisturbed area is sign of lepidopteran infestation.

2. Light traps: Most insects are nocturnal and phototropic. Light traps detect presence of insects and its build up. Light traps with an electrocution net kill insects that are attracted to it and help in controlling the adult insects. The use of a 4W ultraviolet light (peak emission at 250 nm) set at 1.5 m above ground level in the alleyways and corners of godowns accurately detected the presence of *R. dominica* (5).

3. Sticky traps: These help in early detection of insects, especially in the godown or bin headspaces and help in early prediction of infestation (6).

4. Traps for crawling Insects: It provides a hiding place and is available in various designs. It can be used with pheromone-lures for specific insects or food baits to enhance capture of multiple species.

5. Pheromone traps: Unlike light traps, these traps are baited with a synthetic chemical which influences insect's behaviour. These chemicals are species specific and help in better monitoring of particular insect pests. Traps have also been found very effective in early detection of insects at low population levels. Pheromone traps are now available with adhesive glue to which insects get stuck thus, helping in removing a proportion of the population (mass trapping).

B. Detection of insect infestation in seed lots

1. Quantitative sampling

The aim of drawing random samples is to determine the mean value and the variability of the level of infestation or contamination in the seed lot. Ashman (7) devised a tentative "sequel sampling" procedure, involving collecting a number of spear samples from several bags at random and then examine by sieving. It does not account for hidden infestation in the kernels. The

List of synthetic pheromones of major storage insects for monitoring (M) or mass trapping (MT)

Storage insect	Scientific name	Main host	Pheromone component	Purpose
Khapra beetle	*Trogoderma*	Stored *granarium*	Z, E-methyl 8-hexadecenal (92:8) wheat	M/MT
Pulse beetle	*Callosobruchus chinensis*	Stored pulses	'Erection', a mixture of hydrocarbons, dicarboxylic acid	M/MT
Lesser grain borer	*Rhyzopertha dominica*	Stored cereal	Dominicalure 1: (S)-(+)-1-methyl butyl (E)-2, butyl (E)-2, 4-dimethyl-2-pentenoate	M/MT
Grain moth	*Sitotroga cerealella*	Stored cereal seeds	(Z.E.) 7,11- hexadecadienyl acetate	M/MT
Indian meal moth	*Plodia interpuctella*	Stored cereal seed	(Z.E.) 9,12 – tetra decadienyl acetate	M/MT

sample number should not be less than the **square root of the total number of bags** until a 1 kg sample is obtained, and examined for insects by sieving.

2. Direct examination

Seeds are examined in the dry state with the help of a magnifying glass (10X) or stereoscopic microscope aided with light. Two replicates samples of 200 seeds each are visually examined. Live and dead adult weevils, beetles, moth larvae, grubs etc. are separated and counted. They are recorded as number of insects including all stages per weight of the sample.

Insect-damaged seeds are separated and counted including those whose germ (embryo) has been scratched or eaten or have escape hole (s) or eggs adhered to them. Other seeds with no visible symptoms of insect injury are subjected to further tests to detect internal infestation.

3. Detection of internal infestation in seed

The number of internally infested seeds is added to the number of seeds found externally damaged by insect for final calculation. Special techniques to detect internal infestation are employed such as:

1. Dissection method: The seed is cut open or dissected or cracked with or without soaking in water to reveal internal infestation.

2. Alkali or glycerine method: Seeds are submerged in 10% solution of NaOH and boiled for 10 minutes or more depending upon type of seed. After decanting the NaOH solution, seeds are washed with water. The translucent seeds are then examined with a magnifying glass. Those with visible internal infestation are cut open to confirm infestation. Alternatively, seeds can also be made translucent in lactophenol (dissolve 20 g phenol crystal in 20 ml lukewarm distilled water, and then add 20 ml lactic acid and 10 ml glycerine) solution and follow the above mentioned steps.

3. Floatation method: This is also called as speciûc gravity method for detecting hidden infestation in whole seed. The density between sound (un-infested) and infested seed is exploited for the detection of infestation using salt solutions.

