RAMA UNIVERSITY

www.ramauniversity.ac.in

FACULTY OF AGRICULTURAL SCIENCES

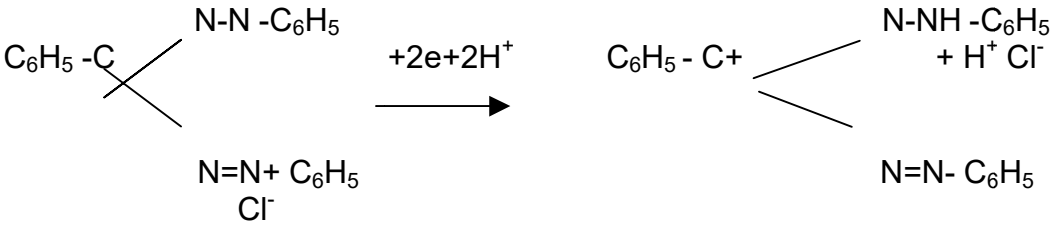
AND ALLIED INDUSTRIES

QUICK VIABILITY TEST

The relative long periods of time required for completion of germination tests delays the seed marketing. This necessitated the development of rapid methods for estimating the germination capacity of seeds. This test was developed by Lakon (1942) in Germany.

Principle

It is a biochemical test, in which living cells are made visible by reduction of an indicator dye. The indicator used is 2,3,5 triphenyl tetrazolium chloride. Within the seed tissues, it interferes with the reduction processes of living cells and accepts hydrogen from the hydrogenases. By hydrogenation of the 2,35 - tri phenyl tetrazolium chloride; a red stable and non difficusable substance, triphenyl formazan is produced in living cells. The reaction is as follows.



2,3,5 triphenyl tetra zolium chloride Triphenyl formazon.

This makes it possible to distinguish red coloured living parts of seeds from the colourless dead ones. Staining of seeds determines whether seeds are to be classified as viable. Completely stained seeds are viable partially and completely unstained seeds are non-viable.

Field of application

This test is not valid for previously germinated seeds.

Method of Tetrazolium testing

A. Testing sample

A representative sample of 50(or) 100 seeds is usually sufficient. However, 200 seeds, in replicates of 100 seeds is recommended.

B. Preparation of solutions

1% solution is used for seeds that are not bisected thro' the embryo, while 0.1% solution is used for seeds in which the embryo is bisected.

The pH of the solution should be between 6 and 8 for best staining. If the pH of the water is not in the natural range, the TZ salt should be dissolved in a phosphate buffer solution. The buffer solution is prepared as follows

Solution -1- Dissolve 9.078 g of $KH_2\ PO_4$ in 1000 ml of water

Solution -2- Dissolve 11.876 g of $Na_2HPO_4.\ 2H_20$ in 1000 ml

water.

Take 400 ml of solution 1 and 600 ml of solution 2 and mix them together. In litre of buffer solution prepared as above, dissolve 10 gms of TZ salt. This gives 1% TZ solution of pH 7.0. This may be further diluted to give lower concentrations.

The solution should be stored in brown bottle to prevent deterioration from light.

Methods of preparation for tetra zolium testing

The seeds are first prepared for staining then stained and evaluated for viability.

Method 1 : Bisect longitudinally

(e.g) maize, sorghum, small grains, large seeded grasses. Soak the seeds in water for 3 to 4 hours. Bisect the seeds by cutting longitudinally thus exposing the mains structures of the embryo. Use one 1/2 of each seed for testing.

Method 2 : Bisect laterally

(e.g.) Small seeded grasses

The seeds are cut laterally near the centre of the seed above the embryo. Place embryo end in TZ solution.

Method 3 : Pierce with needle

(e.g.) Small seeded grasses

Puncture the seeds by piercing thro' the seed into the endosperm near the embryo, but avoid injury to the embryo.

Method 4 : Remove seed coat

(e.g) Dicots with seed coats impermeable to tetrazolium.

Soak the seeds in water for 3-4 hours and then the seed coats and place the seeds in the TZ solution. In some crops like cotton a thin membrane adhering to the cotyledons is also removed in addition to the seed coat.

Method 5 : Conditioning only

(e.g) Large seeded legumes

Seeds of soybeans and other large seeded legumes may swell so rapidly and irregularly when placed directly in water or TZ solution that the seed coats burst. Hence, it is preferable to condition these seeds slowly in moist paper towels overnight before staining, so that they absorb moisture slowly without any damage to the seed.

METHOD 6 : NO CONDITIONING OR PREPARATION

(eg.) Small seeded legumes

Seed coats of these seeds are permeable to TZ and embryos usually will stain without conditioning.

Staining

The prepared seeds should be placed in suitable container (small beakers, petridishes etc.,) and covered with TZ solution. Place the containers in an incubator at dark warm conditions of 40^{o}C.

The staining time varies for different kinds of seeds, different kinds of seeds, different methods of preparation, and different temperatures (< 1 hr to 8 hrs).

When the sample has stained sufficiently the TZ solution should be discarded and the seed sample covered with water immediately. Seed samples can also be kept for 3 days at 10°C for interpretation.

Evaluation of Samples A normal TZ stain appears cherry red.

MONCOTS
NON-VIABLE

1. All structures unstained
2. Shoot largely unstained
3. Scutellar node unstained
4. Major areas of coloeptile unstained
5. Central area of scutellum unstained
6. Insect, mechanical or other injuries causing essential structures non functional.

Dicot seeds Non-viable

1. Embryo completely unstained
2. More than extreme tip of radical unstained
3. More than 1/2 of cotyledon tissue unstained.
4. Deep - seated necrosis at cotyledon and embryonic axis juncture or on radicle
5. Fractured radical.

Advantages of TZ test

1. Quick estimation of viability
2. When the seed is dormant, the TZ test is extremely useful
3. Seeds are not damaged (in dicot) in anlaysis therefore they could be germinated.

Disadvantages of TZ Test

1. It is difficult to distinguish between normal and abnormal seedlings.
2. It does not differentiate between dormant and non- dormant seeds.

Since the TZ test does not involve micro organisms harmful to germinating seedlings are not detected.

SEED BORNE DISEASES AND THEIR MANAGEMENT

Seeds are basic input for crop production. The major world food crop viz., barleys, beans, maize, millet, peanut, pulses, rice, sorghum, sugarbeet and wheat fibre crop cotton and vegetable crops such as cabbage, carrot, tomato are attacked by a number of seed borne pathogens and has their significance as far as seed health is concerned.

Plant diseases cause significance yield losses and the global loss due to plant disease is estimated to be 12 per cent of potential production, which is equivalent to a monetary loss of $ 50 billion at the producer level; in terms of quality, this is approximately 550 million tons. Losses to diseases in different regions of the world vary from 30 per cent in the agriculturally developing countries of Asia to 25 per cent in Europe and 15 per cent in North America. Losses caused by the seed borne inoculum or diseases have not been quantified.

About two third food production of the world comes from wheat, rice and maize among cereals. In wheat important seed transmitted diseases are bunts, Bipolaris seedlings blights and leaf spots, Fusarium blights, Stagonospora glume blotch, smuts and ear cockle. In U.S.A. Stevens (1940) reported 4 per cent national loss due to bunt. In India Mitra (1935) reported in 1932 up to 33 per cent and in 1933 unto 30-40 per cent losses in grain. Sokhi (1974) reported that in individual field losses unto 60 per cent may occur due to leaf blight (*Alternaria tuticina*). Stagonospora nodorum infection in barley and wheat resulted in losses of 1.5 per cent and 1.00 per cent in yields in 1974 and 1975 valued at $ 10 million and S 5 million, respectively, in England and Wales. Average reduction in 1000 gram weight ranging from 32.08 to 40.84 per cent due to Karnal bunt was observed in different varieties of wheat (Karwasara et al., 1991). Wheat plants completely affected with *Anguina tritici* (ear cockle) or *Clavibactor tritici* showed a 100 per cent loss, even partially cockle ears resulted in 52.4 per cent loss and a reduction in 1000 seed weight.

Rice crop is attacked severely by seed borne pathogens viz., Pyricularia oryzae, blast, Bipolaris oryzae, brown spot and Xanthomonas oryzae pv. Oryzae, bacterial blight. In the U.S.A. the first two diseases caused about 2 per cent loss of the total annual rice production (USDA, 1965). In India and other Asian countries, blast and brown spot are common and devasting. Blasting was responsible for a famine in Japan during 1930s and 1940s.

