SEED TESTING MANUAL FOR SPICE CROPS

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SEED TESTING MANUAL FOR SPICES

Introduction

India is known as land of spices. Spices are mainly used for flavouring food and in medicines. India grows over 50 different varieties of spice crops. Cultivation of spice crops in a state depends up on prevailing agro-climatic conditions. The total production of spices is around 2.7 million tonnes among which, about 0.25 million tonnes (8-10 %) is exported to more than 150 countries. During 2000-2003 spices exports quantity touched an all-time high of 2, 64, 107 tonnes. The Indian share of the world trade in spices is 45-50% by volume and 25% in value. Since Spices are of high- value and export oriented in nature, productivity of the crop is of prime importance. This can be achieved only when healthy seeds are used for propagation. Purity test, germination test, tetrazolium test, vigour test and disease testing are the important tests to judge the health of seeds, however, these tests are not applicable to spices, since they are multiplied mainly by vegetative means. The various tests to be used in the case of spices to produce quality-planting materials are dealt with here.

I BLACK PEPPER

Black pepper (*Piper nigrum* L.) (Family: Piperaceae), the king of spices, is one of the oldest and most important spice widely used in cooking and medicine. India is a major producer, consumer and exporter of black pepper in the world. In India, Kerala and Karnataka are major producers of black pepper.

1.1 Propagation

Black pepper can be propagated through seeds and vegetative means. Owing to its heterozygous nature, seedlings will not breed to true to types. Hence black pepper is propagated vegetatively mainly from cuttings. Runner shoots are ideal for making cuttings.

1.1.1 Section of mother vines:

The runner shoots for making cuttings should be selected from uniformly established gardens free from pest and diseases. While selecting elite mother vines, following points should be kept in mind.

a) Age of the elite mother vines should 7 years and above.
b) The vine should have given a stable yield of at least 2 kg dry pepper /vine/year for 4 consecutive years and above.
c) Runner vines may be collected from a particular variety, in which purity may be maintained.

1.2Test for vigour of seedlings

i) The age of the rooted cutting should be 3 months from the date of planting in the polythene bags.

ii) The rooted cutting should be of minimum height of 25 cm with 5 with vigorous growth without exhibiting any nutrient deficiency symptoms, pest and disease infection.

iii) Varietal purity should be maintained.

Black pepper rooted cuttings that fail to meet above requirements should not be considered as a quality planting material of black pepper.

1.3 Test for diseases

1.3.1 *Phytophthora* ***infections***

Phytophthora infections are noticed on leaves, stems and roots of cuttings in the nursery. Dark spots with fimbriate margins appear on the leaves, which spread rapidly resulting in defoliation. The infections on the stem are seen as black lesions, which result in blight. The symptoms on the roots appear as rotting of the entire root system.

1.3.1.1 Detection and diagnosis

- **Based on the propagule formation in water**

The symptomatic plant parts can be immersed in sterile distilled water in a Petri-dish and incubated for 24-48h at 24-28°C under continuous light. Development of characteristic umbellate sporangial formation with long pedicels under the microscope indicates the presence of *Phytophthora.*

- **Based on selective isolation and culture characteristics**

The media for the selective isolation of *Phytophthora* from the soil is called PVPH media (Tsao, 1970). Diseased plant parts suspected to be infected with *Phytophthora* could be plated in the PVPH media after surface sterilization. Isolation of fungi from plant tissues is possible by placing small

portions of infected tissue on to PVPH media in sterile petri dishes under asceptic conditions. Surface sterilization of excised tissue before plating is necessary to avoid surface contamination by saprophytic organisms that commonly grow over plant surfaces.

Materials required

Petri plates, razor blade, surface sterilant such as 0.1% $HgCl_2$ or 10% NaOCl., blotting paper, sterile distilled water.

Mounting media

Lactophenol

Phenol (Pure crystal) 20g
Lactic acid 20g
Glycerol 40g
Water 20ml

Heat the water using a water bath, add phenol and dissolve, and add lactic acid and glycerol

Stain

Lactophenol Cotton blue

Lactophenol 100ml
Cotton blue 0.1g

PVPH Medium

Medium Ingredients :	Difco corn meal agar (CMA)	:	17 g/L
	Pimaricin	:	10 ppm (= 10 ug/ml or 10 mg/ L)
	Vancomycin HCL	:	200 ppm
	Pentachloronitro-Benzene (PCNB)	:	100 ppm
	Hymexazol	:	50 ppm

Procedure

Collect leaves/stem-showing symptoms/Lesions of particular diseases. Wash the specimens thoroughly under tap water. If root tissues are used, prolonged washing may be necessary. This can be achieved by retaining root pieces in a fine sieve placed under a gentle stream of clean running tap water for 30 minutes to 2 hours.

Excise small pieces of diseased tissue from the lesion margin and surface sterilize the tissues for 1-3 minute in 10% NaOCl or 0.1% $HgCl_2$ and then wash in three changes of sterile distilled water. Blot dry on a sterile filter paper. Place on to previously poured PVPH medium in petri plate and incubate at 24-28°C. Characteristic growth appears in the medium is typical for *Phytophthora.* Further identification can be done by microscopic observation of the mycelial growth. The growth obtained in the medium can be incubated under continuous light for 24-48 hrs. The *Phytophthora* causing foot rot namely *P. capsici* can be identified by the microscopic staining procedure.

Procedure for staining

Take clean microscopic glass slides without oil trace and place a drop of lactophenol/water/lactophenol cotton blue. Carefully withdraw a small pinch of fungal growth aseptically from the culture tube/plate with an inoculation needle (Platinum loop holder) by holding the tube against a spirit lamp. Place the fungal pinch on the slide in the drop of lactophenol and gently separate the hyphae using a sterile needle. Place a coverglass over the mount. Remove excess of stain/ mountant using filter paper and observe under the microscope. Permanent preparation may be made in lactophenol/

lacto phenol cotton blue and seal the sides of the cover glass using nail polish.

Detection from soil through baiting

The presence of *Phytophthora* in the soil can be detected and quantified using the baiting technique. *Phytophthora capsici* can be isolated using leaves of *Albizzia* sp. as bait.

Procedure

- Collect soil along with roots from the rhizosphere of black pepper plant affected by foot rot.
- Place around 50 g of the soil in a beaker.
- Add 10ml of distilled water and mix the contents of the beaker thoroughly using a glass rod.
- Put 10-20 leaf lets of Albizzia sp. Cover the beaker with thin polythene sheet to retain humidity and incubate under laboratory conditions of 20-24°C.
- Daily observe for infection of leaves (usually infection starts with in 24h).
- Remove the infected leaves and place it on a glass slide and observe for *Phytophthora* growth under the microscope.

Observation

Positive baiting shows the presence of *Phytophthora* sporangia all along the periphery of the leaves. The infected bits can be placed under the microscope to see the characteristic umbellate sporangial ontogeny typical of *P. capsici* all over the periphery of the leaves.

Identification of *P. capsici*

The *P. capsici* isolate is characterized by the umbellate branching of sporangia with long pedicel having a length of 15-200 µm.

1.3.2 Stunted disease (Viral disease)

Vein clearing, mosaic, yellow specks, mottling and small leaf are the most obvious symptoms for identifying viral infections in the nursery. As viruses are systematic in nature, primary spread occurs through plating material since black pepper is vegetatively propagated. When infected plants are used as source of planting material, the cuttings will also be infected. Hence selection of virus-free healthy mother plants is very important. Secondary spread of the disease occurs through insects such as aphids and mealy bugs. Because of closed placing of seedlings in the nursery, chances of spread through these insects are more.

1.3.2.1 Detection and diagnosis

Two viruses namely, *Cucumber mosaic virus* (CMV) and *Piper yellow mottle virus* (PYMV) are associated with the disease. Use of infected planting material is the main source for virus spread. Hence it is essential to detect planting material for the presence of viruses before they are planted. Direct antibody sandwich **e**nzyme linked immunosorbent assay (DAS-ELISA) and Polymerase chain reaction (PCR) are the two methods used for the detection of these viruses.

Direct Antibody Sandwich ELISA (DAS-ELISA)

In DAS-ELISA, Immunoglobulin G (IgG) extracted from virus specific antiserum are used for coating the solid surface to trap the antigen,

and the same IgG is labeled with an enzyme are employed for detection. The antigen gets sandwiched between IgG's and thus is referred to as the double antibody sandwich (DAS) form of ELISA. The reaction is visualized by addition of substrate of the enzyme used for labeling leading to the development of colour. The intensity of the colour is proportional to the concentration of antigen present.

Requirements

Materials

Microtitre (ELISA) plate: Polystyrene make of any reliable brand may be used Micropipette and tips: Use adjustable volume micropipette.

Multichannel pipette (adjustable100 to 300 µl) (Optional): Multichannel pipettes which can hold four, eight or 12 microtips thus permitting dispensing of volumes simultaneously into several wells can be used to add antigen, antiserum and conjugates.

Incubator, beakers, magnetic stirrer, pH meter

ELISA plate reader

Virus infected and healthy tissue.

Reagents

IgG (against virus of interest)

IgG-alkaline phosphatase conjugate

Coating buffer (Carbonate buffer): Dissolve 1.59 g Na_2CO_3, 2.93 g $NaHCO_3$, and 0.20g NaN_3 in one litre of distilled water.

Phosphate buffered saline (PBS)

PBS-T: Add 0.05% Tween 20 in 1X PBS

Antigen extraction /antibody buffer: Add 2% polyvinyl pyrrolidone (PVP, MW 40,000) and 0.2% albumin in PBS-T.

Substrate buffer: Dissolve 97 ml diethanolamine in 800 ml distilled water, adjust pH to 9.8 with concentrated Hcl, make up the volume using distilled to 1000 ml.

Para nitrophenyl phosphate (PNPP): Commercial preparation available either in powder or tablet forms may be used. Protect from light while storing.

Protocol

1. Add 200 µl of IgG diluted in coating buffer to each well of microtitre plate and incubate at 37^{o}C for 2 h.
2. Well contents are discarded and washed with PBS-T (flooding with three changes of PBS-T for three min each time). After final washing, plates are shaken dry over paper towel.
3. Test samples are extracted in antigen extraction buffer in 1:10 (w/v) using pestle and mortar. Extracts of healthy plants and the extraction buffer are to be used as negative control, whereas, extracts from known infected plant should be used as positive control. 200 µl aliquots of test sample are added and plates incubated overnight at 4^{o}C.
4. The plates are washed as mentioned in (2) and diluted enzyme conjugate (200 µl per well) in antibody buffer is added and incubated at 37^{o}C for 2 h.
5. After washing as described in (2), 200 µl of substrate (p-nitro phenyl phosphate 0.6 mg/ml of substrate buffer) is added to each well and incubated at room temperature to develop color.
6. Results are recorded by measuring the absorbance at 405 nm in an ELISA reader after 30, 60 and 120 minutes after substrate addition.

7. Stop the reaction if required by adding 100 µl of 3 M NaOH solution to each well.

Note:

1. Use appropriate dilutions of antigens, antisera and conjugates. Usually, antigen is used at 1:5 or 1:10 dilution while conjugates are diluted according to their titre value.
2. In each plate include appropriate controls, such as buffer, negative (healthy) and positive (infected).
3. Include at least two or three replications for each of the samples.
4. For better results use, freshly prepared substrate buffer.
5. PNPP is photodegradable. Hence avoid exposure to light. Cover the beaker with aluminium foil to avoid direct contact with sunlight Add as quickly as possible once PNPP solution is prepared.
6. An O D value more than twice that of healthy should be considered as positive for the presence of virus.

1.4.2 PCR for detection of *Piper yellow mottle virus*

As nucleotide sequence of the portion of PYMV genome is available, PCR can be used to detect this virus in planting materials. The important steps in the PCR include: (i) extraction of total DNA from virus infected plant or from purified virus preparations; (ii) synthesis of two (forward and reverse) virus specific primers; (iii) set up the PCR reaction in a vial by adding extracted nucleic acid, primers, nucleotides, magnesium chloride and *Taq* DNA polymerase. Use nucleic acid extracted from a known infected and healthy plants to serve as positive and negative controls respectively; (iii) keep the vials in the PCR machine and start the run as per the pre-

decided program and (iv) identify positive reactions by running contents of PCR on the agarose gel. The presence of bands at the expected position indicate that sample under test is positive.