Accordingly, when seed sample is immersed in a salt solution of appropriate density (normally 1.19 g/cm^3), for about 10 minutes, the heavier un-infested seeds sink to the bottom while the lightly infested seeds ûoat. It can be used for detecting internal infestation in cereals and pulses seeds. The salt solution consists of sodium silicate in water with specific gravity of 1.16, to which methyl chloroform is added after adjusting its specific gravity to 1.30 with deobase oil. When the above solution is placed in a measuring cylinder, a distinct separation layer is formed between the two liquids, the lighter (sodium silicate) solution remain on the top. Seeds containing later stages of weevil larvae float on top of the sodium silicate solution, whereas seeds containing early stages of larvae or light weight seed float at interface of the two liquids. Non-infested, normal seed will sink to the bottom in methyl chloroform solution. The degree of infestation is estimated by the relation between number of floaters and size (number) of sample.

Floatation method for insect-hole bearing seeds: The seed sample is placed in 2% ferric nitrate solution (dissolve 2 g hydrated ferric nitrate in 100 ml water and stir for 30 seconds). Seeds with an insect emergence hole will float while the rest will sink to the bottom. The number of floaters can be counted and infestation can be worked out. This technique is suitable for determining infestation in pulse seed.

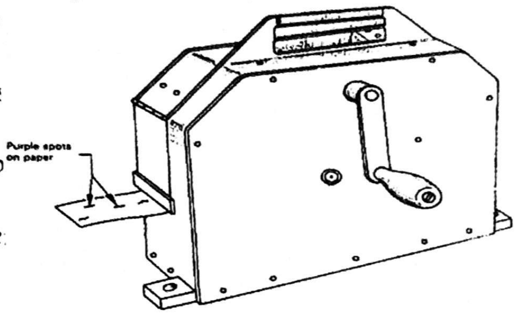
Floatation separation by air : By progressively increasing the intensity of air stream by fan in vertical column, all insect-damaged kernels can be blown out in the first two fractions, from which no emergence had occurred. The detection of insect-damaged seed (i.e. those containing exit holes) can be a relatively quick and efficient procedure in commercial samples by a factor of ten or better.

Floatation method is a qualitative test and does not indicate the insect species or its life stage. The method is simple and quick. Low weight, shrivelled seeds will also ûoat with the infested seed during the test. Hence, it requires confirmation by dissecting the ûoating seed. Seeds with eggs or early larval stages cannot be detected because it will not ûoat.

4. Staining methods

Staining, a chemical indicator technique is a direct method of establishing hidden living infestation in the seed. There are three types of Staining techniques,

The mucilaginous secretions of weevils (*Sitophilus* sp.) are stained with a suitable chemical compound. Weevils deposit their eggs inside the seed and plug th
with saliva. Using suitable
egg plugs in grains can be
The extent of infestation in
by the number of egg p
technique is not applicable
as *R. dominica* and *S. ce*
outside the grain.



The ninhydrin method: The
ninhydrin (triketohydrinden
detection of live insects in
insect body fluid comes
ninhydrin-impregnated filte
"purple" colour.

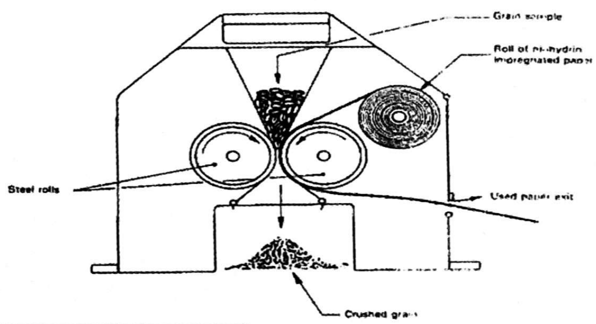


Table : **Detection of Insect**
Techniques

Chemicals used	Color spot	Reference
1. Speciûc for weevil egg plugs in grains		
Acid fuchsin	Cherry red	(8)
Gentian violet	Purple	(9)
Berberine sulfate	Greenish yellow	(10)
2. Speciûc for entry holes in pulses		
Iodine-potassium iodide	Black	(8)
3. General infestation in whole seed/grains		
Ninhydrin	Purple	(11, 12)