Losses cause by *Bipolaris oryzae* (brown spot) is comparable to blast and it is prevalent under poor farming conditions. In 1942, one of the major factors contributing to the Bengal famine in India was the failure of the rice crop because of brown spot. Due to this famine two million people died of Starvation.
Sheath blight (*Rhizoctoria solaui*) is considered of major economic importance in the Peoples Republic of China, Japan, Sri Lanka, Taiwan and the United States.

Loss in yield due to seed borne infection has varied. Bipolaris oryzae, Microdochium oryzae and Phyllosticta cause reduction in seedling size, production of fewer seeds per panicle and empty glumes and seeds are not fully filled.

Bacterial leaf blight is the most serious disease of rice in South East Asia particularly in India, Indonesia, Japan and the Philippines. Yield losses in Japan

generally are 20-30, occasionally reaching 50 % and the Philippines and Indonesia losses are equally high or even higher (Ou, 1972). India too, the losses range from 6 to 60 % (Srivastava, 1967).

The loss differs according to plant growth stage at the time of infection and cultivar. Losses reached 70 per cent for plants inoculated at the seedling stage compared to 40 per cent for those inoculated at flowering.

The barley stripe mosaic virus (BSMV) resulted in yield loss upto 64 per cent and 75 per cent in barley and wheat respectively. The total loss in barley exceeded $ 30 million from 1953 to 1970. In Germany (1945) D. graminea caused 70 and 12 percent loss respectively. Seed certification and routine seed treatment now largely check some of these diseases, which previously have caused heavy losses in many countries although not eradicated.

Oats are attacked by various pathogens but smuts pathogens such as *Ustilago segefum* var*. avenae, loose smut* and *Ustilago segetum* var*. segetum* are important.

Chickpea or gram is another important high protein pulse crop grown world wide. *Ascochyta rabiei Acochyta* blight is one of the important pathogens of this crop. In Spain this pathogen is the most important causing unto 30-50 per cent losses in wet years 5 to 8 per cent being an annual average.

In vegetable crops cabbage is attacked by three important seed borne pathogens such Phoma lingam dry rot black leg and X.C. pv. campestris, black rot and Schlerotinia sclerotiorum, watery soft rot, while blight.

Reduction in market value

a. Discolouration and Shrivelling

Seed borne fungi cause discolouration of part or whole of the seed. Due to discolouration and destoration market value of the seed is reduced. In wheat black discolouration either on germ end or ventral side (groove) of the seed is caused by *Tilletia indica* and brown to dark brown discolouration by *Alternaria alternata* either on germ end or whole seed. Soybean seeds infected with *Cercospora sajina* develop cospicuous light to dark gray or brown areas on the seed coat. Normally the seed coat cracks. Black spots or blemishes appears on the seed coat when soybean seeds are infected with *Macrophomina phaseoli.* Soybean seeds infected with F. *orysporum* appear shrunken, slightly irregular in shape, often with cracks in the seed coat with light to dark pink discoloured areas over most of the surface.

Chickpea seeds infected with *Phoma rabiei* are small and wrinkled with dark brown lesions of various shapes and sizes with pycnidia forming in deep lesions. Dark brown lesions are formed on pea seeds infected with *Macrophomina pinodes*.

Bean seeds infected with *Colletotrichum linde muthaianum* showed brown to light chocolate colour, sunken cankers on seed coats. Less severely infected seeds showed yellowish to brown shrunken lesions, which not always distinguishable from those caused by other organisms. *Ascochyta fabae f. sp.* lentis reduced the quality of lentil seeds due to discolouration and shrivelling. Reduction in seed size was correlated significantly to the level of seed borne

inoculum. Cauliflower seeds cv. Hisar I and Pusa Katki infected with *Alternaria brassicicola*, A. *alternata*, *Aspergillus spp*. *Penicillium spp*. and *Rhizopus stolonifier* showed discolouration and shrivelling (Yadav and Duhan, 1992).

b. Reduction in germination and vigour

Seeds after sowing start germinating and with the activity of germination seeds, seed borne, pathogens become active which may cause seed decay and / or pre or post emergence damping off. Eventually the plant stand is poor in the field. The reduction in germination depends upon the variety / cultivar, type amount and location of inoculum, environmental condition and other factors. In Soybean seedling emergence was reduced 12 per cent *Cercospora kikuchii* and 59 per cent by *Macrophomina phaseolina*. Seed germination in soybean decreased proportionally to the increase of D. phaseolorum var. sojae and phomopsis seed infection. Tomato seeds infected with *Phytophthora nicotinane* var. parasititica either failed to germinate or if they germinated seedlings were killed by the fungus. *Alternaria padwickii* caused decay of rice seeds, roots and coleoptiles ultimately young seedling died (Mathur et. al. (1972).Serve infection of A. *zinniae* in sunflower has caused pre-emergence death and superficial seed infection has caused diseased plants after emergence.

Tilletia indica (Karnal bunt) in wheat seeds reduced germination ranging from

10.75 to 13.50 per cent in different wheat varieties (Beniwal et al., 1990). *Microdochium mivale* infected wheat seeds whither did not germinate or gave rise to abnormal seedlings or visibly diseased plantlets. Smut (*Tolyposporium pemcillariae*) of bajra reduced average germination 21.31 per cent (Yadav and Duhan, 1993). Barley seeds severely infected with *Bipolaris sorokimana* did not germinate or if they germinated, seedlings become infected. Germination of *Bipolaris oryzae* infected rice seeds was lower than healthy seeds.

Alfalfa mosaic virus reduced the germination ranging grom 31 to 35 per cent in alfalfa seeds. Spinach latent ringspot virus in Nicototiana xanthi and N rustica caused a

reduction in germination.Severe infection of Colletrotrichum graminicola lowered the vigour in sorghum seeds.

Seed germination of cauliflower was found inversely proportional to infection, when the seeds were infected with Alternaria brassicicola, A. alternata, Aspergillus spp., Penicillium spp. and Rhizopus stolonifer (yadav and Duhan, 1992). Alternaria brassicae, Alternaria blight in cauliflower reduced average seed germination to 14% (Duhan and Suhag, 1988).

Biochemical changes in seeds

Various seed borne fungi brings about qualitative changes in the physico chemical properties of seeds such as colour, odur, oil content, iodine and sporification values, refractive index and protein content. These changes ultimately afect the commercial value of the seeds.

A. Protein

It has reported that soybean seeds contaminated with Fusarium solani and stored at 80 per cent relative humidity produced rancid, turbid oil, high in free fatty acids.

Seeds obtained from soybean mosaic virus infected plants of soybean showed higher levels of amino acids and protein and a lower level of oil than those obtained from non-infected plants. In barely seeds, protein increased as the proportion of seed infected by barley stripe mosaic virus increased. High protein content in malting barley is undesirable. Southern bean mosaic virus (SBMV) and cowpea mosaic virus reduced the carbohydrate fraction of cowpea sees. SBMV infection increased total N protein, and amino acids, and decreased inorganic phosphorus and nitrate N. A significant reduction in total N content of cowpea aphid borne mosaic infected cowpea seeds was recorded. Carbohydrate content was significantly increased in cowpea seeds harvested from plants infected at seedling stage.

B. Oil

Oil is synthesized by condensation of molecules of glycerin or fatty acids or both.

The glycerin and fatty acids are synthesized from carbohydrates, especially reducing sugars. Naturally occurring mixtures of glycerides in oil contain a small amount of free fatty acids.

Due to high lipase activity of fungi free fatty acids are increased. Wilson (1947) reported that microorganisms produced free fatty acids during the lipolysis of triglycerides. The chemical changes resulting in increased unsaturated fatty acids are undesirable and can cause fatness and cardiovascular disorders in humans. Hence, such seeds are not fit for oil extraction and human consumption or for derived oil cake as animal feed. In seeds of Brassica campestris var. dichotoma. Fusarium infection increased the sponification value but decreased the I value of the oil. Macrophomina phaseolina infection in sunflower seed reduced the oil content and changed the oil colour light yellow to yellowish brown extracted from inoculated seeds and the free fatty acids content increased.

The sponification value of the oil was higher in inoculated seeds than that of naturally infected seeds, while the I-number decreased in both types. The I number indicates the quantity of unsaturated acids present in oil. Vegetables oils are high in unsaturated fatty acids. Decreased I value due to fungal infection has been reported by Raj and Saxena in Indian mustard oil, by Mall and Pateria, in peanut oil, by Singh & Prasad in sunflower oil and by Sharma in sesame oil.