Requirements

Materials

Thermal cycler
Microcentrifuge
Micropipette and tips
Eppendorf tubes
Thin walled PCR tubes
Ice flaking machine and ice
Agarose
Microwave
Gel apparatus with power pack
Transilluminator

Reagents

Template DNA
10 x PCR buffer
$MgCl_2$
dNTP mix
Primers
Taq DNA polymerase
TAE buffer
Ethidium bromide
Gel loading dye

Protocol

1. In a sterile 0.2 ml thin walled PCR tube, add the following

10 x PCR buffer	5.0 µl
10 mM dNTP mix	1.0 µl
100 ng/µl Forward Primer	0.5 µl
100 ng/µl ReversePrimer	0.5µl
Taq DNA polymerase (3Units/µl)	0.5 µl
Sterile water	32.5 -37.5 µl

Template DNA	5-10 µl
Total volume	50µl

Also, set up similar reaction using template DNA from a known template (positive control) and another reaction without any template (negative control).

2. Amplify the nucleic acid using the denaturation, annealing and extension times, and temperatures required for each of the steps. Times and temperatures needed to be set up depend on the primer annealing temperature and the length of fragment being amplified. Extension should be carried out for 1 min for every 1000 base pair of length of fragment being amplified. Denaturing and extension are usually carried out at 94^{o}C and 72^{o}C respectively. Temperature for annealing of primers should be either Tm or Tm-2^{o}C of the primers.

3. Run about 10-15 µl of the reaction mixture in 1% agarose gel for 1 h. through electrophoresis. Include DNA markers of appropriate size.

4. Visualize the DNA by placing the gel on a transilluminator.

Results

A successful amplification reaction should yield a readily visible DNA fragment of expected size. The gel containing samples of positive controls and template DNA under test should contain a prominent band of DNA of appropriate molecular weight. This band should be absent from the lanes containing samples of negative controls.

Reverse transcription polymerase chain reaction (RT-PCR) for the detection of *Cucumber* ***mosaic virus***

RT-PCR is a method to amplify complementory DNA (cDNA) copies of RNA. In this, the first step is the enzymatic conversion of RNA to a single stranded cDNA template. An oligo deoxynucleotide primer is hybridized to the mRNA and is then extended by an RNA-dependent DNA polymerase (Reverse transcriptase) to create cDNA copy that can be amplified by PCR. Either RNA isolated from purified viral preparations or total RNA isolated from infected plant or dsRNA isolated from infected plant can be used as template to initiate cDNA synthesis. The primer for first strand cDNA synthesis can be specifically designed to hybridize to a particular target. Amplification of the desired portion of cDNA can be achieved in PCRs primed by sense and antisense oligonucleotide primers corresponding to specific sequences in particular cDNAs. Positive and negative controls should always be included when setting up RT-PCRs.

Requirements

Materials

Micropipettes, tips, eppendorf tubes, gloves
Thin walled tubes for amplification
Water bath
PCR work station
Thermal cycler
Agarose
Gel apparatus with power pack
Microcentrifuge

Reagents

10X PCR amplificatin buffer
10 mM dNTP mix0.1M Dithreitol
$MgCl_2$
RNase inhibitor

Reverse transcriptase
Taq DNA polymerase
RNase free water
Agarose gel
RNA template (either isolated from purified virus or total RNA isolated from infected plants).
Oligonucleotide primers (both sense and antisense).

Protocol

Assemble RT-PCR reaction components on wet ice and prepare amplification mix into PCR tube in order given below.

10X PCR buffer	10.0 µl
0.1 M DTT	10.0 µl
25 mM $MgCl_2$	5.0 µl
10 mM dNTP mix	1.0 µl
Forward primer	1.0 µl
Reverse primer	1.0 µl
rRNasin (32 units/µl)	0.5 µl
AMV RT (20 units/µl)	1.0 µl
Taq DNA polymerase	1.0 µl
Template RNA*	40.0 µl
Water	to make 100.0µl

*Should be denatured by heating at 75^{o}C for 10 minutes and snap cooling on ice for at least 3 min before adding into PCR mix.

Mix reaction components assembly by inverting and place the tube on a thermal cycler and proceed with the thermal cycle profile chosen for the reaction.

Temperature programme for RT-PCR reaction (25-40 cycles)

Step	Temp (^{o}C)	Duration
Reverse transcription	42	45 min
Denaturation	90-94	30 –60 sec

Annealing*	37-55	30- 60 sec
Extension[$]	72	1- 3 min
Final extension	72	10 min

* depend on the Tm of primers.
[$] Provide one minute for every 1000 bp to be amplified.

Run about 10-20 µl of reaction on 1% agarose gel and visualize the DNA.

Results:
A successful amplification reaction should yield a readily visible DNA fragment of expected size. The gel containing samples of positive controls and template DNA under test should contain a prominent band of DNA of appropriate molecular weight. This band should be absent from the lanes containing samples of negative controls.

1.4 Nematode infestations

Root-knot nematodes (*Meloidogyne* spp.) and the burrowing nematode (*Radopholus similis*) are the two important nematode species infesting rooted cutting in the nursery. The damage caused to roots by nematode infestations result in poor growth, foliar yellowing and some times inter-veinal chlorosis of leaves. The establishment of nematode infected cuttings will be poor when planted in the field and such cuttings develop slow decline symptoms at a later date.

1.4.1 Direct examination of plant material

1.4.1.1 Detection and diagnosis

Select a few rooted cuttings at random from the nursery. Remove the soil and wash the roots in water to observe the root system carefully. Observe the roots keenly for the presence of lesions or galls or rotting. Selected roots

with lesions or galls or rotting may be teased apart using sharp needles or forceps under a stereomicroscope at magnification from 15 to 50 X to confirm the presence of nematodes. Migratory endoparasites like Radopholus emerge in a few minutes and can be found moving about. Presence of galls is a clear indication of root-knot nematode infection. They may be seen attached or embedded in the root tissue.

1.4.1.2 Extraction *of nematodes from roots*

Roots of black pepper rooted cuttings are gently washed free from soil or debris and are sliced into small bits of 2-3 cm size. They are first cleared in diluted sodium hypochlorite bleach (5.25% NaOCl) for about 4 min. Remove all traces of the bleach by thoroughly rinsing in water. Take about 1-2 g roots in a 200 ml glass vial and cover it with acid fuchsin solution (875 ml of lactic acid, 63 ml of glycerol, 62 ml of water, 0.1 g of acid fuchsin). Boil the solution for 30 s in a microwave oven or on a hot plate in a ventilated area. Allow the root tissue to cool and wash off the excess stain in running tap water. Macerate the stained roots in an electric mixer to extract the nematodes from the plant tissue. Drawing aliquots from this suspension and counting under a stereomicroscope can quantify the nematode infection in roots.

1.4.1.3 Extraction of nematodes from soil

Soil collected from polythene bags containing rooted cuttings is mixed thoroughly and about 100 ml is taken in a plastic basin. Add about 3-4 l of water and stir to free nematodes. The suspension is first poured through a 2 mm aperture sieve into another basin avoiding the sediment. The suspension in the second basin is then poured through another sieve of 125 μm aperture

and the process is repeated using 38 or 25 μm aperture sieve. The residues from the above two sieves are pooled and rinsed repeatedly to get a clean suspension that is collected in a 100 ml beaker. The contents are allowed to settle for 1-2 h and the supernatant liquid is carefully decanted. The remaining material is observed under a stereoscope for the presence of nematodes.

1.4.1.4 Storage of samples

Samples are collected in clean polythene bags, sealed and stored under warm (16-18°C) conditions till they are processed. If samples have to be stored for longer duration, the roots may be fixed in hot (60-70°C) formal acetic acid (Formaldehyde – Acetic acid, 4:1)

1.5 Insect pests

1.5.1 Leaf gall thrips (*Liothrips karnyi*)

1.5.1.1 Symptoms and Diagnosis

Infestation by leaf gall thrips (Liothrips karnyi) is more serious at higher altitudes. The feeding activity of thrips on tender leaves causes the leaf margins to curl downwards and inwards resulting in the formation of marginal leaf galls and crinkling and malformation of leaves. The symptoms of pest infestation include presence of marginal leaf galls, crinkled and malformed leaves. The adults are black and measure 2.5 - 3.0 mm in length. The larvae and pupae are creamy white.

1.5.2 Root mealybug (Planococcus spp.)

1.5.2.1 Symptoms and Diagnosis

Root mealybugs infest roots and bases of nursery plants especially at higher altitudes. The symptoms of pest infestation include yellowing, wilting and stunting of plants. The base of stems and main roots are generally infested with mealybug colonies. Many of the plants infested with root mealybugs are often infested by *Phytophthora capsici*, the fungal pathogen and plant parasitic nematodes such as *Meloidogyne incognita* and *Radopholus similis*. In such plants the symptoms are more severe and they succumb to the pest and fungal attack within a shorter period. Root mealybugs are small, oval, soft-bodied insects measuring about 1.5 mm x 1.0 mm in size and the body is covered with white waxy filaments. Mealy bugs are tended by ants and the occurrence of ant colonies at the base of the plants is an indication of the pest infestation.

1.5.3 Scale insects (*Lepidosaphes piperis* and *Marsipococcus marsupiale*)

1.5.3.1 Symptoms and Diagnosis

Among the various scale insects recorded on black pepper, *L. piperis* and *M. marsupiale* sometimes infest older cuttings in nurseries especially at higher altitudes. The pest infestation results in yellowing, wilting and drying of leaves and is more severe during the post monsoon and summer periods. *L. piperis* appear as encrustations on stems and leaves and the females are elongated (about 1mm length) and dark brown. *M. marsupiale* mainly infests leaves and the females are oval to triangular (about 1.5 mm) and yellow brown and also result in sooty mould formation.

1.5.4 Top shoot borer (*Cydia hemidoxa)*

1.5.4.1 Symptoms and Diagnosis

The top shoot borer is occasionally seen in nurseries where the bamboo method is adopted for multiplication of plants. The larvae bore into tender terminal resulting in blackening and decaying of tender terminal shoots of mother plants. The affected shoots are hollow when split open. The adult is a tiny moth with a wingspan of 10-15 mm with crimson and yellow fore wings and grey hind wings. Fully-grown larvae are grayish green and measure 12-15 mm in length.

1.6 Quality requirement under the seed act in terms of tolerance limit for pests/ diseases

Sl. No	Pest/diseases	Tolerance limit (%)
a	*Phytophthora* foot rot diseases	1.0
b	*Radopholus similis*	1.0
c	*Meloidogyne incognita*	5.0
d	Stunted disease	1.0
e	Scale insect	1.0
f	Root mealybug	1.0
g	Leaf gall thrips	5.0
h	Top shoot borer	5.0

II.CARDAMOM

Cardamom (*Elettaria cardamomum* Maton) is propularly known as queen of spices and belongs to family Zingiberace. It is grown in Western Ghats of Kerala, Karnataka and Tamil Nadu. It is a cross pollinated crop and propagated by seeds and suckers. Due to heterozygous nature of seed propagated material, clonal propagation and planting of high yielding clones is presently followed on large scale in Kerala and to a lesser extent in Tamil Nadu and Karnataka where, seed propagation is still in practice for large scale planting.

2.1 Propagation

2.1.1 Seedlings propagation

For raising the seedlings high yielding selections/consistent high yielders have to be selected. The selected plants should have > 2 panicles per yielding tillers. The panicles should posses > 15 racemes with bold capsules having >15 seeds. Seed capsules have to be harvested from 2^{nd} years crop from 3^{rd} or 4^{th} round of harvest (September). After dehusking, seeds are soaked in water, mucilage is removed by repeated washing and floats are discarded. Acid scarification with 25% nitric acid for 10 min helps in early and uniform germination. Early germinated seedlings of 4-5 leaf stages are transplanted to polybags / raised beds provided with 50 % shade. 8-10 months old seedling having minimum 3 tillers are to be selected for field planting.

2.1.2 Rapid clonal propagation

High yielding varieties/selections are generally multiplied in isolated clonal nurseries. Virus free high yielding plants are selected and subcloned

for further multiplication. For rapid multiplication following timely agro techniques has to be followed

1. High yielding plants free from pest and diseases, with characters like bold capsules and retentivity of green colour are to be selected from plantations and part of the clump has to be uprooted for clonal multiplication leaving the mother clump in its original place to induce subsequent suckers for further use.