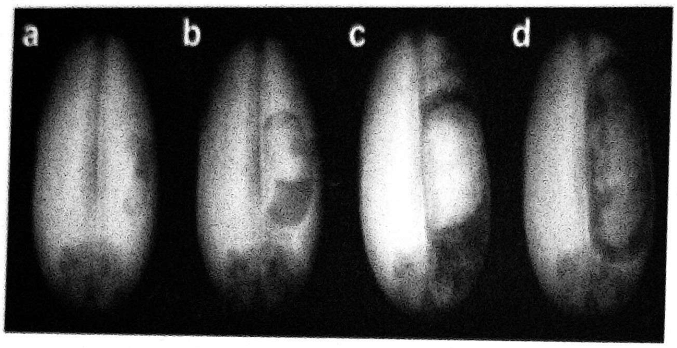
In the United Kingdom, a small portable machine known as the "Ashman-Simon (8) infestation detector" was developed. The counted numbers of seeds are fed into the machine. They pass into the folds of filter paper impregnated with 0.7% ninhydrin acetone solution, and are crushed when they pass through the rollers. The body fluid of insects within seeds oozes out that reacts with the chemical forming purple spots on the tape. Sometimes mild heating is required to develop clear spots. The machine operates at a speed of 45 cm or 300 kernels per minutes and it is claimed that it detects 5-10% of eggs and early larvae, 40-60% of middle age larvae, and
vae in cereals

drin detection of hidden infestation

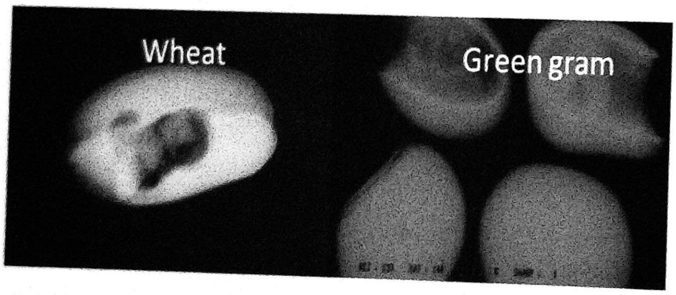
infestation detector"

5. X-ray method

This is an accurate, non-invasive and rapid method of detecting internal insect infestation in seed sample [13]. The healthy seeds can be retrieved from the valuable seed lot. An X-ray machine operates at about 50 KW. Exposure period varies from seed to seed to get a good radiograph which reveals the presence of any insect inside the kernel. It is being extensively used by different countries, especially for quarantine purposes. X-ray manufacturers have developed a compact and suitable unit for this purpose such as X-ray Softex SB-40 manufactured by M/Nissei Commerce Ltd., Tokyo, Japan.



X-ray images of wheat seed infested by different stages of granary weevil (*Sitophilus granaries*): small larva (a), medium larva (b), large larva (c) and pupae (d).

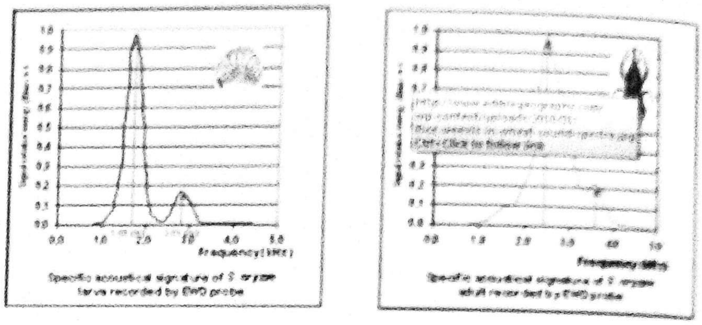


6. Acoustic detection system

An automated acoustic (Sound) detection system that detects the insects in a seed sample by analyzing the spatial and temporal distribution of sounds. The acoustic location-fixing insect detector is an automated system to quantify hidden infestation in 1 kg seed samples. It analyses input from an array of sensors embedded in the sample container walls. It identifies a specific gnawing sound pattern of an insect [14]. The rate of sound detection is inversely proportion to weight of products. Thus, to reliably count insects with varying sound production patterns, the sound production identification needs to be calibrated.

