





Technical Bulletin- 2
(2026/02)

Standard Operating Procedures for disease screening in ginger and turmeric





ICAR-All India Coordinated Research Project on Spices
ICAR-Indian Institute of Spices Research
Kozhikode - 673 012, Keralam, India

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Project Coordinator (Spices)
ICAR-All India Coordinated Research Project on Spices
Kozhikode – 673 012, Keralam, India
Phone: 0495 2731954
email: aicrpspices@gmail.com
Website: www.aicrps.res.in

Contributors

Mohammed Faisal P.
Biju, C. N.
Praveena, R.
Sellaperumal, C.
Meenu Gupta
Sundravadana, S.
Mishra, A.K.
Anamika Debnath
Ajith K.S

Editors

Mohammed Faisal P., Biju C.N., Prasath D., Mukesh Sankar S

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Ginger

Soft rot

Soft rot disease caused by *Pythium* was recorded for the first time from India and presently, the disease occurs in all ginger growing countries throughout the world (Dohroo, 2005). The pathogens responsible for soft rot can infect host plants at any stages of growth and postharvest storage and can lead to severe losses. Stirling et al. (2009) reported more than 50% loss of plants used for seed ginger production in Australia. In most cases these heavy losses have occurred in years when weather conditions were favourable for pathogen growth. The impact of *Pythium* spp. can also be high in storage; losses ranging from 24 to 50% have been reported with rates occasionally exceeding 90% in India. Six species of *Pythium viz*., *P. aphanidermatum, P. butleri, P. deliense, P. myriotylum, P. pleroticum, P. ultimum and P. vexans* are associated with soft rot of ginger. Of these, *P. myriotylum* is the predominant species (Kumar et al., 2008).

Symptomatology

If the rhizomes used for planting are infected, they do not germinate due to the rotting of young buds. If infection occurs after sprouting, the infection occurs through the root or collar region, later spreading to the rhizomes. In matured plants, collar infection results in yellowing of foliage which starts from the leaf tip and proceeds downwards leading to drying of leaves, which droop until the entire shoot dries. The basal part of the stem develops translucent discolouration which then turns water-soaked, leading to the falling of infected shoots. The affected rhizomes firstly become brown, slowly decompose and transform into a wet mass of rotting tissue surrounded within rhizome skin and releasing foul smell. The roots of the infected rhizomes become soft and later rot.



Initial phase



Final phase



Above ground symptoms



Infected rhizomes

Screening for disease resistance under artificial conditions Procedure:

The trial should be laid out in completely randomized design (CRD) and conducted for a period of 2 years. The Pythium myriotylum culture preserved in sterile water stocks should be revived on potato dextrose agar (PDA) medium amended with streptomycin. The *P. myriotylum* inoculum should be prepared following the method described by Ali-Shtayeh et al. (1986), with minor modifications. Briefly, the sand-sorghum substrate consisting of 80 g washed sand, 20 g crushed maize kernels and 20 mL distilled water should be dispensed into 250 mL Erlenmeyer flasks and autoclaved at 121°C for 20 min. Each flask should be inoculated with 5 mm mycelial discs obtained from 3-day-old *P. myriotylum* culture and incubated at 28±1°C for 10 days, with intermittent shaking to facilitate uniform colonization.

Healthy, disease-free rhizomes of the test entries and susceptible check, weighing approximately 20-25 g, should be planted 45 days prior to inoculation in uniform-sized pots containing sterilized potting mixture of soil, sand and farmyard manure in 2:1:1 ratio. The rhizomes should be treated with the recommended insecticide before planting. Ginger plants at 11-12 leaf stage should be used for artificial inoculation. Each pot containing a single plant will be considered as one replication, with a minimum of 10 replications/pots per entry or check.

The optimum inoculum dose for inoculation of rhizome-grown ginger plants will be 1 g of *P. myriotylum* inoculum per 100 g of potting mixture (Praveena et al., 2026). Prior to inoculation, the collar region of each plant should be pin-pricked using a sterile needle, followed by application of *P. myriotylum* inoculum at the plant base. Moist cotton should be wrapped around the inoculated collar region to maintain adequate humidity and facilitate disease development. The inoculated plants should be maintained under greenhouse conditions preferably at 26±2°C and 80-90% relative humidity with regular watering. Plants should be monitored at 1, 2, 3, 4, 5, 6, 7 and 10 days after inoculation (DAI) for the development of initial disease symptoms. Disease intensity should subsequently be recorded on all plants at regular intervals up to 10 days after inoculation.

The experiment should be repeated twice to confirm consistency and reproducibility of the disease reaction. Based on the average disease intensity recorded during a particular year, the entries should be categorized into different resistance/susceptibility classes. The reaction of the entries should be validated using observations recorded during the subsequent year. A minimum of 10 plants per entry/check should be screened and the average disease intensity should be used for final categorization.

$$\text{Disease incidence (DI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Categorization based on reaction towards bacterial wilt	
Category	**Disease Intensity (DI)**
Highly resistant	< 10%
Resistant	11 - 20%
Moderately susceptible	21 - 50%
Susceptible	51 - 75%
Highly susceptible	76-100%
	(Kavitha and Thomas, 2008)

Screening for disease resistance under field conditions Procedure:

The trial should be laid out in randomized block design (RBD) and conducted for a period of three years. The experimental field should be selected in a location endemic to rhizome rot and conducive to natural disease development, where recurring disease incidence has been recorded during the previous years. The test entries, along with known/reported susceptible and resistant checks, should be used for screening for disease resistance, with five replications per entry/check.