2. The minimum planting unit consists of one grown up sucker (rhizome) and a growing young shoot.

3. Trenches having width and depth of 45 cm and convenient length have to be opened filled with jungle soil, compost and topsoil.

4. The rhizomes (planting unit) are placed at a spacing of 1.8 m x 0.6 m in trenches, thus accommodating 9259 plants per hectare of clonal nursery area.

5. Pandal protection, regular watering (once in a week during November to May) and chemical manure @ 48:48:96 g. NPK per plant in two splits have to be applied.

6. On an average 32 - 42 suckers will be produced after 12 months of planting per one planting unit. Taking the barely minimum of 50% of these suckers/clump one can get 16-21 planting units (one grown up sucker along with a growing young shoot *i.e.* sucker) from one mother-planting unit after 12 months.

7. In an area of 1-hectare clonal nursery 1,48,144 to 1,94,439 planting units can be produced after 12 months.

8. Clones thus produced should be free from virus, rhizome rot and root knot nematodes.

2.2 Test for Identification of diseases

2.2.1 Rhizome rot

This disease caused by *Pythium vexans* and *Rhizoctonia solani* takes a heavy toll in the old nurseries. Excesive soil moisture and lack of proper drainage in the nursery are the predisposing factors for infection by *P. vexans* whereas damping off caused by Rhizoctonia appears when warm temperature prevails. When this disease occurs in mature plants it is known as rhizome rot (clump rot). The initial disease symptoms are noticed on leaves, which show slight paleness and yellowing of leaves at the top. Gradually yellowing spreads into leaf blades and leaf sheaths followed by withering of plans. In primary nursery, infected seedlings collapse at collar region and die in patches. In grown up seedlings the infection starts from collar and spreads into rhizome, which first becomes discoloured, and decay. The pseudostems and rhizomes of grown up plants when infected become soft, ultimately resulting in the death of the clump. The Rhizoctonia infection is indicated in the form of brownish discoloration in the collar, whereas pinkish discoloration and soft decay is the symptom of *Pythium* infection.

2.2.1.1 Detection and diagnosis

Presence of *Pythium* or *Fusarium* can be detected by baiting the infected material in water. Characteristic growth of *Pythium* or *Fusarium* formed in water indicates its association with the disease. The subglobose or pyriform, intercalary or terminal 15-20µ long and av 17.9µ broad sporangia can identify *P. vexans* with bell shaped antheridia. *P. vexans* can be identified by the subglobose or pyriform, intercalary or terminal 15-20µ long and av 17.9µ broad sporangia with bell shaped antheridia. The monoclinous bell

shaped antheridia easily distingush *P. vexans* from other species with spherical and aplerotic oospores. *Fusarium oxysporum* can be identified by the delicate mycelium with white, peach or purple tinge. Micro conidia borne on simple phialids arising laterally on the hyphae or from short sparsely branched conidiophores. Micro conidia generally abundant. Macro conidia are thin walled, 3-5 septate fusoid –subulate and pointed at both ends. *Rhizoctonia solani* can be distinguished by the brownish fast growing mycelium with long cells, septa of branch set off from main hyphae and form brown sclerotia.

2.3 Plant parasitic nematodes

Root-knot nematode *Meloidogyne incognita* is a serious problem in cardamom.

They are present both in the nurseries and plantations of the entire cardamom growing tracts of South India. They feed on the roots; make the plants weak and vulnerable to diseases. In their presence the nursery diseases like rhizome rot and damping off due to *Rhizoctonia solani* gets aggravated.

2.3.1 Nature of damage

Poor germination of seeds in the primary nurseries, poor establishment after transplanting to secondary nurseries or main fields, yellowing and drying of leaf tips and margins, stunting and poor growth of the plants, shedding of immature capsules in the main field, heavy galling (root-knots) and abnormal branching of roots. (Galling is prominent in seedlings while in the mature plants the galls are small in size with abnormal branching of roots, which are devoid of rootlets).

2.3.1.2 Detection and diagnosis

2.3.1.2.1 Direct examination of plant material

Remove a few seedlings with their root system intact from nursery beds where symptoms like stunted growth, yellowing etc. are observed. The soil is separated and the roots are washed gently in water. Observe the root system carefully for the presence of galls or any malformation. Dip the roots in Phloxine B (0.15 g/l of water) stain for 15-20 min, rinse and examine in water. The root-knot nematode egg masses will be stained red. Or else the selected roots with galls may be teased apart using sharp needles or forceps under a stereomicroscope at magnification from 15 to 50 X to confirm the presence of nematodes.

2.3.1.2.2 Extraction of nematodes from roots and soil

For extraction of nematodes from soil and roots the methods described under black pepper (A2 and A3) may be followed.

2.4 Viral diseases

2.4.1 **Katte disease** (Mosaic or marble disease)

The first visible symptom appears on the youngest leaf of the affected tiller as spindle shaped slender chlorotic flecks measuring 2-5 mm in length. Later these flecks develop into pale green discontinuous stripes. The stripes run parallel to the vein from the midrib to leaf margin. All the subsequently emerging new leaves show characteristic mosaic symptoms with chlorotic and green stripes. The disease is caused by *Cardamom mosaic virus* (CdMV), spreads through aphid vector *Pentalonia nigronervosa* f. *caladii*

and also by the use of infected planting material. The virus is non-persistent and stylet borne. This disease is not transmitted through seed or soil; however the disease adversely affects seed germination.

2.4.2 Cardamom vein clearing (Kokke kandu) disease

This disease reported so far only from India is of relatively recent origin has become a threat to cardamom cultivation in few endemic pockets of Karnataka. Because of its characteristic symptom, it is locally referred to as *kokke kandu*, The first visible symptoms of the disease include continuous or discontinuous vein clearing. Later resetting, loosening of leaf sheath and shredding of leaves were seen. New leaves get entangled in the older leaves and form hook like tiller hence the name *kokke kandu*. The disease is caused by a virus and transmitted semi persistently by *Pentalonia nigronervosa* f. *caladii*. This disease is not transmitted through seed or soil; however the disease adversely affects the seed germination.

2.4.2.1 Detection and diagnosis for viral diseases

Examine plants for symptoms. As symptoms cannot be the sole criteria for identifying disease free nature of the planting material, it is necessary to perform ELISA or PCR tests. The details of methodology for the same are explained in pages in black pepper.

2.5 Insect pests

2.5.1 Shoot and capsule borer (*Conogethes punctiferalis*)

2.5.1.1 Symptoms and diagnosis

The shoot and capsule borer is a seroius pest in the nursery and the early stages of the larva bores in to un opened leaf buds and the later stage larvae bore the pseudostem and feed the central core resulting in drying of

the terminal leaf. The dried terminal leaf resulting in dead heart is a characteristic symptom of the post infestation. The conspicuous oozing out of frass from the bore-hole on the pseudostem is an indication of the presence of larva inside the plant. The adult is a medium-sized, orange yellow moth with black spots and with a wingspan of 20-25 mm. The fully-grown larvae are dull brown with sparsc hairs and are 15-25 mm in length.

2.5.2 Root grubs (*Basilepta fulvicorn)*

2.5.2.1 Symptoms and Diagnosis

Root grubs cause serious damage to roots of suckers in the nursery resulting in yellowing and drying of leaves, stunting of plants and rotting of roots. The infested plants can be easily pulled off from the soil. Adult beetles are shiny, metallic blue, blusih green, greening brown or brown and measure 4-6 nm length. The mature grubs are short, stout, C shaped, pale white and 1 cm in length.

2.5.3 Shoot fly (*Formosina flavipes*)

2.5.3.1 Symptoms and Diagnosis

The larvae of shoot fly feeds on the growing shoot of suckers in the resulting in yellowing and drying of terminal shoot and dead heart symptoms. The grubs are minute and are seen at the base of the growing central shoot near the rhizome.

6. Quality requirement under the seed act in terms of tolerance limit for pests/ diseases

Sl. No	Pest/diseases	Tolerance limit
a	Rhizome rot	1.0
b	Nematodes (root knot)	5.0
c	Katte	1.0
d	Kokke kandu	1.0
e	Root grub	1.0
f	Shoot borer	5.0
g	Shoot fly	1.0

III GINGER

Ginger (*Zingiber officinale* Rosc.), (Family: Zingiberaceae), a native of tropical South East Asia, is mainly cultivated in tropical and subtropical countries. The underground rhizome is used as spice. Though ginger is cultivated in many states, Kerala, Meghalaya and Arunachal Pradesh are leading producers of ginger in the country.

3.1 Propagation

3.1.1 Selection of mother rhizomes

Seeds should be collected from healthy, disease and pest free beds, which should be marked in the field when the crop is 6-8 months old and still green. Beds having minor disease incidence should be rejected for collecting the seed rhizome.

3.1.2 Preservation of seed ginger

After harvest, big plumpy rhizomes are washed thoroughly in water to remove the soil and dried in shade for a day. The seed rhizomes are treated with a solution containing Quinalphos 0.075% and mancozeb 0.3% for 30 minutes, drain the solution and dry the rhizomes under shade. Dried rhizomes are put in a pit of convenient size (1x1 m) and covered with a plank fitted with 2-3 holes for aeration. The seed rate ranges from1000-1500 Kg/ha. Small rhizome pieces of 2.5-5 cm length weighing 20-25 g each with at least two buds are used for sowing.

3.2 Tests for Diseases

3.2.1 Soft rot or rhizome rot disease

Soft rot is the most destructive disease of ginger that results in total loss of affected clumps. The disease is rhizome and soil-borne, and is caused by *Pythium* spp. The affected rhizome gives a characteristic foul smell.

3.2.1.1 Detection of soft rot causing fungi, *Pythium* sp from rhizomes

Pythium can be detected in rhizome by plating ginger rhizome samples directly onto the medium in the petriplate

Composition of Potato Dextrose Agar medium (gL^{-1})

Potato : 200
Dextrose : 20
Agar : 18
Water : 1000 ml

Potatoes were scrubbed to clean and cut in to small cubes. Two hundred g weighed, rinsed rapidly in tap water and boiled in 1000 ml of water until soft. After filtered through muslin cloth added agar and boiled to dissolve agar. Dextrose was added to this solution stirred to dissolve and made up to 1000 ml. Dispensed 100 ml aliquots in 250ml conical flasks and autoclaved at 120^0C for 20 minutes.

For detection of *Pythium* the method based on direct isolation on selective medium is available. Ten ml of the following P_{10}VP solution was added to 100 ml of molten Potato Dextrose Agar medium and poured it into the sterile Petri plates.

Preparation of P_{10}VP stock solution

P_{10} VP Medium

Medium Ingredients	:	Difco corn meal Agar (CMA)	17 g/L
		Pimaricin	: 10 ppm (= 10 ug/ml or 10 mg/ L
		Vancomycin HCL	: 200 ppm
		Pentachloronitro-Benzene (PCNB)	: 100 ppm

After making the selective medium, the suspected rhizome sample is directly plated onto the medium and incubated for 2-3 days for colony development.

3.3.1 Bacterial wilt

Bacterial wilt caused by *Ralstonia solanacearum* Biovar-3 is also a soil and seed borne disease that results in rotting of affected rhizomes.

3.3.1.1 Detection of bacterial wilt pathogen *Ralstonia solanacearum*

Microbiological methods

The suspected samples can be directly plated onto a medium specific for *R. solanacearum*. Extract from ginger rhizomes is directly plated onto the following specific medium for isolation of *R. solanacearum* and its detection. The colonies developed after 36 hours with fluidal nature, pink center with irregular margins confirmed to be *Ralstonia solanacearum.*

Composition of specific medium (gL^{-1})

Potatoes	200.0
Bactopeptone	10.0
Dextrose	2.5
Cassamino acid	1.0
Distilled water	1.0 L
Agar	18

This basal medium was autoclaved and then cooled to 50°C and the following filter sterilized solutions of antibiotics and vitamin C were added aseptically per liter of medium.

1% Polymyxin B sulphate (100 mg/ml)	10 ml
1% Cyclohexamide (100 mg/l)	10 ml
1% Bacitracin (25mg /l)	2.5 ml
0.1% Penicillin – G (0.5 mg/l)	500 µl
1% Chloramphenicol (5 g/l)	500 µl
1% Crystal violet (5 mg/l)	500 µl
1% 2,3,5 -Triphenyl tetrazolium chloride (TZC 50 mg)	5.0 ml

(2.5ml of vitamin C solution prepared by dissolving a tablet containing 500mg vitamin C in 20 ml of distilled water (62.5mg/l)

Serological methods.