Sound spectra of rice weevil grub and adult in wheat

By NICOLA (14)



7. Carbon dioxide method

The method is based on the fact that insect's activity in the seed lot produces carbon dioxide (CO_2). The extent of internal infestation can be estimated by measuring the quantity of carbon dioxide produced in a given seed sample in 24 h.

Methods		
Gasometric (% CO_2 v/v)	Infrared (ul of CO_2/min)	Level of infestation
<0.2	<1.0	Nil or negligible
0.2	1.0	Low level
0.3-0.5	2.0-3.0	Light to moderate
0.6-0.9	4.0-6.0	Moderate to heavy
>1.0	>6.0	Heavy

Pest infestation levels in seeds according to the different methods of instrumental analysis of CO_2 in 1 kg of material after 24 h incubation is as follows:

This method not only requires long time but high moisture content of seed also interferes with the insect's respiration and its rate (15). It also fails to indicate the presence of dead insects inside the seed.

8. Breeding out

Grain suspected of being infested may be incubated thus allowing insects to complete their life cycle.

The biggest disadvantage is the time factor since even under the optimum conditions of temperature and moisture content (26-30°C and 14-16% m.c.), at least 4-6 weeks will be required to breed out the full population of grain weevils and even longer incubation periods will be required for many other storage insect species

MANAGEMENT OF STORAGE INSECTS

There are several effective methods available for the management of storage insect pests. The Integrated Pest Management (IPM), a blend of proven tactics should be relied as the most effective method. Some of the methods such as use of modified atmosphere, particle film, inert dust, edible and non-edible oils, various physical methods such as use of cold and hot air, Micro wave (MW)/Radio wave (RW) treatments etc. have also been reported very effective. But above all the methods, a good management of seed godown, its inventries and stocks are the major components of IPM.

The good storage practices as preventive measures:

It requires maintenance of storage facilities to an adequate standard and efficient control and handling of stocks. Regular and critical inspection of stores and stocks should be done to maintain good storage hygiene. And finally use of the chemicals should be the last option. The pre-storage preventive measures includes two components such as-

Preparation of Seed stocks:

- Seed stock should be clean, free from broken or damaged seed.
- Dry seed (moisture content <10%) should be stored.
- Pulse seeds carry insect infestation from field and it is detected when adult insects emerge from the seed during pre-processing stage. Therefore, pulse seed should be dried in sun to kill all internal infestation or should be fumigated immediately to avoid insect multiplication inside the go down.
- Ensure new harvest do not carry field infestation in other crop seeds as well. Fumigate if live insect is detected.

Preparation of Seed Store/Shed:

- Clean and repair all the structures, disinfect with malathion 50%EC (dilution 1:100 in water) @ 3L solution per 100 sq. m. on concrete floor using knapsack sprayer. Wet all surface like wall floor, and fill crack, crevices to kill hiding insects. In case of lepidopteran (moth) insects, spray dichlorvous (DDVP 76%EC) (dilution 1:150 in water) @ 3L solution per 100 sq. m. Since dichlorvous has contact cum fumigant action, due precaution should be taken while spraying in the godown. Also paste paper on windows and ventilators for effective treatment.
- Treat old bags with hot water (>50 °C) for 15 minutes and dry them before use or treat it with malathion @ 10 ml/l water or deltamethrin @ 2 ml/l water per 20 m^2 bag surface area or fumigate.