Oil extracted from infected and stored seeds may emit a rancid odour. Oil extracted from A. flavus and A. Alternata infected seeds of safflower emitted a mild, rancid odour within two weeks and as the incubation period increased, the odour to become more intense. Rancity was also observed after four weeks incase of Alternaria carthami. The oil gives unpleasant odour because of increase in free fatty acids if extracted from Fusairum spp. infected seeds of canola. Fungi infections in seeds increase the free fatty acids and cause hydrochloric type of deterioration in oil., which results in oil rancidity.

A rancid taste in peanut oil is observed due to free fatty acids. Oil from Fusarium infected seeds also showed a change in colour, which may be due to pigments synthesized by invading fungi. The colour of the oil was barium yellow and in healthy and diseased kernels respectively. Diseased kernals were lacking four fatty acids eicosanoic, lauric, myristic and palmitoleic.

Changes in physical properties of seeds

Some important physical properties in soybean seeds, viz., density, shape, size, surface area, volume and weight were affected by species of *Alternaria Fusarium* and *Phomopsis* but not by soybean mosaic virus. A reduction in seed density by 4 per cent, volume and weight by 13 per cent was observed in *Phomopsis* infected seeds of soybean. In infected *Phomopsis* symptomatic seeds, breakage of seeds was observed 20 times grater than asymptotic seeds. Any visual physical change in seeds lower the grade or acceptance rate. The reduction in size is increased with the increase in fungal infection severity. Infection by Curvularia lunata (grain / mold), *Fusarium moniliforme* (seed rot) and *Phoma sorghina* (Grain spot, leaf spot) significantly reduced the starch granule size in infected sorghum seeds compared to non-infected seeds. Seeds of soybean infected with *Sclerotinia sclerotiorum* show abnormal colour.

Detection methods

Healthy and viable seed is the first pre-requisite for increasing seed production and to reduce possible seed crop failures. Until and unless we do not know the health status of the seed, it is not possible to manage the disease. To know health of the seed, different testing methods for different pathogens / diseases of different crops have been developed.

Testing methods for seed borne fungi / diseases

1. Examination of dry seeds

It is applied for detection of seed borne fungal pathogens which cause dicolouration of the seed or change the shape and size of the seed. Also for detecting fungal structures present in on or with seed.

Procedure: Working sample 2000 seeds. All parts of seed sample are examined carefully by naked eye for the presence of discolouration and fungal structures and non seed material are removed and identified. e.g., Karnal bunt of wheat *Neovossia indica,* Ergot of bajra *Claviceps fusiformis*

2. Washing test

This method is used particularly for smut and fungi in gramineous hosts except loose smut of wheat and barely. It can also be sued for downy mildew (Pernospora manshurica) of soybean and tumour disease (Protomyces macrosporus) of coriander.

Procedure: Sample taken by weight / number of seed and put in conical flask containing sufficient water. The flask is shaken for 5-10 minutes. Drops from the washing water are examined under microscope for identification fungal spores.

e.g., Flag smut of wheat - *Urocystis agropyri,* Smut of pearl millet - *Tolyposporium penicillariae*

3. NaOH seed soak method

Applied for Karnal bunt of wheat and bunt of rice. Procedure: Working sample - 2000 seeds.

Seeds are soaked in 0.2 % NaoH for 18-24th, at 20-25^{o}C. After this swollen seeds are spread over blotter paper to remove excess water / moisture. Infected seeds giving jet- black appearance can be separated from healthy seeds.

4. Blotter method

This method is widely used. All kinds of cereals, vegetables, crucifers, legumes, ornamentals and forests seeds are tested by this method.

Procedure: Seeds are placed on well water soaked filter paper and incubated at 20$\pm$ 2^{o}C usually for 7 days in alternating cycles of 12th light and 12th darkness. Then individual seed is examined under stereo-microscope and fungi are identified based on habit characters.

In fast germinating seeds 2,4-D (2,4-dichlrophenoxy acetic acid) @ 0.10 to 0.20 per cent solution is used to check the growth of the seedlings.

In case of cereals this can be replaced by deep freeze blotter method (10^{o}C for the days, then at 20^{o}C for four days, then at 20^{o}C for overnight and at 20^{o}C for five days.

e.g., Black and gray leaf - *Alternaria brassicicola* , Spot of crucifers - A. *brassicae*, Ascochyta blight of gram *Ascochyta rabiei*.

5. Agar plate method.

This method is used for detection of same type of pathogens as in blotter method. Those fungi which are not easily detectable in blotter method can be detected by this method.

Procedure: Working sample - 400 seeds

Seeds are planted on specific medium after treating with 1 to 2% sodium hypochlorite (NaOCl) and incubated in the same way as in blotter method. Fungi are identified based on colony characteristics. Colonies with doubtful identity should be examined under compound microscope.

e.g.,	Pathogen	Medium
	Alternaria triticnia	Nutrient agar
	Fusarium oxyporum	Nutrient agar.

6. Seedling symptom test.

This test is applicable for those fungi which are capable of producing symptoms on the root and shoot of the young seedlings. This test for certain pathogens provides information pertaining to field performance of the seed lot.

Procedure: Seeds are sown in autoclaved soil or sand or any type of other media and incubated at 20^{o}C for 14 days under 12h of alternating cycles of artificial light and darkness. After incubation, individual seedling is examined and per cent infection is calculated.

e.g., *Alternaria* spp. in crucifers an d wheat, *Fusarium* spp. in a number of hosts.

7. Embryo count method.

This method is specifically used to detect loose smut of wheat and barely. Downy mildew, Seclerospora graminicola of bajra can also be detected by this method.

Procedure: 2000 seeds are soaked in 5 per cent solution of NaOH and 0.01 per cent (100 ppm) trypan blue solution for 24 h at 25-30oC. Pass the material through different sieves of 3.5, 2.0 and 1.0 mm size along with showers of tap water. Dehydrate the embryos with methylated spirit or 95 % ethyl alcohol for 2-3 minutes. Transfer the embryos in 200 ml of lactic acid + glycerol + water mixture (1:2:1). After that transfer the embryos into a 250 ml beaker containing 75 ml of lactic acid + 150 ml glycerol (1:2) and then embryos are boiled for 2 minutes. Then the mixture is allowed to cool down. Observe the embryos under steromicroscope for the presence of mycelium. Calculate the per cent infection.

8. Non destructive seed health test.

This test is conducted on high valued germplasm that can not be sacrificed as in conventional method. This test is easily applicable in large seeded crops such as corn, soybean and common bean, however, it can also be applied in small seeded crop like alfalfa, cabbage and lettuce. It consists of extracting tissue from dry seed with a metallic drill or cork borer. (1 to 3 mm) and testing extracted tissue for the disease. This test does not decrease germination rate and also help in detection of the disease.

e.g., *Ustilago segetum* in wheat, *Phoma betae* in beet

9. Fluorescence method.

The fungus to produce a fluorescent substance under Nuv light. e.g., *Ascohyta pisi* in pea seeds exhibits yellow green fluorescence.

CROP	DISEASE	PATHOGEN
Wheat (*Triticum aestivum*)	Loose smut Karnal bunt Flag smut	*Ustilogo segetum var. tritici Neoovossia indica Urocystis agropyri*
Chickpea (Cicer arietinum)	Ascochyta blight Wilt	*Ascochyta rabiei Fusasrium oxysporum f. sp. Ciceri*

Crucifers	Grey and black leaf spot	*Alternaria brassicae* *A. brasscicicola*
Rice (*Oryza sativa*)	Bunt False smut Stack burn	*Neovosssia hoorrida* *Ustilaginoidea virens Pyricularia oryzae*

		Trichoconiella padwickii
Cotton (*Gossypium spp.*)	Anthacnos e Wilt	*Colletotrichum indicum C. oxysporum f. sp. Vasinfectum*
Maize (*Zea mays*)	Black kernel rot Cob rot Southern leaf blight	*Botryodiploida theobromae Fusarium moniliforme Drechslera maydis*
Pealmillet (*Pennisetu m vulgare*)	Downy mildew or smut	*Sceleospora graminicola Tolyposporium Penicillariae*
Sorghum (*Sorghum vulgare*)	Antharacnose Kernel or grain smut Purple seed stain	*Collectrotrichum graminicola Spehaelotheca sorghi Peronosclerospora sorghi*
Soybean (*Glycine max*)	Anthracnose Pod & stem blihgt Purple seed stain	*Collectrotrichum dematium Phomospsis sojae Cercospora kikuchii*
Cucumis spp.	Anthracnose	*Colletotrichum lagenarium*
Brinjal (*Solanum melongen a*)	Fruit rot	*Phomopsis vexans*
Carrot (*Daucus carota*)	Black root rot or seedling blight Leaf blight	*Alternaria radicina* *A. dauci*
Onion (*Allium cepa*)	Damping oof Downy mildew Purple blotch Stemphylium blight	*Botrytis allii Peronospora destructor Alternaria porri Stemphylium vesicarium*
Pepper chilli *(Capsicum annum*)	Anthracnose or Ripe fruit rot	*Collectotrichu m capsici*
Radish *(Raphanus sativus*)	Grey leaf spot Leaf spot	*Alternaria brassicae A. raphani*
Tomato (*Lycopersico n esculentum*)	Blue eye rot Damping off Early blight Late blight or Fruit rot	*Phyttophthora parasitica Pythium aphanidermatum Alternaria solani Phytophthora infestans*

Management of seed borne pathogens / diseases in seed quality programmes

Disease cane have direct effects on seeds that mean that the seed itself is diseased, thus viability and appearance of the seed us affected and or the pathogen is transmitted tot he plant grown from the seed.