Serological method is available for detection of bacterial wilt pathogen in ginger. Various techniques including serological methods have been developed for the detection of *R. solanacearum* in soil, plant materials and irrigation water (Seal, 1997)

Latently infected rhizomes have been implicated in the transmission and spread of bacterial wilt of crop plants especially in ginger. The sensitivity, short time, high speed and low cost are the main advantages of serological methods. The DAS-ELISA methodology is furnished below

Enrichment of *R. solanacearum* extracted from ginger rhizomes

Composition of Enrichment Broth

Same as *R. solanacearum* specific medium except that it does not contain the solidifying agent, agar.

Enrichment broth (4.5 ml) was dispensed in 30 ml sterile vials and 1ml of 10^{-5} dilution of the rhizome wash and vascular extract was transferred to separate vials. This was incubated for 48 hours at 30^0C with constant agitation (180 rpm) in an incubator shaker.

Coating the microtitration plate with R solanacearum specific rabbit immunoglobulins (IgG)

To 12.5 ml of coating buffer, 100µl of *R solanacearum* – specific rabbit IgG is added.

Composition of coating buffer (g)

Na_2CO_3	:	0.159
$NaHCO_3$	:	0.293
NaN_3	:	0.020
Distilled water	:	100 ml

125 µl of the coating solution was added to each well of the microtitration plate and the plate was covered with a piece of parafilm to prevent evaporation and it was incubated at 37^oC for 4 hours.

Application of the samples to the wells of the microtitration plate.

The excess coating solution was discarded from the microtitration plate and the plate was washed with washing buffer.

Composition of washing buffer

Tween 20	:	250 µl
PBS buffer	:	500 ml with sodium azide

Composition of PBS buffer (gL^{-1})

NaCl	:	8.0
KH_2PO_4	:	0.2
KCl	:	0.2

Na_2HPO_4	:	1.15
NaN_3	:	0.2

The washing is done in microplate washer (Immunowash, BIORAD, Model 1575) as follows: The wells are filled with washing buffer and it is left for 3-4 minutes in the carrier of immunowash. The plate is then emptied and refilled with washing buffer and the process is continued for two more times. After last washing the plate is put upside down on a paper towel and tapped it several times to remove all buffer.

With a micropipette, a 125 µl sample of one of the enriched sample is transfixed to each of two wells of the microtitration plate. This is repeated for all samples. The positive and negative control is also added to the plate.

Note: The positive controls are boiled suspensions of R *solanacearum* at 10^8, 10^7 $10^6$and 10^5 bacteria/ml and the negative control is an enriched extract of R *solanacearum* - free soil supplied along with the kit. The plate is sealed with parafilm and incubated at 4^oC in a standard refrigerator for 18 hours (overnight).

Addition of enzyme-labeled *R solanacearum* -specific rabbit immunoglobulins (conjugated IgG)

After 18 hours, the plate is washed as previously described until plate is completely colorless. After discarding the last washing buffer, 125µl of conjugate solution is added to each well of the plate. The plate is sealed as before and incubated it at 37^oC for 4 hours.

Conjugate solution

R *solanacearum* specific rabbit IgG conjugated to alkaline phosphatase	100 µl
Conjugate	12. 5ml

Conjugate buffer

PVP-4000	0.250 g
Non-fat powdered milk	0.025 g

Development of colour

The conjugate solution from the plate is discarded and the unbound conjugated IgG is removed by washing the plate with washing buffer as previously described. After discarding the last washing buffer, 125 µl of the color development solution is added to each well of the plate.

Preparation of colour development solution

One tablet (5 mg) of substrate P-np (P-nitrophenylphosphate) is dissolved in 12.5 ml of substrate buffer and pH is adjusted to 9.8 by adding 1N NaOH.

Composition of substrate buffer (pH 9.8)

Diethanolamine	:	10.9 ml
HCl (37%)	:	1.50 ml
Distilled water	:	6.00 ml

After adding solution to all the wells as quickly as possible, the plate is left for 60 minutes at room temperature (20-25^{o}C) for the colour to develop. The colour formation of the positive samples is observed and measured using spectrophotometer (µQuant, Microplate Reader, Bio-Tek, USA) at 405 nm wavelengths.

Note: The samples are considered positive if the absorbance is three times as much as the average of the enriched soil solution free of *R. solanacearum* used as negative control.

NCM-ELISA methodology: This is one the qualitative method for detection of the pathogen in suspected sample with out the aid of Plate Reader.

Rhizomes harvested for seed purpose can be tested for *R. solanacearum* in post enrichment NCM-ELISA. Enrichment is performed by incubating the rhizome extract as described above in selective medium at 30°C with constant agitation (180 rpm). An 8 x 12 cm of 0.45 mm pore size nitro cellulose membrane (NCM) (Biorad) is immersed for 5 minutes in 30 ml Tris Buffered Saline buffer (TBS) (0.02 mol l -1 Tris-HCl [pH 7.5], 0.05 mol l -1 NaCl, 0.01% NaN 3) for NCM-ELISA. Twenty µl of the enriched suspension are put on the membrane manually, transferred to a dry filter paper and air-dried for at least 60 minutes. The NCM dotted with the samples is incubated for 1 h in 30 ml of the blocking solution (2% non-fat powdered milk in TBS buffer) in a Petri dish (15 cm diameter), with gentle rotary agitation (50 rpm) and the membrane is incubated for 2 h with gentle agitation in 30 ml of the antibody solution (100 ml of *R. solanacearum*-specific antiserum diluted 1:1000 is added to another 30 ml of the same blocking solution). The membrane is washed to remove the unbound *R. solanacearum* antibodies with 30 ml

T-TBS (TBS with 0.05% Tween-20) three times for 3 minutes each with constant agitation at 100 rpm. Then, the membrane is incubated for 1 h with gentle agitation with 30 ml of the conjugated solution, containing goat-antirabbit antibodies conjugated to alkaline phosphatase (Biorad), diluted 1: 4000 in 30 ml of the blocking solution. The membrane is rinsed three times for 3 minutes each with T-TBS, with constant agitation (100 rpm) to remove the unbound conjugate. During the last washing, the colour development

(substrate) solution is prepared by adding drop by drop while agitating, first 100 ml of NBT (p-nitro blue tetrazolium) solution and then 100 ml of BCIP (p-toluidine salt of 5 bromo, 4-chloro, 3-indolyl phosphate) solution in a dark flask containing 25 ml of substrate buffer (0.1 mol l -1 Tris base, 0.1 mol l -1 NaCl, 0.005 mol l -1 $MgCl_2$, $6H_2O$, pH 9.6). The membrane is then incubated with 25 ml of the substrate solution with gentle agitation for 5 to 30 minutes. The reaction is stopped by discarding the substrate solution and by rinsing the membrane thoroughly with tap water. The membrane is then placed on filter paper sheets to dry. Development of colour can be compared to positive control strips for *R. solanacearum*.

It is recommended to test at least 100 samples in a lot of one ton of ginger. If 10% of the samples test positive for the pathogen, then the particular lot can be rejected.

Detection of *Ralstonia solanacearum* through PCR

This is one of the most confirmatory assays for detecting the presence of the pathogen in rhizome (or even in soil**).** DNA from rhizomes of ginger can be isolated using CTAB method. However it is recommended to use DNA isolation kits PCR amplification for detection of *R. solanacearum* in soil is performed using the DNA isolated from rhizome. Reaction volume (25µl) contained PCR buffer (Mo Bio, USA), $MgCl_2$: 1.5 mM (Promega Corporation, USA), dNTP mix: 0.05mM (Mo Bio, USA), Polymerase enzyme: 0.5u, Template DNA: 100 ng, BSA: 10µg, Primers: 20 pmoles each (Rs Primer1: 5'-gTC gCC gTC AAC TCA CTT TCC-3'; Rs Primer 2: 5'-gTC gCC gTC AgC AAT gCg gAA TCg-3').

PCR is performed in thermal cycler at the following PCR conditions as shown below and the final PCR products are resolved in 1.5 or 2.0 % agarose in 1x Tris Acetate EDTA buffer at 4^{o}C for 6 hours at 4V/cm. The gel is stained with Ethidium bromide and photographed on an UV transilluminator and the results are documented in Alpha imager 2002 for analysis.

Thermal cycling conditions for ***Ralstonia solanacearum*** specific primer

***Ralstonia solanacearum* specific primer**	
Initial denaturation at 94°C for 3 min, annealed at 53°C for 1 min and extended at 72°C for 1.5 min	
94 °C for 15 s 60 °C for 15 s 72°C for 15 s	X 30 cycles
Final extension 72°C for 5 min	

Primer sequence mentioned above is known to amplify 281bp sequence in the genomic DNA of *Ralstonia solanacearum*, which has been exploited in the detection assay for *R. solanacearum* using PCR.

3.4.1 Tests for nematode pests

Root knot (*Meloidogyne* spp.), burrowing (*Radopholus similis)* and lesion (*Pratylenchus* spp.) nematodes are important nematode pests of ginger. Stunting, chlorosis, poor tillering and necrosis of leaves are the common aerial symptoms. Characteristic root galls and lesions that lead to rotting are generally seen in roots. The infested rhizomes have brown, water soaked areas in the outer tissues. Nematodes infestation aggravates rhizome rot disease.

3.4.1.1 Detection and diagnosis

Direct examination of plant material

Collect seed rhizomes at random and examine for symptoms like dry rot. Using a sharp knife cut the rhizomes across and observe for brown patches or necrotic areas. Such pieces are teased apart in water under a stereoscope. Active nematodes can be seen wriggling out of the tissue.

3.3 Insect pests

3.3.1 Rhizome scale (*Aspideilla hartii)*

Symptoms and Diagnosis

The rhizome scale infests rhizomes in storage feeding on sap resulting in shriveling and desiccation of rhizomes. Adult (female) scales are circular (about 1mm diameter) and light brown to grey and appear as encrustations on the rhizomes.

Quality requirement under the seed act in terms of tolerance limit for pests/ iseases

Sl. No	Pest/diseases	Tolerance limit (%)
a	Rhizome rot	1.0
b	Bacterial wilt	1.0
c	Rhizome scale	1.0
d	Nematodes	5.0

IV. TURMERIC

Turmeric (*Curcuma longa* L.) (Family: Zingiberaceae) is cultivated mainly for its culinary and medicinal values and the dried rhizomes or underground stems yield the spice of commerce. India is a leading producer of turmeric in the world. Andhra Pradesh, Tamil Nadu and Orissa are the major turmeric production states in India.

4.1 Propagation

4.1.1 Preservation of seed rhizome

Depending upon the variety, the crop becomes ready for harvest in 7-9 months after planting during January-March. Bold and healthy rhizomes for seed purpose are stored by heaping in well-ventilated rooms and covered with turmeric leaves**.**The seed rhizomes can also be stored in pits with sawdust, sand, leaves of *Glycosmis pentaphylla,* etc. The pits are to be covered with wooden planks with one or two openings for aeration.To protect the rhizomes from scale and fungi , rrhizomes are dipped in quinal phos 0.075% solution and Mancozeb 0.3 % for 30 minutes and dried before storage.

4.2 Test for diseases

4.2.1 Rhizome rot

The disease is caused by *Pythium spp.* Infected rhizomes appear soft resulting in rot.

4.2.1.1 Detection and diagnosis

Methodology used for the detection of rhizome rot of ginger is employed (refer pages19, 20).

4.3 Test for nematode pests

Root knot nematodes (*Meloidogyne* spp.) and burrowing nematodes cause damage to turmeric. Root lesion nematodes (*Pratylenchus* spp.) are of common occurrence in Andhra Pradesh.

4.3.1 Direct examination of plant material

Collect seed rhizomes at random and examine for symptoms like dry rot. Using a sharp knife cut the rhizomes across and observes for brown patches or necrotic areas. Such pieces are teased apart in water under a stereoscope. Active nematodes can be seen wriggling out of the tissue.

4.4 Test for insect pests

4.4.1 Rhizome scale (*Aspideilla hartii)*

4.4.1.1 Symptoms and Diagnosis

The rhizome scale infests rhizomes in storage feeding on sap resulting in shriveling and desiccation of rhizomes. Adult (female) scales are circular (about 1mm diameter) and light brown to grey and appear as encrustations on the rhizomes.