After adopting various preventive measures, insect pests do infest stored seed and affect its health. To ensure insect free storage system, various options are available for the management of storage insects. They can be used individually or in combination in most appropriate manner to keep the insect damage (ID) below Indian Minimum Seed Certification Standard. These options are described in brief as below:

Thermal regulation

Temperature control is widely used in post-harvest to slow down development of pathogens and insects. For control of insects, both high and low temperatures are reported effective. Temperature, rate of temperature change, and duration of exposure are contributing factors. Extreme temperatures are very effective against most of the storage insects; it may also cast effect on the heat sensitive crop seeds. Therefore, its use in seed sanitization may remain restricted to those crop seeds that tolerate high temperatures and their germination and vigour are not influenced. However, heat treatment for short period such as solar heat may prove helpful over high temperature for long spell of time.

The Response of Stored-Product Insects to Temperature (16)

Stage	Temp. zone (°C)	Effects
Lethal	> 62	death in less than 1 min
	50 to 62	death in less than 1 h
	45 to 50	death in less than 1 d
	35 to 42	populations die out
Sub-optimum	35	development stops
	33 to 35	slow development
Optimum	25 to 33	maximum rate of development
Sub-optimum	20 to 25	slow development
	13 to 20	development slow or stops
Lethal	03 to 13	death in days (un-acclimatized) and movement stops
	-10 to -5	death in weeks to months if acclimatized
	-25 to -15	death in minutes, insects freeze

1. Species, stages of insect development and moisture content of material will influence the response to temperature; d= days. Temperature above 45 °C may be injurious to seeds.

Solar Heating

A solar heater made of dark cloth and translucent plastic sheet has been tested for control of pulse beetle. The solar heater temperature reaches >60 °C. All stages of the cowpea beetle, *Callosobruchus maculatus*, inside the seed are destroyed at temperature >60æ% C within around two h. All stages of *Callosobruchus* spp. were found killed when pigeon pea, *Cajanus cajan*, was solarised in polyethylene bags in India (17). Solar heating raised the temperature in seed bags (polythene) to 65°C each day. Solarised pigeon pea seeds remained free from bruchid damage even after 41 weeks of storage. On the contrary untreated control had up to 91% bruchid damaged seeds and their germination was reduced to 42%. No reduction in seed germination was observed in the solar-heated seeds. Solarisation is a safe and relatively inexpensive method for disinfesting seeds of pigeon pea.

Cold Storage

Low sub-optimum temperatures can slow down the damage from mold and insects, and can increase the shelf life of the commodities. Insects have a narrower band of suboptimum temperatures. Generally, seed held between 13°C and 20°C will slow, but not stop, the development of most of storage insects. The susceptibility of insects to lethal cold temperatures varies greatly between species and life stages, and is often dependent upon factors such as temperature, length of exposure and sex. In general, species that are most easily controlled by cold temperatures include *Tribolium castaneum*, and *Oryzaephilus spp.*, whereas *Trogoderma granarium, Sitophilus oryzae, Ephestia kuehniella* and *Plodia interpunctella*. The most susceptible life stage is typically the egg, which can be killed by exposure to 10°C for two weeks. Acclimatization appears to be very important factor in increasing the survival of insects at low temperatures.

Inert Dusts

Inert dust particularly use of sand, cow dung ash etc. in wheat storage has been in use since time immemorial in many parts of India. During the last two decades lot of work has been done on its use, resulting in commercial use of several inert dust formulations. There are many kinds of inert dusts such as lime, common salt, sand, kaolin, paddy husk ash, wood ash, clays, diatomaceous earths (90% SiO_2), synthetic and precipitated silicates (98% SiO_2), and silica aerogels (18). Because of their low mammalian toxicity, they are used to protect stored grains against a number of coleopteran pests.

Rice husk ash (RHA) was found to be effective against pulse beetles that attack stored legume seeds. Mixing 1% in weight of the ash in the seeds before storage effectively protects the seeds from pest infestation.

In the multi-location trials conducted under National Seed Programme (Seed Technology Research) good control of storage insects with diatomaceous earths (DE) @ 5 g/kg seed was obtained.