The test entries and check varieties should be planted in 3 × 1 m beds at a spacing of 20 × 30 cm (rhizome-to-rhizome × row-to-row), accommodating a minimum of 30 plants per bed, with five beds per entry/check maintained as five replications. Disease-free healthy rhizomes weighing approximately 20-25 g should be planted during the recommended planting period after treatment with recommended insecticides. The recommended package of practices should be adopted uniformly for raising the crop across all centres. Timely sprays and/or soil application of recommended plant protection chemicals should be undertaken to manage insect pests and diseases other than rhizome rot, so that the natural incidence of rhizome rot can be assessed without interference from other diseases.

Natural incidence of rhizome rot should be monitored during the peak disease period, particularly July and August. Disease incidence should be assessed by recording the number of infected tillers/plants among all plants in each bed, with a minimum of 30 plants per bed and expressed as percentage.

For assessment of disease index, disease severity should be recorded using the standardized rhizome rot disease rating scale. A minimum of 20 plants per bed/replication should be scored and the mean disease index should be calculated for each replication.

Based on the average disease incidence and/or disease index recorded during a particular year, the entries should be categorized into different resistance/susceptibility classes. The disease reaction observed in the first year should be validated using observations recorded during the subsequent two years. A minimum of 450 plants per entry/check should be screened over the three-year period and the mean disease incidence/index should be used for final classification of the entries.

Disease rating scale for rhizome rot (0 to 9 scale)	
Category	**Description/Symptoms**
0	No symptoms
1	Up to 25% tillers yellow or fallen
3	26-50% tillers yellow or fallen
5	51-75% tillers yellow or fallen
7	>75% tillers yellow or fallen
9	100 % tiller death
	(Kavitha & Thomas 2008)

$$\text{Percent Disease index (PDI)} = \frac{(n\,x0 + n\,x\,1 + n\,x\,3 + n\,x\,5 + n\,x7 + n\,x9)}{N\,x\,9} X\ 100$$

Where, n = Number of plants in each score, 5 = Maximum disease grade, N = Total number of plants under observation.

Categorization based on reaction towards bacterial wilt	
Category	**Disease Intensity (DI)**
Highly resistant	< 10%
Resistant	11 - 20%
Moderately susceptible	21 - 50%
Susceptible	51 - 75%
Highly susceptible	76-100%
	(Kavitha and Thomas, 2008)

Selected references:

- Ali-Shtayeh MS, Lim-Ho CL & Dick MW 1986 An improved method and medium for quantitative estimates of populations of *Pythium* species from soil. Transactions of the British Mycological Society 86: 39–47.

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Bacterial wilt

Bacterial wilt caused by *Ralstonia pseudosolanacearum* race 4 biovar 3/4, is one among the most important soil/rhizome-borne diseases of ginger. In India, biovar 3 causes rapid wilt in ginger in 5-7 days under artificial stem inoculation and in 7-10 days under soil inoculation. The rhizome-borne inoculum is primarily responsible for disease initiation which further spreads horizontally, aided by high rainfall and cool weather under field conditions. Generally the disease appears and proliferates during June to August. The pathogen survives in the soil as well as infected planting material and spreads through rhizome/soil-borne inoculum, rain splash and run-off water. High rainfall and warm weather favours disease development and subsequent dispersal.

Symptomatology

The initial symptom of bacterial wilt is mild drooping and curling of leaf margins of lower leaves (green wilt). In the advanced stage, plants exhibit severe yellowing and wilting symptoms. The vascular tissues of affected pseudostem exhibit dark streaks. The affected pseudostem and rhizome when pressed gently extrudes milky ooze from the vascular strands. The infected rhizome putrefies, emit foul smell and the affected plants withers within 2-3 weeks.



Green wilt (initial stage) | Advanced stage

Screening for disease resistance under artificial conditions Procedure:

The trial should be laid out in completely randomized design (CRD) and the duration would be 2 years. The *Ralstonia pseudosolanacearum* isolated from symptomatic ginger plants (preferably the local isolate) should be used for artificial inoculation experiments. If the pathogen is stored in water stocks, the culture should be revived on CPG medium (Cassamino acid 1; Peptone 10; Glucose 5 gL-1) amended with 2, 3, 5, triphenyl tetrazolium chloride (50 mg/mL) and the screening experiments should be conducted only after confirming both identity and virulent nature of the pathogen. The phenotypic characters of R. pseudosolanacearum isolate indicating its virulence such as irregularly shaped fluidal colonies with spiral pink center formed on CPG medium amended with 2, 3, 5, triphenyl tetrazolium chloride should be confirmed before inoculation. The isolate should also be subjected to colony PCR to confirm the identity as described by Kumar et al. (2006).