Quality requirement under the seed act in terms of tolerance limit for pests/ diseases

Sl. No	Pest/diseases	Tolerance limit (%)
a	Rhizome rot	1.0
b	Bacterial wilt	1.0
c	Rhizome scale	1.0
d	Nematodes	1.0

V. VANILLA

Vanilla (*Vanilla planifolia* Andr.), (Family: Orchidaceae), a native of Mexico, is cultivated to a limited extent in Kerala, Karnataka and Tamil Nadu. The crop has gained considerable importance in recent years due to increased demand for natural vanillin that is extracted from its pods.

5.1 Propagation

5.1.1 Selection of mother vines

Vigorously growing healthy disease and pest free vines are selected from yielding plants for the collection of cuttings. Vines of current years growth with dormant auxiliary vegetative buds can be selected for taking stem cuttings. Longer cuttings having 60-120 cm in length, with 15-20 internodes 13-15 leaves are ideal for planting. Over matured part of vanilla plant and flowered one may not be used for the selection of cuttings.

5.2 Test for Diseases

5.2.1 Stem Rot

5.2.1.1 Detection and diagnosis

The disease usually appears during the post monsoon periods of November-February months, when the temperature is very low and humidity relatively very high. The disease appears in the form of water soaked lesions extending to both sides of the stem forming a yellow coloration. When the basal or middle portions of the vines decay and shrivel, the remaining distal portions of the vines show wilting symptoms. Vine rot and drying are generally observed at the basal portions above the ground level. The infected areas showed decaying symptoms and the remaining portions wilt

off as they have insufficient number of aerial roots. The causal organisms were identified as *Fusarium oxysporum f.sp.vanillae* & *Phytophthora meadii.* The method of identification of the organisms is the same as described for capsule rot & rhizome rot of cardamom

5.2.2 Root Rot

5.2.2.1 Detection and diagnosis

Initially the disease appears in the form of browning and death of both underground aerial roots. Aerial roots may be produced in large numbers but most of them die before entering the soil and results in flaccidity and shriveling of the stem and finally the vine droops. During the periods of drought the vines become less capable of withstanding the infection due to inadequate uptake of water and minerals, excess sunlight and excess bearing of fruits due to over pollination. The causal organisms of the disease include *Phytophthora meadii* and *Sclerotium rolfsii Sclerotium rolfsii* can be identified by the formation of pure white thick mycelial mat and white to brownish mustard like sclerotia over the infected areas. Culturing of the infected portions in Potato-Dextrose agar will also show the pure white mycelial mat with in 48hrs and white to brown sclerotial formation in seven days.

5.2.3 Tip rot and die back

5.2.3.1 Detection and diagnosis

Visible symptoms of rotting develop on the petiole and lower portions of the youngest leaf. Within a few days the rotting extends to the whole funnel like leaf and the shoot apex become soft brown and droop down. During rainy season, the rotting and dieback extends downwards until the next

node. The causal organism(s) of the disease include *Phytophthora meadii* or *Fusarium oxysporum.*

The method of identification of the organisms is the same as described in rhizome rot of cardamom. (Isolation procedures were the same as described for foot rot disease of black pepper. For fungi other than *Phytophthora* or *Pythium*, Potato Dextrose agar can be used instead of PVPH or $P_{10}VP$ medium.)

5.2.4 Test for viral infection

5.2.4.1 Mosaic and necrosis diseases

5.2.4.1.1 Detection and diagnosis

Various kinds of mosaic such as mild mottle, mild mosaic and mild chlorotic streak (could be seen when the leaf is held against light) are observed. In a few cases, such mosaics are also associated with leaf distortion with wavy margin. The size of the leaves also gets reduced and in advanced stages, leaves become brittle and show severe crinkling. The association of *Cucumber mosaic virus*, *Cymbidium mosaic virus*, *Vanilla mosaic virus* have been reported with the disease. The necrosis disease is characterized by the appearance of brown necrotic patches on the stem region with shriveled appearance. The affected stem shows distinct necrotic lesions of varying length (few mm to several cm). This disease is different from the fungal induced stem rot. Stem necrosis can be distinguished from stem rot caused by fungi by the following:

- Stem rot affected region will be totally blighted and very soft which can be easily felt by touching the affected region, while stem necrosis

(caused by viruses) affected region when touched appear very dry and hard and gives cracking sound when attempted to break open. Fungal diseases are commonly seen during the wet monsoon period whereas stem necrosis is seen all though the year.

- A close look at the stem rot affected region show a white cottony growth on the upper surface of the affected region while no such growth is seen with stem necrosis affected region.

- In case of stem rot the portion above the lesion often wilts yellowing of leaves whereas in stem necrosis no wilting would be seen.

In a few cases, necrosis is also seen on the leaves at the lower surface in the form of scab. This often gives the appearance of sun scorch. The disease initially starts as a necrotic spot on the stem and slowly gets enlarged and encircles the stem. In an affected plant, necrosis may be seen only at one or few regions on the stem. Rest of the stem region looks apparently healthy without any visual symptoms. A few of the necrosis affected plants also show mosaic symptoms on leaves.

The major means of spread of the virus is though the use of infected stem cuttings. Insects may also play an important role in the transmission and spread of the disease in nature.

The presence of viruses in the planting material may be confirmed through sensitive laboratory tests such as enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). The detailed procedure of both these techniques have been given in pages 5-8.

5.3 Quality requirement under the seed act in terms of tolerance limit for pests/diseases

Sl. No	Pest/diseases	Tolerance limit (%)
a	Fungal diseases (Stem/root rot)	1.0
b	Viral diseases (Mosaic and necrosis)	1.0

SEED TESTING FOR SEED SPICES

The seed spices have emerged as one of the important group of spices crops in India. All most all states in India grow one or more seed spices. But major growing area lies in arid to semi-arid regions mainly in the states of Rajasthan and Gujarat and some extent in Tamil Nadu, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh and Chattisgarh. About 50 varieties of different seed spice crops have been released but sufficient quantity of quality seeds are not available to the farmers. The quality seed of seed spices is a pre-requisite for successful raising of these crops. The quality seed production requires information on seed certification standards particularly the field and seed standards. The minimum seed certification standards and necessary guidelines on the quality seed production of seed spices are warranted by the seed producers and related agencies time and again.

6.1 Seed quality parameters:

There are four parameters considered important in quality seed production such as physical purity, germinability, health and genetic purity.

The seed spices crops *viz.,* coriander, cumin, fennel ajowain and dill belong to family Apiaceae and are mostly cross pollinated in nature. The cross pollination occurs mostly through insects (entomophilous). The coriander and cumin are considered as often cross pollinated, whereas fennel, ajowain and dill are cross pollinated crops. The fenugreek belonging to Leguminosae family is self pollinated in nature. The isolation distance to

be kept between the fields of two varieties to produce foundation and certified seed, respectively are given in Table 1.

6.1.1 Isolation distance for seed production in seed spice crops

Name of crop	Distance in meters	
	Foundation seed	**Certified seed**
Fenugreek (highly self pollinated)	50	25
Coriander (often cross-pollinated)	800	400
Cumin (often cross pollinated)	800	400
Fennel (cross pollinated)	1000	800
Ajowain (cross pollinated)	1000	800
Dill (cross pollinated)	1000	800

The specific requirement for seed crop of seed spices showing maximum permitted limits for off types, objectionable weeds, and designated diseases are given in Table 2.

6.1.2 Purity Standards of Seed Spices

Name of crop	Maximum permitted limits (%)					
	Foundation seed			Certified seed		
	Off type (un wanted seed)	Objectionable weed	Diseased plants	Off type/ Un-wanted seed	Objectionable weed	Diseased plants
Coriander	0.10	-	0.10	0.50	-	0.50
Cumin	0.10	0.01	0.10	0.20	0.02	0.50
Fennel	0.10	-	0.10	0.20	-	0.50
Fenugreek	0.10	0.01	0.10	0.20	0.02	0.50
Ajowain	0.10	0.01	0.10	0.20	0.02	0.50
Dill	0.10	0.01	0.10	0.20	0.02	0.50

The objectionable weeds and designated diseases as reported for various seed spices crops are given here.

6.1.3 Objectionable weeds in seed spices:

Cumin : *Plantago pumila* (jiri)
Fenugreek : *Melilotus* (Senji)
Ajowain : *Trachyspermum roxumburghai*

6.1.4 Designated seed borne and other diseases in seed spices:

Coriander **:** Fusarium wilt (*Fusarium oxysporum* f.sp.*coriandrii*), stem gall (*Protomyces Macrosporus),* powdery mildew *(Erisiphae polygoni)*

Cumin **:** Fusarium wilt (*Fusarium oxysporum* f.sp. *cumini),* Cumin blight (*Alterneria Burnsii),* powdery mildew (*Erisiphae polygoni)*

Fennel **:** Alterneria blight (*Alterneria tenuis*) and *Ramularia* blight (*Ramularia foeniculi*)

Fenugreek **:** Root rot (*Rhizoctonia solani*), downy mildew (*Peronospora trigonellae*), leaf spot (*Cercospora traversiana*), powdery mildew (*Erisiphae polygoni*)

Ajowain **:** Collart and root rot (*Sclerotium rolfsii*), blight *(Alternaria* spp.)

Dill **:** Root rot F (*Fusarium oxysporum*), powdery mildew (*Erisiphae polygoni*)

6.1.5 Seed standards in seed spices:

The seed spice crops meeting field standards for certification should be harvested, threshed and processed as per guidelines issued by the certification agency. Soon after the completion of seed processing the composite sample is taken for analysis of the seed standards such as

percentage of pure seed, inert matter, other crop seed, total weed seed, germination and moisture. In order to ensure quality seed production, it is necessary to strictly follow the seed standards. The prescribed permittable seed standard limits of seed spices crops is given in Table 3. On receipt of Seed analysis Report and the result of grow-out-test, the packing, tagging, sealing follows the issuance of certificate.

6.1.6 Seed standards for Seed Spices

Name of crop	Seed standard (%)											
	Pure seed (minimum)		Inert matter (maximum)		Seed of other crop (maximum)		Total weed Seed (maximum)		Germination (%)		Moisture (%)	
	FS	CS	FS	CS	FS	CS	FS	CS	FS	CS	FS	CS
Coriander	98	97	2	3	0.10	0.20	0.10	0.20	65	65	10	10
Cumin	95	95	5	5	0.05	0.10	0.10	0.20	65	65	8	8
Fennel	95	95	5	5	0.05	0.10	0.10	0.20	70	70	8	8
Fenugreek	98	98	2	2	0.10	0.20	0.10	0.20	70	70	8	8
Ajowain	95	95	5	5	0.05	0.10	0.10	0.20	65	65	8	8
Dill	95	95	5	5	0.05	0.10	0.10	0.20	70	70	8	8

FS - Foundation Seed; **CS -** Certified Seed

Note:

1. Insect damaged seeds should not be more than 0.5% in each sample.
2. The moisture % for vapour proof container should be 2% less than the prescribed limit.

The seed quality control is not an easy task. It requires lot of planning and efforts on the part of government. The production and marketing agencies are also equally responsible to maintain and preserve the seed quality to the highest possible standard.

The detailed procedure / methodology for seed testing is given in Annexure – I.

6.2 SUBMITTING SEED SAMPLES FOR TESTING

6.2.1 Timing

Timing of seed stocks one to two months prior to planting should reflect seed quality following storage and handling.

6.2.2 Obtain representative sample:

Take several grab samples or use a grain spear to collect a representative sample of the grain. Avoid the temptation to take the whole sample from the soil or augerhopper. These samples can give a biased indication of seed viability. Contact a seed-testing laboratory that can recommend a sampling procedure for your particular situation.

6.2.3 SEED SAMPLING

The first step in seed testing is sampling. ***Seed lot***: It is a uniformly blended quantity of seed either in bag or in bulk.

Seed size	**Maximum quantity per lot**
Larger than wheat and paddy	20,000 kg
Smaller than wheat and paddy	10,000 kg
Maize	40,000 kg

Details are to be attached with the samples.

- Name and address
- Kind of seed and variety
- Year of harvest
- Type of test required (e.g. germination, purity or tetrazolium), and then forward the sample to be tested to the seed laboratory of your choice.