The aim of disease management is to prevent economic losses and to increase the value of seed crop. Control of seed borne - pathogens and diseases are attained through integrated disease management system directed against the pathogen in favour of the host and for modification of the environment.

1. Selection of seed production area

The first opportunity for management of seed-borne diseases is selection of areas where the pathogens of major concern of seed crops are unable to establish or maintain themselves at critical levels during periods of seed development. Areas with low rainfall and low relative humidity generally are favourable for production of high quality seed with low inoculum level.

The incidence of smut and ergot diseases of pearl millet and Karnal bunt of wheat can be reduced to the great extent by growing these crops in dry areas. Infection of cotton seed by *Alternaria alternata, Fusarium oxysporum* and other fungi is reduced by growing cotton in low rainfall coastal plains in Georgia, U.S.A.

Selection of site / field

With in small areas, selections of plant field are important. Many seed borne fungi can be avoided by proper selection of field infested with pathogens like wilt and arhar, smut and ergot of pearl millet and ear cockle of wheat should not be selected for seed production. In Denmark, crucifer seed crops are grown on certain small islands and coastal areas, where ample ventilation provided by wind reduces the development of A. *brassicicola*. Karnal bunt disease free seed can be produced in the fields of dry districts of Haryana.

2. Eradication or reduction of soil borne pathogens

It can be achieved through the following practices.

i. Crop rotation: Crop rotation is essential to avoid soil-borne inoculum because many seed borne fungi survive between crops, on or in crop debris for a limited number of years.

Example: Three years crop rotation against Phomopsis vexans in eggplant (USA); 3-4 years crop rotation Karnal bunt of wheat and 3 years for downy mildew of pearl millet.

ii. Fallow: Fallow helps reduction of inoculum of soil invaders in absence of host. In India, summer fallow decreases Fusarium wilt of cumin.

iii. Burning : Now a days cereal crops are generally harvested by combine. By burning the straw and stubble of crops, the inoculum of the fungi can be destroyed.

iv. Water management : Irrigation time and amount of water influence disease development.

In Florida soils, flooding continuously or at 3 days intervals for a period of 3-6 weeks can destroy *Sclerotia* of *Sclerotinia sclerotiorum* in rice. The incidence of covered smut of barley decreases to a lowest level, if flooding of soil is followed by broadcasting of the seed one our later.

3. Modification of cultural practices

a. Preparation of the seed bed

Preparation of seedbed plays a vital role in controlling diseases of various crops. Seed should be sown in well-aerated soil not in packed soil. Packed soil reduces the emergence of seedling / plants. In barely crops, packed soil causes a significant reduction in emergence of seedlings and an increase in the amount of blight and stunting produced by *Drechslera sorokiniana*.

b. Date of sowing

The selection of the sowing date depends upon the prevailing temperature and humidity requirements of the host and parasite. In Madhya Pradesh, soybean seed quality in some cultivars is improved by planting the crop from June 1 to September 2. In Haryana incidence of downy mildew and ergot of pearl millet is negligible or less if crop is sown in the end of June or Ist week of July. Sowing the crop in the last week of July (< biblio >) can reduce disease severity of smut. Similarly less disease severity of Stemphylium blight of onion was observed by sowing the crop on 30th October as compared to 20th September (Jakhar et al., 1994).

c. Depth of sowing

Depth of sowing influences germination of seedling and transmission of disease. Shallow sowing gives rapidly growing vigours plants and susceptible phase is shortened. Heavy soils require shallow sowing. Shallow sowing usually leads to decrease the loose smut. (U*. avenae*) in oats and covered smut. (U. *hardei*) of barley.

d. Rate of sowing

The seedling rate may be cardinal factor, where it adversely affects the plant growth and favours development and spread of the pathogen in the field, which ultimately can result in higher disease incidence and seed infection. In flax crop, damping off caused by *Colletotrichum lini* is increased by thick sowing.

e. Proper dose of fertilizers

Adequate and balance dose of fertilizers are important in reduction of seed infection, improving the seed quality and maximizing yield. Plants are more susceptible to diseases in soils with excessive or deficient nutrients than those grown in soil with well- balanced fertility. Excessive application of nitrogenous fertilizer increases the susceptibility of rice plants to *Pyricularia* blast (P. *oryzae*).

f. Selection of disease resistant /tolerant varieties

The use of disease resistant or tolerant cultivars is the most economical and efficient way of controlling diseases. However, resistance to a pathogen may not be available in all crops. If resistance is not available, cultivars that escape infection should be considered. In Denmark, early maturing cultivars of cauliflower escape from attack of *Alternaria blight*. (A.*brassicicola*), while late cultivars often are severely attacked because of prevailing humidity.

4. Reduction or elimination of seed-borne inoculum

a. Certification and field inspection of seed crops

Certification ensures that seed lots meet certain quality standards and that the history of each lot. Through certification, certain seed-borne pathogens have been controlled and spread to new areas has been checked. Loose smut of wheat and barely is controlled through seed certification programme in India and Sweeden. Seed crops are

periodically inspected for the presence of diseases and diseased plants are removed. Field inspection also indicate the initiation of chemical spray programme.

b. Biological control

The pearlmillet smut infection occurs through young emerging stigma. The pollination plays a role in reducing smut. The priority of pollen or sporidum entry determined that either grain setting or heavy smut infection. The pollen being natural to plant is preferred and consequently there is little in a inflorescences scope for sporidium to create infection (Yadav et al., 1996). Similarly, infection of ergot in this crop can be reduced through pollen management (Kumar et al., 1997).

c. Seed treatment

Seed treatment is usually given as a precautionary measure in seed production. The main aim of seed treatment is to control unnoticed infections or contamination particularly in breeder and foundation seed to keep the inoculum to lowest level or zero. Seed treatment of basic seed is less expensive, less hazardous and more effective and causes less pollution as compared to bigger quantities of seed in subsequent generations. Raxil, vitavax and bavistin seed treatment gives 98-100 per cent diseases control for loose smut of wheat and barley. Seed treatment with dithane M 45 and ceresan gives complete control of brown spot and stack burn of rice. Carbendazim (Bavistin) and carboxin (Vitavax) gave between 72-76 per cent disease control of root rot of cotton (Jakhar et al. 1998).

d. Isolation distance

Proper isolation distance between seed production plot and commercial plots should be maintained for production of disease free seeds. It varies from region to region depending upon weather conditions. In Germany minimum isolation distance for wheat and barley seed crop against loose smut is 50 m whereas in Holland, it is 100 m.

e. Eradication of other hosts.

Many diseases perpetuate on weed hosts or wild plants in the absence of the main cultivated host. Eradication of such host from the field or localities helpful in preventing perpetuation and spread of diseases. In India, eradication of collateral hosts such as *Panicum antidotale, Cenchrus ciliaris* and *Seteria verticillata* around pearl millet crop is recommended for reducing the incidence of ergot.

5. Chemical protection of seed crops

Chemical protection is inseparable from integrated control planning against seed- borne diseases unless true resistance in the crop is assured. The aim of the use of chemical in plant diseases control is to create, a toxic barrier between the host surface or tissues and the pathogen, present at a particular site on the host.