Oils

Many edible and non-edible oils such as mustard, ground nut, neem, castor, karanj etc. have been used to control storage insect pests particularly pulse beetles successfully. The oils act primarily at contact sites by obstruction of the respiratory system (hypoxia) and also as an oviposition repellent, because eggs do not adhere on the surface of grain. However, dose requirement of oils for seed is different from the grains. Under National Seed Programme (Seed Technology Research) edible and non edible oils viz. neem, karanj, Indian mustard, groundnut and castor were tested against pulse beetle infesting different pulse seeds during nineties. All the oils were effective against pulse beetle infestation but different pulses have shown varying degree of susceptibility to oil treatment. Chickpea was most tolerant and 10 ml oil/kg of seed did not show any deleterious effect on its germination and vigour. Green gram and pigeon pea were found susceptible to oil treatment and 2.5ml/kg seed dose was found safe. Sometimes, varietals variation in response to oil treatment was observed. Therefore, while using oil, care should be taken to test it because crop, variety, moisture content, type of oil, its dosage and ambient conditions are important factors that influence efficacy of oils and safety to seed.

Mechanical Disinfestations

Mechanical seed processing removes large fraction of insects, insect infested and diseased seeds from seed lots. Of different machines, screen-grading machines separate out all seeds which are broken or damaged by insects in the field, besides removing live insects and some of its stages. The gravity separator is extensively used to remove internally infested seed where the >25% of endosperm has been eaten away by the insect. Similarly, removal of cut seed or damaged seed reduces infestation by secondary feeders.

Controlled Condition Seed Storage

It is known that the life span of seed is influenced by storage conditions specifically the temperature and relative humidity (RH). Harrington rules stated that the life of the seed is halved (1) for each 5° C increase in seed storage temperature and (2) for each 1-per cent increase in seed moisture content (19). In addition to it, the arithmetic sum of storage temperature in degree Fahrenheit and the RH per cent should be around 100. It holds well only in the range of 5 to 14 % seed moisture. The hot and humid environment is congenial for the activity and growth of microorganisms and insects, which can diminish or destroy the seed quality rapidly. Hence, the seed quality preservation for longer time can be achieved by lowering the temperature and RH of store. The requirement of controlled condition seed store arises when the ambient conditions preclude the use of open store, particularly if ambient temperature & RH are more than 300C and 70%, respectively for significant period. Cold storage technology is suitable in seed industries where low volume high value seeds are produced and stored for medium term storage. In high volume storage of wheat, paddy such facility would be uneconomical. Cold storage technique is normally used in combination with dehumidifier to create control atmosphere such as 20° C+ 40% RH for better storage of carry over stock (for 1-2 years) or 15° C + 30% RH for storage of vegetable and nucleus seeds for 3-5 years period.

Seed Storage under Modified Atmosphere (MA)

The use and manipulation of natural components of the atmosphere, e.g. oxygen, nitrogen and carbon dioxide, to preserve seeds, food grains and food products is known as "MODIFIED" or "ALTERED" atmosphere storage. The normal gases of the atmosphere can be changed to achieve control of storage pests

Modified atmosphere systems depend on either depletion of oxygen to suffocate the organisms or the addition of carbon dioxide to act directly and kill them. In these treatments the new atmospheres are maintained for an adequate period of time to kill all stages of the organism. MA should have no adverse effect on the commodity. In many respects the requirements for carbon dioxide rich MA application for insect control are closely related to chemical fumigation because it requires gas-tight enclosures capable of holding the gas at required concentration for a particular period. Various factors like abiotic factors (temperature and humidity), insect species and their stages, dosages and exposure period etc. influence carbon dioxide toxicity/efficacy. Carbon dioxide is also reported to increase toxicity of MBr and PH_3 to insects.

(1) The carbon dioxide gas is applied to storages structure from a vessel of liquid carbon dioxide with appropriate vaporizers and pressure regulators to control flow rate. Dry ice has also been used for the treatment of grain in freight containers and in conjunction with fumigation with methyl bromide.