Sampling intensity

For seed lots in bags (or container of similar capacity that are uniform in size)

Up to 5 containers	Sample each container but never < 5 primary samples
6-30 containers	Sample atleast one in every 3 containers but never < than 5 primary samples
31-400 containers	Sample atleast one in every5 containers but never < than 10 primary samples
401 or more containers	Sample atleast one in every 7 containers but never < than 80 primary samples

When the seed is in small containers such as tins, cartons or packets a 100 kg weight is taken as the basic unit and small containers are combined to form sampling units not exceeding these weight e.g. 20 containers of 5 kg each. For sampling purpose each unit is regarded as one container.

For seeds in bulk

Up to 500 kgs	At least 5 primary samples
501 to 3000	Primary samples for each 300 kg but not less than 5 P.S
3001-20,000	Primary samples for each 500kg but not less than 10 P.s
20,001 and	Primary samples for each 700 kg but

above not less than 40.

6.2.6 METHODS OF SAMPLING

6.2.6.1 Hand sampling

This is followed for sampling the non-free flowing seeds or chaffy and fuzzy seeds such as cotton, tomato, grass seeds etc., In this method it is very difficult to take samples from the deeper layers of bag. To over come this, bags are emptied completely or partly and then seed samples are taken. While removing the samples from the containers, care should be taken to close the fingers tightly so that no seeds escape.

6.2.6.2 Sampling with triers

By using appropriate triers samples can be taken from bags or from bulk.

Bin samplers: Used for drawing samples from the lots stored in the bins.

Nobbe trier: This is suitable for sampling seeds in bag, not in bulk.

Sleeve type triers or stick triers: It is the most commonly used trier for sampling. There are two types *viz., w*ith compartments and without compartments. This trier is used for drawing seed samples from the seed lots packed in bags or in containers.

6.2.7 Weight of submitted sample

The minimum weight for submitted samples for various tests are as follows:

Moisture test

100 grams for those species that have to be ground and 50 grams for all other species.

6.2.8 For verification of species and cultivar

Crop	Lab only (g)	Field plot & lab (g)
Peas, beans, maize, soybean and crop seeds of similar size	1000	2000
Barley, oats, wheat and crop seeds of similar size	500	1000
Beetroot and seeds of similar size	200	500
All other genera	100	200

6.2.9 For other tests like purity and count of other species

Crop	Size of seed lot (kg)	Size of submitted sample (g)	Size of working sample for purity (g)	Size of working sample for count of other species (g)
Coriander Fennel	10,000 10,000	400 180	40 18	400 180
Fenugreek	10,000	450	45	450

The samples taken may be packed in bags, sealed and marked for identification. For moisture testing the samples should be packed separately in moisture proof polythene bag and kept in the container along with submitted samples.

6.2.10 Information to accompany the sample

Date, Kind, Variety, Class of seed, Lot No., and Quantity of seed in lot (kg), Sender's name and address. Test(s) required: (1) Purity (2) Germination and (3) Moisture.

6.2.11 Types of sample used in seed testing lab

Service sample : Sample received from the farmers.

Certified sample : Sample received from certification agencies or officers.

Official sample : Sample received from the seed inspectors.

6.3 MIXING AND DIVIDING OF SEEDS

6.3.1 Mechanical dividing

The reduction of sample size is carried out by the mechanical dividers suitable for all seeds except for chaffy and fuzzy seeds.

Soil divider: It is suitable for large seeds and chaffy seeds.

6.3.2 Random cups method

This method is suitable for seeds requiring working sample upto 10 grams provided that they are not extremely chaffy and do not bounce or roll (e.g) *Brassica spp*. Six to eight small cups are placed at random on a tray. After a preliminary mixing, the seed is poured uniformly over the tray. The seeds that fall into the cup is taken as the working sample.

6.3.3 Spoon method

This is suitable for samples of single small seeded species. Sufficient portions of seed are taken to estimate a working sample of approximately but not less than the required size.

6.3.4 Hand halving method

This method is restricted to the chaffy seeds. The halved portions are arranged in rows and alternate portions are combined and retained. The process is repeated until the sample of required weight is obtained.

6.4 PHYSICAL PURITY ANALYSIS

6.4.1 Purity test

A purity test is the analysis of a representative sample of the seed tonnage for contaminants, such as weed seeds, other crop seeds, broken seed, soil, insects and any other contaminants. Any prohibited matter (Seeds and live insects) found during the purity analysis are identified and entered on the report. Seed must not be sold until prohibited material is removed from the seed lot. The purity test does not cover the genetic purity of the sample.

Pure ***seed:*** The seeds of kind / species stated by the sender. It includes all botanical varieties of that kind / species. Immature, undersized, shrivelled, diseased or germinated seeds are also pure seeds. It also includes broken seeds, if the size is > 1/2 of the original size except in leguminosae and cruciferae, where the seed coats entirely removed are regarded as inert matter.

Other crop seed**:** It refers to the seeds of crops other than the kind being examined.

*Weed seed***:** It includes seeds of those species normally recognized as weeds or specified under seed act as a noxious weed.

*Inert matter***:** It includes seed like structures, stem pieces, leaves, sand particles, stone particles, empty glumes, lemmas, paleas and chaff. Awn stalks longer than florets, spikelets are to be removed and treated as inert matter.

Method:

The Working sample:

The purity analysis is done on the working sample of prescribed weight drawn from submitted samples. The analysis may be made on one working sample of the prescribed weight, or on two sub-samples of at least half this weight, each independently drawn.

Weighing The working sample

The number of decimal places to which the working sample and the components of the working sample should be weighed as below:

Weight of the working sample in gram	Number of decimal places required	Example
<1	4	0.7534
1-9.999	3	7.534
10-99.99	2	75.34
100-999.9	1	753.4
1000 or more	0	753.4

Physical separation:

The working sample after weighing is separated into its components *viz*., pure seed, other crop seed, weed seed and inert matter.

Method of purity separation:

Place the sample on the purity work board after sieving / blowing operations and separate into other crop seeds, weed seeds and inert matter.

After separation identify each kind of weed seeds, other crop seeds as

to genus and species. The names and number of each are recorded.The type of inert matter present should also be noted.

Calculation:

All the four components must be weighed to the required number of decimal places. The percentage of the components are determined as follows:

$$\% \text{ of component} = \frac{\text{Weight of individual component}}{\text{Total weight of all components}} \times 100$$

If there is a gain or loss between the weight of the original samples and the sum of all the four components is in excess of one percent, another analysis should be made.

Duplicate tests

Analysis result near the borderline in relation to the seed standards, one more test is done and the average is reported. However, if a duplicate analysis is made of two half samples, or whole samples, the difference between the two must not exceed the permissible tolerance.

Determination of inseparable other crop seeds and objectionable weed seeds (by Number /Kg)

Whole submitted sample is used and the number per kg may be calculated and reported even if the working sample is less than a Kg.

Determination of other distinguishable varieties **(ODV)**

Ten times, the size of working sample is used. It is determined based on the morphological characters of the seeds. The authentic samples should

be available for comparison. The number of ODV should be calculated and reported as No./kg of seeds.

Calculation of results:

The % by weight of each of the component should be calculated to one decimal place.

Reporting results:

The results of each component are given in one decimal place and the total of all components must be 100. Components of < 0.05% shall be reported as Trace. If the result for a components is nil, this must be shown as '-0.0-' in the appropriate space.

6.4.2 Equipment used for purity analysis

Seed Blower

There are 2 plastic columns one for larger seeds and the other for smaller seeds. The plastic column is provided with a semi-circular outlet where the terminal velocity of wind can be adjusted. A time clock is also provided for the automatic running of the bowler. The inert matter is separated by stratification using the terminal velocity of air.

Purity work board

This is used for effective separation of different components. At the centre of the board, there is an illumination by which the emptiness of the seed is easily identified.

6.4 GERMINATION TEST

Germination tests shall be conducted with the pure seed fraction. A minimum of 400 seeds are required in four replicates of 100 seeds each or 8

replicates of 50 seeds each or 16 replicates of 25 seeds each depending on the size of the seeds and size of containers of substrate. The test is conducted under favourable conditions of moisture, temperature, suitable substratum and light if necessary. No pretreatment to the seed is given except for those recommended by ISTA.

Medium

The medium serves as moisture reservoir and provides a surface or medium for which the seeds can germinate and the seedlings grow. The commonly used substrata are sand, paper and soil.

Sand

Size of sand particle: Sand particles should not be too large or too small. The sand particles should pass through 0.80 mm sieve and retained by 0.05 mm sieve.

Toxicity: Sand should not have any toxic material of any pathogen. If any pathogen is found, then the sand should be sterilized in an autoclave.

Germination tray: When we use the sand, germination trays are used to carry out the test. The normal size of the tray is 22.5 x 22.5 x 4 cm. The tray may be either zinc or stainless steel.

Method of seed treatment

Seeds in sand (s): Seeds are planted in a uniform layer of moist sand and then covered to a depth of 1 cm to 2 cm with sand.

Top of sand (TS): Seeds are pressed into the surface of the sand.

Spacing: We must give equal spacing on all sides to facilitate normal growth of the seedling and to avoid entangling of seed and spread of disease. Spacing should be 1-5 times the width or diameter of the seed.

Water: The amount of water to be added to the sand will depend on size of the seed. For cereals, except maize, the sand can be moistened to 50% of its water holding capacity. For large seeded legumes & maize sand is moistened to 60% WHC.

PAPER

Most widely used paper substrates are filter paper, blotter or towel (kraft paper). It should have capillary movement of water, at vertical direction (30 mm rise / min.) .It should be free from toxic substances and free from fungi or bacteria. It should hold sufficient moisture during the period of test. The texture should be such that the roots of germinating seedlings will grow on and not into the paper.

Methods

Top of paper (TP): Seeds are placed on one or more layers of moist filter paper or blotter paper in petridishes. The petridishes are covered with lid and placed inside the germination cabinet. This is suitable for those seeds, which require light.

Between paper (BP): The seeds are germinated between two layers of paper.

Roll towel method: The seeds are placed between two layers of paper and rolled in towels. The rolled towels are placed in the germinator in an upright position.

Inclined plate method: Germination on glass plate with germination paper and kept at an angle of 45^{o}.

Soil

Should be non-caking, free from any large particles. It must be free from weed seeds, bacteria, fungi, nematode or toxic substances. Soil is not recommended for reuse.

Temperature: Required temperature is maintained (most seeds germinate between 20-30^{o}C)

Light: Light should be provided for seeds requiring light for germination (e.g.) lettuce and tobacco.

6.5.2 Germination apparatus

Germination cabinet / Germinator: This is closed chamber where in temperature and relative humidity are controlled. We can maintain the required temperature.

Room germinator: It works with same principles as that of germinator. This is modified chamber of larger one and the worker can enter into it and evaluate the seedlings. Provisions are made to maintain the temperature and relative humidity. This is used widely in practice.

Counting board: This is used for accurate counting and spacing of seeds. This consists of 2 plates. The basal one is stationary and top one is movable. Both top and basal plates are having uniform number of holes *viz*., 50/100, when the plates are in different position. After taking the sample, the top plate is pulled in such a way that the holes are in one line so that the fixed numbers of seeds fall on the substratum.

Vacuum counter: Consists of head, pipe and wall. There are plates of 50 or 100 holes which can be fitted to the head. When vacuum is created the plate absorbs seeds and once the vacuum is released the seeds fall on the substrate.

Impression board: Made of plastic / wood with 50 or 100 holes per pins. Here the knobs are arranged in equal length and space. By giving impression on the sand it makes uniform depth and spacing for seed.

6.5.3 GERMINATION REQUIREMENTS FOR DIFFERENT CROPS

Crop	Substratum	Temp. (^{0} C)	Light	First count days	Final Count days	Recommendation for breaking dormancy
Coriander (*Coriand*um *sativum* I)	TP,BP	20-30, 20	L	5	10	7
Fennel (*Foeniculam vulgar*e Mill)	TP,BP,TS	20-30	L	5	14	Light
Fenugreek (*Trigonella foenumgraecum* L)	TP,BP	20-30, 20	-	4	14	Light
Cumin (*Cuminum cyminum*)	TP,BP	20-30	-	5	7	-

TP- TOP of paper
BP- Between paper
TS- Top of Sand

6.6 Seedling evaluation

ISTA classified the seedlings into different categories based on the development of essential structures.