For effective spray, selection of fungicides, time of spray and weather conditions are important. Spraying rice crop with the fungicides like Kitazin, Zineb and Mancozeb reduces seed infection of *Alternaria padwickii, Cuvularia launata, Drechslera oryzae, phoma spp.* and *Sclerotium spp*. Application of chlorothalonil (1.5 kgha-1), carboxin (2.5 kgha-1) reduce A. padwickii and D. oryzae, when applied before dough stage in rice crop. Foliar spray of copper oxychloride in maize reduces seed borne infection of Fusarium moniliforme and Curvularia

pallescence. Three sprays of iprodione (50 per cent a.i.) at 05 to 1 kg a.iha-1 on cabbage (*Brassica oleracea*) seed crops at 3-week intervals from the

young green pod stage until cutting control pod and seed infection and seed quality is improved. Stemphylium blight of onion was controlled by spraying dithane M45 (0.2%) effectively when 6 sprays were given starting 20th October to 10th May on seed crop (Jakhar et al., 1994).

6. Management of storage fungi during storage

Storage fungi (*Aspergillus* and *Penicillum spp.*) invades grains and seeds stored at moisture contents in equilibrium with ambient relative humidity ranging from 65-90% and can cause major losses in seed viability. Effective management of storage fungi invasion is obtained by drying of seeds to below the minimum moisture content for storage fungi by aeration. The effectiveness of this practice often breaks down, however, when seed is held in storage facilities with poor environmental controls.

7. Plant quarantine.

The importance of plant quarantine increased because of the increase in exchange of seeds or grains for consumption along with better means of transportation. The International exchange of plants or their parts is practiced widely to improve the crops of a country and their genetic base. The basic principle of plant quarantine is to check the entry and spread of potentially dangerous plant pathogens and insects imported along with germplasm. Phytosanitary certificates should be issued by the exporting country as per the International plant protection convention 1951.

Seed health examination

Seed health testing is to determine the health status of a seed lot, which in turn establishes the sanitary condition of the seed in commerce.

Seed health testing

Science of determining the presence of absence of disease causing agents such as fungi, bacteria and viruses and insects in the seed samples.

The pathogen may be carried with the seeds in three ways.

1.Admixture

Pathogens are independent of seeds but accompany them. Ergot sclerotia are mixed with healthy seeds during threshing.

2. External

The pathogen may be present on seed surface as spores, oospores and chlamydospores as in case of karnal bunt of wheat, covered smut of barley, downy mildew of pearl millets etc.,

By surface sterilization external Seed borne disease is killed and if symptoms produced then internal and no.

3. Internal

Pathogens establish within the seed with definite relationship with seed parts.

PROCEDURE

WORKING SAMPLE

The entire submitted sample, or a portion of it, depending on the test method, may be used. Normally the working sample shall not be less than 400 pure seeds.

Methods

1. EXAMINATION WITHOUT INCUBATION

Such tests give no indication as to the viability of the pathogen

I . Direct examination

The submitted sample, or a sub-sample from its is examined, with or without a steroscopic microscopic and searched for ergots and other sclerotia, nematode galls, smut balls, insects, mites and evidence of diseases and pests in seed or in inert matter.

II. Examination of Imbibed seeds

The working sample is immersed in water or other liquid to make fruiting bodies symptoms of pests etc., more easily visible, or to encourage the liberation of spores. After imbibition the seeds are examined either superficially or internally preferably with the help of stereoscopic microscope.

III. Examination of Organisms removed by washing

The working sample is immersed in water with a wetting agent or alcohol and shaken vigorously to remove fungal spores, hyphae, nematodes, etc., intermingled with or adhering to the seeds. The excess liquid is then removed by filtration, centrifugation or evaporation and the extracted material examined with the help of compound microscope.

2. Examination after incubation

After incubation for a specific period, the working sample is examined for the presence of or symptoms of disease organisms, pests, and evidence of physiological disturbances in the seeds and seedlings. The examination maybe superficial or internal. Three types of media are commonly used.

1. Blotters

These are used when pathogens are to be grown from the seeds or when seedlings are to be examined. The seeds with or without pretreatment are so spaced during incubation as to avoid secondary spread of organisms. Lighting is provided to stimulate sporulation of fungi when needed. Some pathogens can be identified without magnification but a stereoscopic microscope or a compound microscope is often helpful in identifying spores.

2. Sand, artificial composts and similar media can be used for certain pathogens. The seeds, usually without pre-treatment, are sown suitably spaced in the medium so as to avoid secondary spread of organisms and then incubated in conditions favourable for symptom expression.

3. Agar plates are used to obtain identifiable growth of organisms form seeds. Precautions should be taken to ensure their sterilization. The seeds, normally after pre treatment are spaced on the surface of sterilized agar and incubated. Characteristic colonies on the agar can be identified, either macroscopically or microscopically. Lighting is often useful and germination

inhibitors may be used.

3. Examination of plants

Growing plants from seed and examining them for disease symptoms is sometimes the most practicable method for determining whether bacteria, fungi or viruses are present in the sample. Seeds from the sample under test may be sown or inoculam obtained from the sample may be used for infection tests with healthy seedlings or parts of plants. The plants must be protected from accidental infections from elsewhere and conditions may require careful control.

4. Other techniques

Specialized methods involving serological reactions, phage-plaque formation, etc., have been developed for some disease organisms and may be used preferably in consultation with the seed pathologist.

Calculation and expression of resutls

Results are expressed as percentage by number of seeds affected or as number of organisms in the weight of sample examined. The result must be accompanied by statement of the test method used, including any-pre treatment applied, and of the amount of the sample or fraction examined. The absence of statement concerning the health condition of the seed does not necessarily imply that the health condition is satisfactory.

Specific methods for some diseases

1. Burnt of Paddy causal organism : *Neovossia horrida*

Procedure : Sodium hydroxide (NaOH) seed soak method.

i.) Paddy seeds are soaked in flask beaker containing 250 ml of 0.2 percent sodium hydroxide solution (2g/NaoH/1000 ml water) for 24 hr at 20-30 ^{0} C.
ii) After 24 hours the solution is decanted
iii) Seeds are thoroughly washed in tap water
iv) Seeds are spread over a blotter paper so that excess water on the surface of seed is absorbed.
v) Seeds are examined visually aided with light.
vi) The seeds exhibiting shiny jet black are separated.
vii) Such shiny jet black seeds are ruptured separately in a drop of water by puncturing and observed visually for the release of stream of fungal spores.
viii) The number of seeds releasing stream of fungal spores are counted as infect seed.
ix) Results is reported in percentage.

The seeds looking only shiny jet black have been found to contain burnt infection whereas those with brown to dull black discolouration do not reveal burnt infection. This is a symptom of brown spot or brown discolouration of rice.

II. Ergot of pearl millet , causal organims ; Claviceps fusiformis (=Clavieceps microcephela)

Ergot of sorghum, causal organism - Sphacelia sorghi and clavieeps spp., and ergot of triticale, causal organism. Claviceps purpurea.

Procedure

i) Seeds are examined visualy aided with light in dry state for the presence of ergot sclerotia. These sclerotia are purplish brown in colour, irregular in shape, hard structures and these may be two to three times bigger than healthy seeds.

A seed lot of sorghum mixed with well developed and broken sclerotia is called an ergotted seed lot. However, a seed lot mixed with honey dew lumps containing mycelial mat with conidia should not be called an ergotted seed lot.

ii) The sclerotia are separated and counted

iii) Result is reported in percentage (by number).

Seed pests and their management

The growth of agriculture based economies of the world depends upon the sustained supply of quality seeds. The productivity led growth in agriculture is based on the application of advanced technology, which in turn is dependent on cultivar's access to the seed of desired genetic composition and adequate purity. Quality seed also offer the highest economical and social return among all the agricultural inputs. It also ensures the optimum utilization of the other production inputs viz., fertilizers, pesticides etc., in India, ecological conditions are diverse in different regions and consequently a number of crops are cultivated. A large insect fauna is associated with each of these crops from production to storage because of the congenial conditions for the survival and multiplication of insect pests. The extent of losses caused by these anthropods in total seed yield and quality depends upon their feeding behavior / life cycle. Besides, these pests also lower the seed recovery, which has a significant impact on the success of seed industry in the region.

Seeds are required to be stored since seeds harvested in the preceding season are usually used for sowing in the next season after an interval of six months or more. Also buffer seed stock needs to be maintained which may extent from 2 to 3 years. In tropical and sub-tropical world, the losses in typical village stores as well as warehouses are more due to seed damage by biotic (insects, rodents, micro-organisms, etc.,) and abiotic (temperature, humidity) factors. Among these factors, largest proportion of preventable (post harvest) wastage is due to moisture, insects and rodents in storage.