(2) The studies on the effect of carbon dioxide (CO_2) on the control of storage insect pests and the seed quality attributes under ambient condition at different NSP centres and at the IARI, New Delhi suggested that

- Storing paddy seeds in 40% (v/v) CO_2 rich atmosphere recorded nil insect infestation and 92% seed germination, after twelve months of storage under ambient conditions prevailing at the TNAU, Coimbatore. The containers with normal air and 10 pairs insect (*R. dominica*) recorded 20% infestation; and without insect it was 13%, respectively. Similar results were obtained in 2006-07 and 2007-08.
- The result obtained in 2015 with 25L capacity containers at the TNAU further revealed that large scale CO_2 treatment would be equally effective in controlling storage insect.
- Based on studies conducted in 2015 at MPKV, Rahuri, ANGRAU, Hyderabad and TNAU, Coimbatore it was evident that 50% CO_2 treatment can provide complete protection from insects like *Rhizopertha dominica* in wheat, *Sitotroga cerealella* in paddy and *Callosobruchus* spp. in green gram without affecting seed quality up to 6-12 months storage period. Even the hardiest insect *like Trogoderma granarium* can be controlled *by* using 50% CO_2. Thus CO_2 rich modified atmosphere can be a good alternative to use of chemical treatments including fumigants for preventing storage pests in wheat, paddy and green gram.

However, the success of CO_2 rich modified atmosphere depends up on hermetic (air tight) sealing of container, correct dose of CO_2, and temperature >25°C.

Fumigants

A fumigant is a chemical vapour or gas that, when released, penetrates objects or enclosed areas in concentrations that are lethal to pest organisms. This excludes aerosols, which are particles suspended in the air, often referred to as smokes, fogs or mists. The most important and useful properties of fumigants are, itpenetrates into the material being fumigated and diffuses afterward. This gas may account for part of the

Important Fumigants, their dosages and exposure period:

Fumigant	Dose ml or g/cu.m. space	Dose ml or t seed	Exposure period (h)	Repetition (number)	Ovicidal toxicity
ED: CT mix.	480	740	24	2-3	Low
EDBr	32	56	24	02	Normal
CS_2	480	740	24	01	Low
MBr	32	56	12	02	High
PH_3	03	09	5-7	3-4	Moderate

toxic action of these applications. This is called the fumigation effect.

There are number of chemical fumigants recommended for the control of storage insects. A list of such fumigants is given below but most of them are not being used or banned due to environmental and human health hazard reasons. Many insects have also been reported tolerant to some of them. Among all, only phosphine (PH_3) fumigant was found safe to all kind of seed with up to four fumigations. Hence some of its properties are described for proper usage.

- AlP tablets is available in the names of 'Celphos, Quickphos' etc. It weighs 3 and 1 g Pellets (used against rodents) and liberates phosphine gas 1/3 of its weight.
- Ammonium carbonate, ammonium bicarbonate, urea and paraffin, are also added. The chemical reaction is
- $AlP + 2NH_4 OC (O) NH_2 + 3H_2O= -PH_3 + Al (OH)_3 + -4NH_3 + -2CO_2$
- CO_2 suppresses flammability of PH_3 while diffusing from the tablet in presence of moisture. Ammonia is a warning gas and it reduces fire hazards.
- Aluminium phosphide produces carbide or garlic type odour. It is heavier than air and has low water solubility. It is highly inflammable *per se;* a safe and convenient method to evolve gas.
- The larvae and adults succumb more easily. While the eggs and pupae are usually hardest to kill. The tolerance of eggs and pupae can be overcome by relatively long (10-day) exposure periods.
- Phosphine does not affect the germination of seeds of cereal, legume with one or two fumigations at comparatively high concentrations. Up to four repeated applications, it showed no adverse effect on viability and vigour of different crop seeds in a multi -location trial carried out under the National Seed Project in India.