6.6.1 Categories of seedlings

Normal seedlings

Seedlings which show the capacity for continued development into normal plant when grown in favourable conditions of soil, water, temperature and light.

Characters of normal seedlings

a. A, well-developed root system with primary root except in certain species of graminae which normally producing seminal root or secondary root.
b. A, well-developed shoot axis consists of elongated hypocotyl in seedlings of epigeal germination.
c. A, well-developed epicotyl in seedlings of hypogeal germination.
d. One cotyledon in monocotyledons and two in dicotyledons
e. A, well-developed coleoptile in graminae containing a green leaf.
f. A, well-developed plumule in dicotyledons.
g. Seedlings with following slight defects are also taken as normal seedlings. Primary root with limited damage but well developed secondary roots in leguminaosae (Phaseolus, Pisum) graminae (maize), cucurbitaceae (cucumis) and malvaceae (cotton).
h. Seedlings with limited damage or decay to essential structures but no damage to conducting tissue.
i. Seedlings, which are decayed by pathogen but it, is clearly evident that the parent seed is not the source of infection.

Abnormal seedlings

Seedlings which do not show the capacity for continued development into normal plant when grown in favourable conditions of soil, water, temperature and light.

Type of abnormal seedlings:

Damaged seedlings: Seedlings with any one of the essential structures are missing or badly damaged so that the balanced growth is not expected. Seedlings with no cotyledons, with splits, cracks and lesions on essential structures and without primary root.

Deformed seedlings: Weak or unbalanced development of essential structures such as spirally twisted or stunted plumule or hypocotyl or epicotyl, swollen shoot, stunted roots etc.,

Decayed seedlings: Seedlings with any one of the essential structures showing diseases or decayed symptoms as a result of primary infection from the seed which prevents the development of the seedlings.

Hard seeds

Seeds which do not absorb moisture till the end of the test period and remain hard (e.g.) seeds of leguminosae and malvaceae.

Fresh ungerminated seeds

Seeds which are neither hard nor have germinated but remain firm and apparently viable at the end of the test period.

Dead seeds.

Seeds at the end of the test period are neither hard nor fresh or have produced any part of the seedlings. Often dead seeds collapse and a milky paste come out, when pressed at the end of the test.

6.6.2 Retesting

If the results of a test are considered unsatisfactory it shall not be reported and a second test shall be made by the same method or by alternative method under the following circumstances.

a. Replicates performance is out of tolerance
b. Results being inaccurate due to wrong evaluating of seedlings or counting or errors in test conditions.

c. Dormancy persistence or phytotoxicity or spread of fungi or bacteria. The average of the two tests shall be reported.

6.6.3 Use of tolerances

The result of a germination test can be relied upon only if the difference between the highest and the lowest replicates is within accepted tolerances. To decide if two test results of the same sample are compatible again the tolerance table is used.

6.6.4 maximum tolerated ranges between replicates

Average percentage germination		Maximum range	Average percentage germination		Maximum range
1	2	3	1	2	3
99	2	5	87 to 88	13 to 14	13
98	3	6	84 to 86	15 to 17	14
97	4	7	81 to 83	18 to 20	15
96	5	8	78 to 80	21 to 23	16
95	6	9	73 to 77	24 to 28	17
93 to 94	7 to 8	10	67 to 72	29 to 34	18
91 to 92	9 to 10	11	56 to 66	35 to 45	19
89 to 90	11 to 12	12	51 to 55	46 to 50	20

This table indicates the maximum range that is the difference between the highest and lowest in germination percentages tolerable between replicates, allowing for random sampling variation only at 0.025 probabilities. To find the maximum tolerated range in any case, calculate the average percentage to the nearest whole number of the four replicates. If necessary,

From 100- seed replicates by combining the sub-replicates of 50 or 25 seeds which were closest together in the germinator. Locate the average in

column 1 or 2 of the table and read off the maximum tolerated range opposite in column 3. This table indicates the tolerance to be used in deciding if two tests are compatible, allowing for random sampling variation only at 0.025 probability.

To determine if two tests are compatible, calculate the average percentage germination of the two tests to the nearest whole number and locate it in column 1 or 2 of the Table. The tests are compatible if the difference between the germination percentages of the two tests does not exceed the tolerance in column 3 opposite.

6.6.5 Compatibility of tests

Average percentage germination		Tolerance
1	2	3
98 to 99	2 to 3	2
95 to 97	4 to 6	3
91 to 94	7 to 10	4
85 to 90	11 to 16	5
77 to 84	17 to 24	6
60 to 76	25 to 41	7
51 to 59	42 to 50	8

6.6.6 Reporting results

The result of the germination test is calculated as the averages of 4 x 100 seed replicates. It is expressed as percentage by number of normal seedlings. The percentage is calculated to the nearest whole number. The percentage of abnormal seedlings, hard, fresh and dead seeds is calculated in the same way. These should be entered on the analysis certificate under appropriate space.

6.7 Types of germination

Two types of seed germination occur and neither appears to be related to seed structure. These two types can be illustrated by observing the germination of bean and pea seeds. Although these seeds are similar in structure, their germination patterns are quite different. Epigeal germination in beans and hypogeal in peas.

Epigeal germination

During germination, the cotyledons are raised above the ground. During root establishment the hypocotyl begins to elongate in an arch which breaks thro' the soil, pulling the cotyledon and enclosed plumule (epicotyl) thro' the ground and projecting them in the air. (eg.) bean, castor, cucurbits and other dicots and onion.

Hypogeal germination

During germination the cotyledons remain beneath the soil while the plumule pushes upward and emerges above the ground. Here the epicotyl (plumule) elongates (e.g.) Peas, grams, mango, grasses and many other species.

6.8 Quick viability test

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 comparatively unstained seeds are non-viable. This test is not valid for previously germinated seeds.

6.8.1 Method of Tetrazolium (TZ) testing

Testing sample

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

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_2PO_4 in 1000 ml of water.

Solution 2: Dissolve 11.876 g of $Na_2HPO_4.2H_2O$ in 1000 ml of water.

Take 400 ml of solution 1 and 600 ml of solution 2 and mix them together. In a 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 of TZ testing

The seeds are first prepared for staining, then stained and evaluated from light.

Method 1: Bisect longitudinally

Soak the seeds in water for 3 to 4 hours. Bisect the seeds by cutting longitudinally thus exposing the main structures of the embryo. Use1/2 of each of seed for testing. (e.g.) maize, sorghum, small grains, large seeded grasses.

Method 2: Bisect laterally

The seeds are cut laterally near the centre of the seed above the embryo. Place embryo end in TZ solution. (e. g) small seeded grasses.

Method 3: Pierce with needle

Puncture the seeds by piercing thro' the seed into the endosperm near the embryo but avoid injury to the embryo. (e.g) small seeded grass.

Method 4: Remove seed coat

Soak the seeds in water for 3-4 hours and then remove 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. (e.g) dicots with seed coats impermeable to tetrazolium

Method 5: Conditioning only

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 preferably to condition these seeds slowly in moist paper towels overnight before staining, so that they absorb moisture slowly without any damage to the seed. (e.g) Large seeded legumes

Method 6: No conditioning or preparation

Seed coats of these seeds are permeable to TZ and the embryos usually will stain without conditioning. (e.g.) Small seeded legumes

Staining

The prepared seeds should be placed in suitable container (small beakers, petridishes) 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 methods of preparation, and different temperatures (>1 hr to 8 hrs). When 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^{o}C for interpretation.

Evaluation of samples

A normal stain appears cherry red.

Monocots - Non viable

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

Dicot - Non viable

1. Embryo completely unstained
2. More than extreme tip of radicle 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 radicle

6.9 DETERMINATION OF MOISTURE CONTENT

The moisture content of a seed sample is the loss in weight when it is dried. It is expressed as a percentage of the weight of the original sample. It is one of the most important factors in the maintenance of seed quality.

6.9.1 Methods of moisture determination

Air oven method

In this method, seed moisture is removed by drying at a specified temperature for a specified duration.

Moisture meters

Moisture meters estimate seed moisture quickly but the estimation is not as precise as by the air oven method.

Weight of the submitted sample

100 gm for species that have to be ground. 50 gm for all other species. The sample should be submitted in polythene bag of 700 guage.

6.9.1.1 Air oven method for seed moisture estimation

Materials required

Grinding mill

It should be constructed of non-absorbent material. It should grind evenly and should be operated at such a speed that during grinding, it should not cause heating of the ground material. Air currents that might cause loss of moisture must be reduced to a minimum. The finess of grinding should be adjustable.

Container

Containers of glass or non-corrosive metal (e.g. Stainless steel) should be used.

Oven

A good quality electric air oven with a thermostatic or electronic temperature control for maintaining temperature within = 1^0C is required.

Desiccator

Analytical balance

Sieves : A set of wire mesh sieves with meshes of 0.5 mm, 1,0 mm and 4.0 mm.

Grinding

For some seeds (e.g. cereal and cotton), fine grinding is essential before the moisture content is determined. In such cases, at least 5% of the ground material should pass through a wire sieve with meshes of 0.5 mm and not more than 10 remain on a wire sieve with a mesh of 1.0 mm. For leguminous seeds, coarse grinding is recommended. At least 50% of the ground material shall pass through a wire sieve with meshes of 4.0 mm.

Pre drying

If the species is one for which grinding is necessary and the moisture content is more than 17 (or 10 in the case of soybean and 13% in rice) pre-drying before grinding is necessary. For this purpose, two 50 gm portions are weighed and placed on to open trays at 130^{o}C for 5-10 min. If seed moisture content is about 25.0% or more it should be pre-dried at 70^{o}C for 2-5 hours, depending on the initial water content. The pre dried seeds should be kept in a closed desiccator for cooling. Then each of the duplicate quantities is weighed separately and about 20 g is ground. The ground material is then subjected to the moisture testing using an air oven as described below.

Moisture estimation

It should be carried out in duplicate on two independently drawn 5-10g working samples, weighed with an accuracy of 1 mg. Most species are dried for 1 hr at 130^{o}C, cereals for 2 hrs (130^{o}C) and maize for 4 hrs (130^{o}C). Seeds containing high percentage of oil should be dried at 103^{o}C for 17 hours.

Steps:

1. Empty container along with its cover should be weighed.
2. The submitted sample should be mixed thoroughly and two small portions of seed sample are weighed directly into the containers.
3. After weighing remove the cover or lid of the container and the open container should be kept in the oven which has already been heated to the prescribed drying temperature.
4. At the end of the drying period, container should be closed with its cover or lid.
5. The container should be transferred into a desiccator. The desiccator should be closed and the sample should be allowed to cool.
6. The sample should be weighed again and the moisture content may be calculated to one decimal place by the following formula.

$$m = \frac{m2 - m3}{m2 - m1} \times 100$$

Where m =seed moisture content.

m1 =weight of the empty container with its cover

m2= weight of the container with its cover and seeds before drying.

m3= weight of the container with its cover and seeds after drying.

The duplicate result of the determination may not differ by more than 0.2% otherwise the analysis should be repeated. If the material is pre-dried, the moisture content is calculated from the results obtained in the pre-dried and dried stages using the following formula.

$$M = S1 + S2 - \frac{S1 \times S2}{100}$$

M = moisture content
S1= moisture percentage lost in pre drying stage
S2= moisture percentage lost in drying stage.

6.9.2 moisture meters: universal Osaw digital moisture meters

The principle involved in these moisture meters is that wet grains are good conductors while dry grains are less conductors of electricity. So the moisture content is directly proportional to the electrical conductivity of the seed.

It consists of a compression unit to compress the sample to pre-determined thickness. The thickness setting is very easily read on a vertical and circular scale. The seed material on test is taken in a test cup and is compressed. Then press the push type switch till the reading comes in the display. Here no temperature reading and correlated dial are required. The computer versions of digital moisture meter automatically compensate for temperature corrections.

6.10 SEED HEALTH TESTING

It is done for determining the presence or 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 the way.

Admixture

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

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 pearlmillet etc. By surface sterilization external seed borne diseases are killed.

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

Examination without incubation

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

Direct examination

The submitted sample, or a sub sample from it is examined, with or without a stereoscopic microscope 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.

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.

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 a compound microscope.

Examination after incubation

After incubation for a specific period, the working sample is examined for the presence of symptoms of disease organisms, pests and evidence of physiological disturbances in the seeds and seedlings.

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 is often helpful in identifying spores.