Damage

Insect pest infestation during storage leads to poor health status of seeds. It is well-known that insect pests infesting stored products are tropical in origin. The seed quality is affected on the basis of insect damage to endosperm or embryo. If embryo portion is damaged, seed fails to germinate and if endosperm is damaged , seed may germinate and develop into a seedling but the vigour of the seedling will depend upon the extent and intensity of the damage. The degree and type of insect infestation and insect species involved depend upon the crop seed and prevailing ecological conditions. Other factors viz., sanitation, bulk or bag storage etc., generally influence of the seed safety. In bulk storage seeds continuously release heat and moisture by respiration, slowing down the air movement through the bulk of the seeds, which tends to develop hot spots and caked seeds. Insects also damage indirectly by contaminating the seed with their waste, casteskins, webbing and body parts. Heating also takes place due to feeding by some insect pests which leads to the development of moulds. "Weevilized" seeds are not accepted as (I) seeds may fail to meet the certification standards and (ii) they may lead to contamination of new stores.

Losses

In India, insects and rodents alone polished off the monthly food requirement of
760 million people during 1998-99. The total preventable (post harvest) loss of food grain was 10 per cent of the total production or about 20 million tonnes per year.

No such extensive estimate of rejection of seeds due to insect-pests either during seed production or subsequent storage have been made in India. Insect infection of seeds would assume serious proportions if seeds were produced for marketing abroad because of economic liberalization. In global marketing insects or fungi in seeds during storage and transport greatly affects its quality. Low levels of insets, infestation can develop into damaging / above minimum permissible certification levels before the seed reaches its final destination. In a study, the data (1989-90 to 1993-94) provided by Seed Testing Laboratories, working in diverse ecological conditions., reported that the seed samples are not assayed for insect damage (ID) in 40 per cent laboratories, as per rules provided in Indian Minimum Seed Certification Standards. Rejection of revalidated seed samples as per certification standards do not take into account either the loss of seed yield inflicted by insect pests during production or low seed recovery from raw seed yield after cleaning and grading process.

In Haryana, both primary and secondary seed feeders infest wheat seed. Lesser grain borer *Rhizopertha dominica* was most prevalent (61.5%) followed by *Sitophilus oryzae* (59.3%) in farmer's saved wheat seed samples. *Trogoderma granarium*-essentially a germ feeder, was recorded from 15.5 per cent samples-mostly from dry zones. On an average 67.6 per cent samples could not meet the minimum certification standards; maximum damage in a sample was by T. *granarium* in which 43 per cent seed embryo was eaten by the beetle.

Temperature and seed moisture

Most insect pests of stores have a short period from egg to adult and their reproduction rate is high. The two abiotic factors, which influence these characteristics, are temperature and moisture. Since cool and dry conditions are sage for seed storage, it is considered that the seed with moisture less than 10 per cent and temperature below 20^{o}C remains free from pests. (The optimum range of temperature for most insect species lies between 28 to 32^{o}C). Temperature less than optimum may decrease their feeding activity, prolong developmental period and may cause mortality in many individuals due to starvation. At higher temperature (25-35^{o}C) and humidity, seed deterioration is accelerated since the conditions favour insect's multiplication and formation of hot spots besides biochemical and nutritional changes in seeds.

High seed moisture content is the greatest single factor for the loss of seed viability. With high moisture content, the spoilage increases due to seeds' own metabolic activity and also increased pest incidence. The moisture requirement differs from one insect species to another. However, all of them need more than 10 per cent seed moisture. Therefore, seeds having less than 10 per cent moisture are safe for storage. Storage above

16 per cent seed moisture content not only deteriorates the seed quality but also occupies more space as its bulk increases. The seed moisture content of 12-14 per cent heating starts and at 45 per cent seed moisture, germination starts. The process initiate chain reactions resulting in the loss of seed viability. Insect-pests of stores obtain water primarily from the seed itself. If the moisture content of seed is low, generally less than 10 per cent, the insects must obtain water by breaking down the seed components or by using their own energy reserves. Under such situations, fewer insects survive. It is important to note than fluctuations of moisture as small as 0.5 to 1.0 per cent, can significantly affect the rate of extent of insect infestation.

Harvesting conditions

The harvesters and threshers available in the market are designed for grain and not for seed crops. Threshing efficiency is the main criteria for grain crops whereas seed breakage is the concern of a seed grower. Grain crops are harvested at low grain moisture content, though at such a moisture content, the seed damage would be more, which in turn would affect the storability of the seed. However, harvesting and storing seed at high moisture content would not only lead to quick multiplication of insects but also invasion and development of various fungi. The development of insects however aided if broken seeds and dockage are present in a seed lot. For the store insects infesting the crops in the field after harvesting, threshing must be done quickly so that control measures could be applied in stores before damage occurs in serious proportions. Regions where *Sitotroga* occurs prompt harvesting and threshing is best since it helps to prevent infestation in field and in bin.

Seed residues in processing plant especially conveyers, seed treaters, transport vehicles viz., trolleys, trucks etc., harvesters and threshers etc., are particularly susceptible to stored seed insects and storage fungi. Favourable temperature and moisture conditions provide the suitable environment for the build-up of large pest populations. Therefore, inspecting, removing and treating these seed residues is advisable to prevent contaminating newly harvested seed crop.

Seed certification requirements

The Indian Minimum Seed Certification Standards, seed standards for "Insect Damage" have been provided in Chapter 1 (General Seed Certification Standards) and sub section XXV. The rule states that "A seed lot under certification shall not have apparent or visible evidence of damage by insects for both foundation and certified seeds classes in excess of 1.0 per cent for seeds of maize and legumes and 0.5 per cent for the seeds other than maize and legumes unless otherwise prescribed".

Thus, it is essential to determine the insect infestation of a seed sample/ lot submitted to Seed Testing Laboratory. The information obtained is reported to be useful in several ways.

- Insects can be cause of poor germination and weak seedlings
- Latent / hidden infestation can lead to increase infestation of a seed lot during transit / storage.
- Spread of insect pests to newer areas and
- It helps in adoption of proper remedial measures.

For assaying the seed sample from "ID" point of view, it is essential to test the sample immediately at the time of its lifting or preserve it in a way so that insects do not multiply.

Seed storage

A. Requirement of Store

In India, farmers store the seed by various methods to prevent losses, but they succeed only partially owing primarily due to use of traditional (old) receptacles (pit / type
/ underground structures, paddy straw structures, *Kachha kothi,* earthen pots, oil drums, *Parchhatii* and *thekha* storage structure) where maximum loss occurs. Safe storage is aided by absence of cracked seeds and other inert material which is not only provide food to insects and fill up the spaces between seeds but also interfere with the natural movement of air through the seeds if stored over long periods and seed quality could not be maintained. In a survey of Haryana State, it was observed that most farmers do not distinguish between seed and grain. In fact, the produce is consumed as grain from threshing onwards and the same lot is used as seed for planting in the next season.

Though on commercial scale: Silos" are being used, yet "Pusa metallic bins" are commonly used by the farmers for on-farm storage. Seeds kept in dark, damp stores and improper receptacles absorb moisture from either ground or atmosphere particularly during monsoon. The warm season and high seed moisture content are highly conducive to the development, survival and rapid multiplication of insect pests. Thus, to save the seeds from insect damage /infestation the storage structure should have the following characteristics.

- It should be moisture proof.
- It should be controlled aeration to cool the seeds and thus limit insect development.
- It should be capable of being made sufficiently air tight, for distribution of fumigants and
- It should be easy to clean and inspect and also allow smooth in and out movement of seed.

  Besides, it should protect the seed from rodents, birds, objectionable odors, theft etc.,

B. Sources of insect infestation

The harvested seeds most likely have optimum temperature and moisture conditions for insect development. Before strategies are adopted for the control of these insects, the sources of insect-pest infestation in stores should be looked into to prevent the multiplication of insects. The sources of insect infestation are:

- Leftover seed from bins/ stores or spilled seeds under the stacks
- Cleanings of the processing unit
- Old infested stores
- Old gunny bags
- Insect infested trolleys, wagons, trucks etc.,
- Storing insect infested seeds with fresh stock or vice-versa
- Entry of insects from neighbouring stores and
- Carryover of field infestation

C. Seed inspection during storage

Frequent inspections and constant vigilance are the most important steps of pest management in seed stores. Foul odour, flour spots on gunny bags or dockage generally indicate foci of infestation. Small plastic traps, with or without pheromone bait have been successfully used in the bulk seed to scout for insects.