Two methods are commonly followed for fumigation of seed lots namely, space fumigation and cover fumigation. Space or room fumigation is less effective than cover fumigation because fumigant used is determined by g phosphine/cu m of space while in case of cover fumigation it is calculated on the basis of g phosphine/tonne of material to be fumigated. Proper air tight condition can be created under cover whereas in room fumigation leakage is a common problem. Dosages are decided on the basis of extent of infestation, prevailing temperature and type and stage of insects. Normally, under Indian condition 7-10 days exposure period is ideal to kill all type of insects and their stages. PH_3 concentration should not be less than 200mg/cu m during the entire exposure period. Phosphine fumigation is less effective during winter season, particularly when temperature is $< 20^{\circ}$ C. Therefore, longer exposure period may be required.

Seed protectants and seed treatment

Seed treatments, in broad terms, are the application of biological, physical and chemical agents and techniques to seed that improve its qualitative attributes, provide protection to seeds and plants from their diseases and insect-pests and improve the establishment of healthy crops.

- Today's modern seed treatment products offer control of target insect-pests and diseases and ensure the establishment of healthy and vigorous plants.
- The newest active substances and formulations provide long-lasting, broad spectrum, control of pests and diseases (depending on the specific active ingredient).
- They are precisely blended products consisting of several active ingredients, special wetting agents, colorants and sometimes bird repellents which are rigorously tested for their safety to the seed, the users and the environment.
- The seed industry and seed treatment applicators use an array of quality management systems to optimize the application process. These quality systems define the treatment process, handling of treated seed, worker/environmental

protection, stewardship and provide the information required to facilitate the safe and legal treatment of seed and the disposal of treated seed.

- Earlier DDT and then malathion were commonly used as seed protectants but due to environmental hazards associated with DDT and development of insect resistance against malathion, intensive screening of newer insecticide molecules was carried out under NSP. Deltamenthrin, an insecticide was first identified and used as seed protectant in place of malathion. Now under NSP regular screening of newer molecules is done at different centres to identify safe and effective seed protectant(s) against stored seed insect pests. The results of seed entomology trial on seed protectants of 2015 revealed that emamectin benzoate (Proclaim 5SG) @ 2 ppm (40.0 mg/kg seed), spinosad (Tracer 45 SC) @ 2 ppm (4.4 mg/kg seed) followed by flubendiamide (Fame 480 SC) @ 2 ppm (4.2 mg/kg seed) and thiodicarb (Larvin 75 WP) @ 2 ppm (2.7 mg/kg seed) were at par with deltamethrin (Decis 2.8 EC) @1.0 ppm and provided control of storage insects infesting wheat, pearl millet, maize and paddy under different agro-climatic conditions up to six months. Similarly, all the above insecticidal treatments irrespective of the dosages were effective against pulse beetle (bruchid) without impairing the seed viability of different pulse seeds for the same storage period. The seed protectants suffer from several limitations such as it provides short term remedies, toxic if seed moisture is above prescribed limit, treated seed if loses viability becomes unfit for food and feed purpose leading to loss to the seed growers/traders.

CONCLUSION

- Seed health testing against storage insects is important for seed quality assurance because insects affect both quality as well as quantity of stored seeds, besides good will in the market, seed crop health and grain production. The comparative evaluation of five detection techniques namely breeding out, CO_2 method, floatation method, ninhydrin method, and x-ray method of hidden infestation of *S zeamais*, *R dominica*, *S cerealella*, and *C chinensis* in cereals and pulses revealed the following order (from high to low) in terms of accuracy in detection: breeding out > ninhydrin method > x-ray method > floatation method, and the CO_2 method was rapid but not quantitative (20).

- The best seed store insect management is IPM in seed godowns. It includes primarily store hygiene, ideal storage conditions and practices, proper conditioning of seeds, regular monitoring of storage insect and detection of its infestation in seed lot, selection and proper application of remedial measures for control of pests. Use of carbon dioxide rich modified atmosphere has shown great promise in the safe storage of seeds without any adverse effect on seed quality attributes.

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