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.

In seed spices, Altenaria blight caused by Alternaria burnsii in cumin and Alternaria blight as well as Ramularia blight in fennel are reported to be a partial seed borne diseases. For the pathogenic test of these diseases, the following methods are used.

1. Standard blotter paper method:

400 seeds of each sample will be taken at random. Used sterilized blotter paper at the bottom of each petri dish aseptically and moisturized by sterile water. Ten seeds are placed at equal distance in each petri dish and incubate at 20-25^{0}C under room temperature. The petri dishes will be examined after 4-7 days of incubation for the presence of seed borne mycoflora and count the percentage of infected seeds.

Agar plates are used to obtain identifiable growth of organisms from seeds. Precautions should be taken to ensure their sterilization. The seeds, normally after pre-treatment, are placed 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.

2. Agar plate Method

Four hundred seeds of each sample will be taken at random. Prepare PDA medium and ten seeds are placed at equal distance aseptically per petridishes containing 20 ml of PDA and incubate at 20-25^{0}c under room temperature.

The fungal growth on the seed will be examined regularly and on seven days of incubation, count the percentage of infected seeds. Also transferred the individual fungal growth emerged from seeds on slant test tube by needle for pathogenecity. For sterilized condition, seeds will be treated with 0.1% $Hgcl_2$ solution for one minute followed by three washing of sterile distilled water, for unsterilized condition, seed will be kept as such without any treatment. This method is more reliable than standard blotter paper method.

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 inoculum obtained from the sample may be used for infection tests with healthy seedlings or parts of plants. The plants must be protected from accidental infection from elsewhere and conditions may require careful control.

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 results

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 a statement concerning the health condition of the seed does not necessarily that the health condition is satisfactory.

6.11 seed storage

Seed storage is important to get adequate plant stands in addition to healthy and vigourous plants.

Factors affecting seed longevity in storage

Genetic factors

The storage is influenced by the kind/ variety of seeds. Some kinds are naturally short-lived (e.g.) onion soybeans, groundnut etc. Within a crop the storage period varies between varieties. Also the storage periods of hybrid and parent are differing.

Effects of weather

Fluctuating temperature during seed formation and maturity will affect seed storage pre harvest rain may also affect the viability.

Pre harvest sanitation spray

In pulses, insect infestation comes from field (e.g.) bruchids.

Seed structures

The presence or absence of glumes (lemma and palea) in grasses influence the storage period. Husk, chaff or both have shown an inhibitory effect on the growth of mould and an increase in life span of cereals seeds. Generally small seeds escape injury; where as large seeds are more likely to be extensively damaged (e.g.) bean, lima bean and soybean.

Initial quality of the seed

Seed lots having vigourous, undeteriorated seeds store longer than deteriorated lots.

Environmental factors

Moisture content

The amount of moisture in the seeds is the most important factor influencing seed viability during storage. Generally if the seed moisture content increases the storage life decreases. If seeds are kept at high moisture content the losses could be very rapid due to mould growth very low moisture content below 4% may also damage seeds due to extreme desiccation or cause hard-seediness in some crops.

Since the life of a seed largely revolves around its moisture content it is necessary to dry seeds to safe moisture contents. The safe moisture contents 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 upto 10% moisture content

appears quite satisfactory. However, for storage in sealed containers, drying upto 5-8% moisture content depending upon particular kind may be necessary.

Relative humidity and temperature during storage

Relative humidity is the amount of H_2O present in the air at a given temperature in proportion to its maximum water holding capacity. Relative humidity and temperature are the most important factors determining the storage life of seeds. Seeds attain specific and characteristic moisture content when subjected to given levels of atmospheric humidity. This characteristic moisture content is called equilibrium moisture content. Equilibrium moisture content for a particular kind of seed at a given relative humidity tends to increase as temperature decreases.

Thus the maintenance of seed moisture content during storage is a function of relative humidity and to a lesser extent of temperature. At equilibrium moisture content there is no net gain or loss in seed moisture content.

Temperature

Temperature also plays an important role in life of seed. Insects and moulds increase as temperature increases. The higher moisture content of the seeds the more they are adversely affected by temperature. Decreasing temperature and seed moisture is an effective means of maintaining seed quality in storage. The following are thumb rules by Harrington are useful measures for assessing the effect of moisture and temperature on seed storage. These rules are as follows:

For every decrease of 1% seed moisture content, the life of the seed doubles. This rule is applicable between moisture content of 5-14%.

a. For every decrease of 5^{o}C in storage temperature the life of the seed doubles. This rules applies between 0^{o}C to 50^{o}C.

b. Good storage is achieved when the percentage of relative humidity in storage environment and the storage temperature in degrees Fahrenheit add upto one hundred but the contribution from temperature should not exceed 50^{o}F.

c. Nomograph

Roberts (172) developed formulae to describe the relationship between temperature, seed moisture content and period of viability. From these relationships it was possible to construct a seed viability nomograph. These nomographs are helpful in predicting the retention of seed viability in defined storage environment for a particular period or to determine combination of temperature and moisture content which will ensure the retention of a desired level of seed viability for a specific period.

d. Gas during storage

Increase in O_2 pressure decreases the period of viability.

N_2 and CO_2 atmosphere will increase the storage life of seeds.

Microflora, insects and mites.

The activity of all these organisms can lead to damage resulting in loss of viability.

The microflora activity is controlled by relative humidity, temperature and moisture content of seed.

6. 12 Seed treatment

Treated seeds with fungicides can be stored for longer periods. Fumigation to control insects will also help in longer period of storage.

Fumigation

Once the seed storage is free of completely free of insects, the most serious source of reinfestation is infested seed, which is brought in. Seed may be brought from the field already infested, or it maybe transferred from infested storage. Such infestation is controlled by fumigation. Fumigation is effective only in gas-tight storage. Numerous effective fumigants are available.

Fumigant	*Dosage*	*Exposure period*
Methyl bromide	16 to 32 mg / cubic meter	24 hours
Hydrogen cyanide	32 to 64 mg/cubic meter	24 hours
Hydrogen phosphide	5 to 10 tablets per tone of seed	3 to 7 hours.

It must be borne in mind that fumigation, particularly repeated fumigation, may seriously reduce the vigour and even the germination capacity of seeds. This is particularly true of seeds with high moisture content. Seeds with moisture content greater than 14 per cent should be dried to below this value before fumigation.

Type of packing materials

Moisture vapour proof containers can help in longer storage than the moisture pervious containers.

Use of desiccants

Desiccant like silica gel can maintain the moisture content in equilibrium with the relative humidity of 45%. It is kept @ 1 kg/10 kg of seeds. When the blue silica gel turns to pink colour it should be dried at 175^{o}C in oven and then again placed in the container.

6.13 SEED PACKING MATERIALS

Classification of packaging materials or containers as detailed below:

Moisture and vapour pervious containers

These containers allow entry of water in the form of vapour and liquid. These are suited for short-term storage. The seeds in these containers will attain seed equilibrium moisture with the surrounding atmosphere (e.g.) cloth bags, gunny bags, paper bags etc.

Moisture impervious but vapour pervious containers

These allow entry of water in the form of vapour and not in liquid. The seeds in these containers can't be carried over for long period in hot humid conditions. (e.g.) polythene bags of > 100 gauge thickness and urea bags.

Moisture and vapour proof containers

These containers will not allow entry of moisture in the form of liquid or vapour. These are used for long-term storage even in hot humid conditions if the seeds are sealed at optimum moisture content (e.g.) polyethylene bags of >700 gauge thickness, aluminum foil pouches, rigid plastics etc. Certified seeds of cereals, pulses and oil seeds are normally packed either in gunny bags or cloth bags. However, paper bag, aluminum foil pouches and polyethylene bags are used for packaging flower and vegetable seeds.

6.13.2 Types of storage requirements

The types of storages are based on the time of storage. It can be classified into 4 types as given below:

Commercial seeds: The largest storage need is for the storage of seed from harvest until planting. The storage period ranged from a few days to 8 or 9 months. Here seeds must be dried to a m.c of < 14% for starchy seeds and less than 11% for oil seeds.

Carryover seeds: About 20-25% of stored seeds may have to be carried over through one growing season to the second season. This storage period is usually between 1-year &11/2 years.

Seeds can be stored in steel bins with tight fitting lids or in moisture proof bags.

Foundation seed stocks*:* This can be stored for several years, since reproducing foundation or stock seeds minimize genetic drift is. This seeds can be stored at expand about 25% and temperature at 30^{o}C or a relative humidity of 45% and temperature of 20^{o}C. This can be achieved by using a dehumidifier. Store the seeds with polythene bags of > 700 gauge thickness.

Germplasm seeds: These seeds are to be stored for many years. Basic requirements for such very long-term storage are coldest temperature and seed moisture content in equilibrium with20-25% R.H. storage rooms can be, maintained at 5^{o}C and 10^{o}C and 30% R.H. Here the seeds should be dried to lower level.

6.13.3 Agar plate method

400 seeds of each sample will be taken at random. Prepare PDA medium and ten seeds are placed at equal distance aseptically per petridishes containing 20 ml of PDA and incubate at 20-25^{0}C under room temperature.

The fungal growth on the seed will be examined regularly and on seven days of incubation, count the percentage of infected seeds. Also transferred the individual fungal growth emerged from seeds on slant test tube by needle for pathogenicity. For sterilized condition, seeds will be treated with 0.1% $HgCl_2$ solution for one minute followed by three ashing of sterile distilled water, for un sterilized condition, seed will kept as such without any treatment. This method is more reliable than standard blotter paper method.

Seed storage sanitation or godown sanitation

1. Storage environment should be free from insects and rodents
2. Chemicals such as insecticides, fertilizers should not be stored along with seeds.
3. Storage room should be kept cool and dry.
4. Fumigation may be done whenever needed.
5. Use wooden pallets for arranging the bags in cris-cross manner for effective ventilation on all sides of the bags.
6. Seed bags should be stacked upto 6-8 tires depending upon density of seeds.
7. Restacking once in 3 months or less is important for prolonging seed viability.
8. Before storage disinfect the godowns by spraying malathion 50% E.C. @ 0.5%.
9. If old gunnies, cloth bags and containers are to be used these should be fumigated with aluminium Phosphide.
10. Size of the stack should be 30 x20 feet to facilitate fumigation under gas proof or polythene covers.

Periodical inspections should be carried out and control measures to be taken i.e. Malathion 50 E.C. @ lit /100 m should be applied in every 3 weeks. It must be borne in mind that fumigation; particularly repeated fumigation may seriously reduce the vigour and even the germination capacity of seeds. Seeds with moisture content greater than 14% should be dried to below this value before fumigation.

6.13.4 Maintenance of viability in storage

1. Store well matures seeds.

2. Store normal coloured seeds.
3. Seeds should be free from mechanical injury.
4. Seeds should be free from storage fungi or microorganisms.
5. Seeds should not have met with adverse conditions during maturation.
6. Storage environment or godown should be dry and cool.
7. Seeds should be dried to optimum moisture content
8. Required relative humidity and temperature should be maintained during storage.
9. Seeds should be treated with fungicides before storage.
10. Storage godown should be fumigated to control storage insects, periodically.
11. Suitable packaging materials should be used for packing.

Format of Seed Analysis Report

MINI SEED TESTING LAB ANALYTICAL REGISTER (To be maintained by ADSCN)

Sl.No.	Name and address of the applicant	Crop/Variety	Class of the seed	Details of the seed source	Date of expiry	Quantity of the lot	Date of receipt	Date of sampling

Date of test	Normal seedlings	Analytical result abnormal seedlings	Hard seed	Dead seed	Date of Despatch or result	File No.	Seed Rate recommended	Initial of ADSCN

SEED SAMPLES AND SEED ANALYTICAL REGISTER
(To be maintained by ADSCN)

Sl.No.	**Name and address of the Producer**	**Kind/ Variety**	**Class**	**S.C.No.**	**Lot No.**	**Quantity of the lot**	**Date of test**
Purity	OCS	Analytical test results			Hard seed	Moisture	Remarks
		WS	ODV/GP	Germination			

Name of the SCO	**Date of drawing sample by SCO**	**Date of receipt of Sample by ADSCN**	**ADSC Code No.**	**Date of receipt of analytical report**	**Kind of sample Fresh Resample Validation**	**STL Code No.**	**Date of communication of result to the producer**	**Initials of ADSCN**