Appropriate inspections of a seed store including outside areas are essential to check the insect population build-up. The behavior of the insect must be kept in mind when looking for signs of their probable presence. The inspections must be made by well equipped and trained staff who should look for the raw seed, equipment used during transportation, processing, cleaning etc and maintain a complete record of the insect control. Regular periodic inspections throughout seed storage must be made to detect initial insect infestation or heating. Such inspections are particularly important during monsoon or in coastal regions where seed moisture and ecological conditions are congenial for rapid insect multiplication.

Inspections of possible insect infestation in stored seeds have become pivotal with shift towards integrated pest management. In fact, inspections have become a routine schedule / part of any efficient and effective pest management programme. Inspections should be pre planned to the extent possible and basic tool and equipment prepared prior to the inspection. During the inspection, checking should be made everywhere including all static areas and equipment. Clear and concise report should be provided. For inspection we need a seed probe and seed physical purity board to separate the insects(s) and damaged seeds.

The build-up of the pest population changes according to ambient temperature and moisture levels during the storage. It is, therefore, advisable to inspect the stores / bins at 3 or 4 weeks intervals. During monsoon period and in coastal areas inspection should be made a fortnightly interval. Seed samples should be drawn systematically and inspected for insect population, seed damage and dockage. During the inspection, be alert to the presence of odours, caking and crushing as these are clear indicators of insect and moisture problems.

Insect pests of seed stores

A large number of insect species are associated with seeds during storage (Table 1). These insects mostly belong to orders Coleoptera (beetles / weevils) and Lepidoptera (moths). These insects, depending upon their ability to damage sound seeds can be divided into Primary and Secondary seed feeders. The primary consumers breach the pericarp or testa making feeding possible by a much wider range of insects, mites and fungi. The primary consumer obtains water metabolically from seeds and releases it into their immediate environment along with waste heat. At the same time the accumulating frass, seed dust and carcasses restrict air movement, contributing to the formation of hot spots. Amongst primary pests, adults and larvae of *Trogodema granarium*, *Sitophilus oryzae* and *Rhizopertha Dominica* are most important pests. Grain moth, *Sitotroga cerealella* is not a serious problem in North India but is the major pest in coastal as well as South India. In legumes, *Callosobruchus spp* is very serious.

Table 1. Insect pests of seed stores

Common Name	Local Nam	Scientific Name	Seeds Attacked	Mode of damage	Optimal climatic requirements

	e				

Rust Red Flour Beetle	Suri	*Tribolium castaneum*	All already damaged seeds	Secondary feeder; Adults & larvae feed on seeds already damaged.	30-35^{o}C RH > 70% Seed M.C. 11-16%
Khapra beetle	Pai Khapra	*Trogo-derma granarium*	All seeds	Primary feeder; larvae feed on germ portion and reduce seed into frass	34-37^{o}C RH > 50% Seed M.C. 11.5%
Lesser grain	Ghum	*Rhizo-pertha dominica*	All seeds	Primary feeder; adults and larvae feed on seed and reduce them to mere shells with many irregular holes.	33-36^{o}C RH > 60% Seed M.C. 11.14%
Rice weevil	Sundwali sursi	*Sito-phiulus oryzae*	Cereals	Primary feeder; larvae feed and pupate inside the seed. Seedling vigour is adversely affected.	26-30^{o}C RH > 70% Seed M.C. 14%
Pulse beetle	Dhora	*Calloso-bruches chinensis*	Pulses	Primary feeder; larvae feed and pupate inside the seed. Seed quality is adversely affected.	30-35^{o}C RH > 50% Seed M.C. 12 -14%
Grain moth	Patanga	*Sitotroga cerealella*	Cereals	Larvae feed seed from inside which partly get filled with the excreta. Damaged endosperm results in reduced seedling vigour.	30^{o}C RH > 70% Seed M.C. 14 - 16%
Indian meal moth	Patanga	*Podia inter-punctella*	Cereals & pulses	Primary feeder; larvae feed inside seed making a silken web.	29^{o}C RH >70% Seed M.C. 14.- 16%.

The generation time is often short and in a few months, the population can explode. The weevils complete their life cycle inside individual seeds and may

develop into substantial population before their presence is even noticed.

Secondary seed feeders attack the already damaged seed where testa is cracked, holed and broken either due to mechanical damage during harvesting, threshing and processing or by too rapid seed drying or by prior feeding damage by primary seed feeders. Such insects are primary pests of flour / processed products. Most common secondary pests on seeds include the larvae of *Ephestia, plodia* and the beetles of *Oryzaephilus, Crytolests, Tribolium* and other *Tenebrionidae*. As per the climatic requirements, *Trogoderma* is a serious pest of warm and dry regions while *Sitophilus* and *Rhizopertha* of humid and moderately warm regions. In seed stores insects can be categorized in to two groups.

Group I. Insects that infests the seed in the field and do not multiply in transit / store e.g. pink boll worm in cotton, midges in millets etc., The number of seeds damaged in field remains constant. Pests are transmitted through these infested seeds from place to place and year to year. They resume their life activity in field or other media under favorable conditions.

Group II. Insects that infest the seed in field / store or any other place where the seed is handled. The arthropod pests continue life-cylce in ambient conditions. Number of damaged seeds as well insects continue to increase with the passage of time e.g. weevils, beetles, moths etc., Thus, after lifting a seed sample containing such insects, it should immediately be tested.

Proper pest control is predicated on knowing what we are trying to control. Thus, identification of insect pest species is important. Knowing the insect species in a potential problem can help us decide what the risks are, what actions are indicated and how prompt these actions need to be adopted.

MANAGEMENT

For the management of the insect pests in a seed store, the management strategy should be well- defined, since not only insect pests are to be controlled but the viability of the seeds is also the preserved. Five key steps in dealing with insects in a seed store or processing plant as reported earlier, in chronological order are:

1. Have an inspection or surveillance system for the storage which would yield prompt awareness of a possible problem;
2. Determine the extent and nature of the possible problem - what species, how many and when;
3. Device a plan for the control of the problem. Make use of your own basic knowledge and information. Consult experts, literature etc., or whatever combination is required.
4. Implement the device plan being willing to modify is as indicated and
5. Monitor the results of the effort.

The strategy of insect management must keep into consideration the following key aspects of insect control.

A. Prevention

1. Keep facility clean (sanitation)
2. Do not bring the insects into the processing plant
   - Check conveyances
   - Check raw seed including packaging supplies
   - Maintain building tightness
3. Have clearly defined inspection programme for early detection
   - Use checks list(s) of where to look
   - Use traps as monitoring devices

B. Presence noted - Assessment of Problem

1. Define magnitude of "problem" species, number / locations etc.,
2. Select control treatment (s) based on;
   - Seed damage in bulk or bag

- Potential for contamination
- Physical facilities
- Risk to operator & others
- Cost (control measures, possible of seed or seed quality etc.,)

Insects in seed stores are controlled by avoidance, physical manipulation of the store and finally toxic chemicals. Right type of storage structure play a pivotal role in keeping the seed insect free in on-farm storage in tropical and sub-tropical regions of the world. Generally the control measures are grouped in to (i) preventive and (ii) curative measures. The curative methods are categorized into chemical and non-chemical methods. The chemical methods include fumigation of stores as well as spraying the store surfaces / bags and also mixing of insecticide dusts with seeds. Non-chemical methods include biological control, host resistance to pest modified atmosphere system, temperature manipulations, radiation physical barrier etc.,

Preventive

Prompt harvest, drying and thorough cleaning of the seed help prevent entry of insects into the seed stores.

Good sanitation in the processing plant and harvest machinery is crucial, since seed pests, often breed in small remnant deposits of old seeds in accessible places viz., conveyor belts, screens, lift hoppers etc.,

Hard to reach spots should be disinfected by fumigation.

Destroy (burn or bury deep) the sweepings, clear trash, litter from outside the store / processing hall and remove the spilled seeds from under the stacks.

Remove all the leftover seeds from store / bin; sweep down the walls, ceilings, sills, ledges and floor etc.,

Make necessary repairs of crack and crevices.

Use new gunny bags for fresh harvest. However, if old bags are to be used dip them in 0.1 % Malathion 50 EC (1 part Malathion 50 EC + 500 parts of water).

Drying followed by cooling and ventilation of bulk stored seed, as well as temperature monitoring is important for long term storage.

Bagged seed must be kept at distance from walls to allow inspections, fumigation and avoiding the seed to absorb moisture from moist surfaces.

To disinfect the stores, spray 0.5 % Malathion 50 EC 1:100) on floor, wall and ceiling or fumigate with aluminium phosphide tablets (3 g each) @ 7-10 tablets or 10.0 L EDCT per 1000 cubic feed.

To avoid insect damage to store seeds mixing of Malathion 5D @ 250 g/qtl or deltamarthrin 2.8 EC @ 4 ml/qt. seed is recommended. These treatments besides checking the insect infestation in seeds do not have any deleterious effect on its quality.

Spray inside and outside pits surfaces of the store after cleaning with the following insecticides to kill any insect that has remained in the store or insects that may crawl across these areas to damage the stored seed.

1. 0.1 % Malathion 50 EC (1 part of insecticide + 500 parts of water) or
2. 0.1 % Fenvelrate 20 EC (1 part of insecticide + 200 parts of water) or
3. 0.1 % Cypermathrin 25 EC (1 part of insecticide + 2500 parts of water)

Care must be taken to treat all the cracks, crevices and areas around doorways and other places where insects could enter from outside.

Note:

- Always look for the expiry date on the label of the insecticide container; use as directed on the label; do not use spray material that have sat overnight after mixed with water.
- To keep pulses free from bruchid infestation, keep a 7.0-cm layer of sand at the top of seed stored in bulk.
- Where empty godown is infested with *Trogoderma*, it must be fumigated with EDCT or aluminium phosphide before use.

Fumigation

Under tropical and sub-tropical climate conditions, inadequate methods of seed storage as well as high risk factors of insect infestation warrants the use of fumigants in seed stores or application of insecticides to save the seed from insect ravages. Fumigation with fumigants is widely practices, for curative or preventive action, as they are cost effective, efficient and easy to use against target pests besides can penetrate in to places when other control methods become impossible or impractical.

Fumigant is a chemical substance, with at specified temperature and pressure can exist in gaseous from in sufficient concentration to be lethal to the pest organisms. Apart from other characteristics a fumigant should not affect the viability of seeds, should be non-persistent, non-corrosive and highly diffusible. Fumigation with fumigants viz., aluminium phosphide, methyl bromide, ethyl di-bromide and EDCT are most common world over. EDB has been found to reduce the seed germination of many cereals and legume crops. Though no adverse effect of fumigation with aluminium phosphide have been observed yet varietal differences have been observed in wheat, rice, green gram and broad beans for germination and other quality parameters. AT higher seed moisture content and phosphine doses, complete loss of viability, mutagenic effects and chromosomal aberrations have been reported in wheat and onion seeds. In rice, high phosphine dose resulted in increased dihydrogenase activity, seed leachate electrolytes along with decreased respiratory enzymes. In most of the crops, the germination and vigour is affected with excessive fumigation doses and prolonged exposure period if the seed moisture content is high. Hence, even fumigation has implications during revalidation of seed lots.

Aluminium phosphide should thus be used with care in seed stores as phosphine gas react differently with seed of crops having different moisture contents. The storage facilities should be sound to avoid movement of moisture in the store, as seed quality is reported to be affected if phosphine fumigation is done more than12 per cent seed moisture content.

Curative

- Fumigate with aluminium phosphate @ 7 tablets (3 g each) or EDCT mixture (3:1) per 1000 cubit feet space with exposure period of at least 7 days.
- Never keep the fumigants at the bottom of the floor / bin as the gas is heavier than air and travel downwards. Since the gas can penetrate downwards unto 8 feet, the fumigant should be placed accordingly.

- Spray the stores with DDVP @ 0.25 % (1:300). For 100m^2 area 3 liters of spray material is required. Spray on all the walls, ceiling and floor of the store. Spray other surfaces / structures (see checklist) where presence of insect is suspected.
- Surface of the bags should be sprayed with Malathion 50 EC (1 ml Malathion in 100 ml water after 3 or weeks).

Precaution

- Fumigants are to be done cautiously by trained persons / under technical guidance.
- It should be ensured that the fumigated structures are air tight
- Never use EDB in seed stores, as it would affect seed quality.
- Never mix BHC or DDT with seeds.

Non Chemical Methods Biological control

Stored product insects are attacked an array of parasites and predators that exert some degree of natural control. Augmentation or manipulation of these natural enemies offers new means of controlling storage pests in situations where the use of other means of control is objectionable. A parasitic wasp, *Bracon hebetor* attacks late stage larvae of *Cautella* and *Plodia interpunctella* and suppresses their populations. The potential of this method is still rudimentary in stores.

Pheromones

Synthetic pheromones have produced for the almond moth, red and confused flour beetles, lesser gain borer, khapra and few other insects species. The studies have shown that pheromone traps are effective in detecting hidden infestation.

Host resistance to insect-pest

Though much effort by plant breeders has been made to develop insect resistant lines in various crops, yet little research has been performed to develop lines whose grains
/ seeds resist attack by stored product insects.

Temperature manipulation

The application of high or low temperatures offers a non-chemical means of disinfecting stored commodities. Sublethal temperatures can affect reproduction, growth, development, feeding and movement. However, temperature adverse to survival of storage insect population must not affect the seed viability.

Controlled or modified atmospheres

Killing of insects with modified atmosphere of oxygen, carbondioxide and nitrogen has long been recognized. Laboratory studies have shown that atmospheres deficient in oxygen are effective in controlling all life stages of principal insect species that infest stores. For instance, air with only 2 per cent oxygen will asphyxiate Sitophilus stores. Further, continuous storage in these atmospheres for periods unto 1 year did not adversely affect germination of wheat, rice, barley, malt and almonds. Further tests to compare the economic

feasibility of modified atmospheres and chemical control methods are needed. In addition, basic information on population growth and development of surviving insects subjected to these conditions are needed.

Radiation

Effect of Gamma-Radiation has been studies extensively for disinfestation o insects in stores. However, its affect on seed viability has not studied in detail.

Physical barriers

One of the demonstrated non-chemical methods for the control of stored pests is the use of physical barriers placed around the commodity. For example, a mutli-wall paper bag has been developed to protect the seeds. Another physical barrier is the packaging material, which prevents the insects from infesting the seeds in bags. The packaging material identified to be resistant to insects are; ethylene tetrafluorethylene, polyester, polyvinylchloride, polypropylene and polycarbonate. Insecticides have also been inserted into packaging material to make them insect resistant. Plastic sheet with high tensile strength is also being tried as barrier for the insect infiltration. Also, physical disturbance or turning of seeds vacuums conveyance and Entoleters has been reported to reduce the population of external feeding insects only. Inert dusts derived from the shells of diatoms are being used to mix with seeds. However seed moisture must be kept in mind while using these dusts.

INTEGRATED PEST MANAGEMENT

Now a days, IPM can be applied for the management of insect pests in stores. Computer models are being used to predict when the control strategy is to be adopted and recommended which control methods(s) to be used. These models use the knowledge of insect ecology and previous store history in the best management programmes. IPM requires a complete understanding of insect pest's species, their monitoring, biology, behavior and reaction towards various management practices. In IPM reduced use of pesticides encourage biological and physical methods of pest control besides decreasing the chances of insecticide resistance. Frequent sampling is most important component in such management progammes.

Table. Yearly fluctuations of temperature and relative humidity in selected areas of India.

Location	**Below 45% RH**	**Above 60 % RH**	**Above 30°C**
Poor storage places			
Trivandrum	none	12 mo (all < 70%)	none
Chennai	none	11 mo	4 mo
Calcutta	none	11 mo	2 mo
Cuttack	none	10 mo	4 mo
Mumbai	none	12 mo	none
	none		
	none		

Fair storage places Coimbatore Bangalore Hyderabad	none 1 mo 3 mo	9 mo 8 mo 5 mo	none none 3 mo
Better storage places Srinagar	none	12 mo	none

Ahmedaba d Delhi Jaipur	7 mo 7 mo 7 mo	3 mo 3 mo 3 mo	6 mo 4 mo 3 mo
Best storage places Abu Siml a	 8 mo 6 mo	 4 mo 3 mo	 non e non e

* Below 45 % RH : Seeds can be dried to moisture levels safe for storage without heat

** Above 60 % RH : Mold growth, rapid loss of germination.

*** Above 30^{o}C : Difficult to dry seeds artificially because of limitations in heating the air to reduce RH of drying air.