For preparation of the inoculum, inoculate a loopful of *R. pseudosolanacearum* colonies cultured on CPG medium on to 100 mL of sucrose peptone broth (Sucrose 20; Peptone 5; MgSO4.7H2O 0.25; K2HPO4 0.5 gL-1; pH 7.2) and incubate with continuous agitation in shaking incubator maintained at 28oC and 150 rpm. After attaining sufficient growth (OD600 = 1.00), centrifuge the broth at 8000 rpm for 20 minutes at 4°C. Harvest the bacterial pellet in 100 mL sterile distilled water to obtain a bacterial concentration of approximately 1 × 108 cfu mL-1 (Kumar, 2006, Praveena et al., 2026).

Disease-free healthy rhizomes of the test entries and susceptible check weighing about 20-25 g should be planted 45 days prior to inoculation in pots with uniform size containing sterilized potting mixture (soil, sand and farmyard manure in 2:1:1 proportion). The rhizomes should be treated with recommended fungicides/insecticides prior to planting (Suseela Bhai et al., 2019). Each pot with plants will be considered as a single replication (minimum 10 replications or pots).

The bacterial inoculum (100 µL of sterile distilled water containing 1 × 10^8 cfu mL-1) should be placed using micropipette inside the bottom most leaf sheath of 45-days-old plants after making slanted pin pricks with sterile needle reaching up to the center of pseudostem. The inoculation site, specifically the lowermost leaf sheath and the plant base should be covered with coir pith to facilitate inoculum retention and ensure prolonged exposure for effective infection establishment. After pseudostem inoculation, the plant base should be drenched with 50 mL of the same inoculum. The inoculated plants should be maintained in greenhouse at ambient temperature (preferably 26-28°C) with regular watering. The plants should be monitored for symptoms of wilting and yellowing and the disease intensity should be calculated (Indrasenan et al., 1982).

$$\text{Disease intensity (DI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \text{ X } 100$$

For disease scoring, observations on disease intensity should be recorded on all the plants up to 60 days after inoculation. Based on average DI during a particular year, the entries can be delineated into different categories of susceptibility or resistance which needs to be validated based on the observations recorded during the subsequent year. A minimum of 10 plants should be screened per entry/checks and the average score should be recorded.

Categorization based on reaction towards bacterial wilt	
Category	**Disease Intensity (DI)**
Resistant	0 to 5%
Moderately resistant	6 to10%
Mildly susceptible	11 to 25%
Moderately susceptible	26 to 40%
Highly susceptible	More than 40%
	Indrasenan *et al*. (1982)

Screening for disease resistance under field conditions Procedure:

The trial should be laid out in randomized block design (RBD) and the duration would be three years. The area under the experiment should be maintained under uniform shade level. Recommended package of practices should be adopted to raise the crop which should be uniformly adopted in all the centers. The test entries shortlisted along with known/reported susceptible and resistant checks [five replications each; one replication consists of three beds] should be used to screen for disease resistance. The test entries and the check varieties should be planted in 3 X 1 m2 size beds with a spacing of 20 X 30 cm (rhizome to rhizome X row to row) to comprise a minimum of 30 plants per bed. The location/field should be endemic/conducive to natural infection of bacterial wilt disease (sick plot) wherein recurring infection is recorded/observed during the period of experiments and previous years. Timely sprays/soil application of recommended plant protection chemicals should be adopted to manage insect pests and diseases other than bacterial wilt. Disease-free healthy rhizomes weighing about 20-25 g should be planted during the second fortnight of April and the rhizomes should be treated with recommended fungicides/insecticides before planting (Suseela Bhai et al., 2019). Three beds would constitute a single replication and five replications (total of 15 beds per entry/check) should be maintained for each entries and checks. The disease intensity should be assessed at 90th day after planting and disease intensity should be calculated (Indrasenan et al., 1982).

$$\text{Disease intensity (DI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \text{ X } 100$$

For disease scoring, observations on disease intensity should be recorded on all the plants (minimum of 30 plants per bed/replication) from all five replications. Based on average DI during a particular year, the entries can be delineated into different categories of susceptibility or resistance which needs to be validated based on the observations recorded during subsequent two years. A minimum of 150 plants should be screened per entry/checks and the average score should be recorded.

Categorization based on reaction towards bacterial wilt	
Category	**Disease Intensity (DI)**
Resistant	0 to 5%
Moderately resistant	6 to10%
Mildly susceptible	11 to 25%
Moderately susceptible	26 to 40%
Highly susceptible	More than 40%
	Indrasenan *et al.* (1982)

Selected references:

- Indrasenan G, Sreekumar V, Mathew J & Mammen MK 1982 Reaction of different types of ginger to bacterial wilt caused by *Pseudomonas solanacearum* (Smith) Smith. Agric. Res. J. Kerala 20: 73–75.

- Kumar A 2006 Methods for screening ginger (*Zingiber officinale* Rosc.) for bacterial wilt resistance. Indian Phytopathol. 59: 281–286.

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- Suseela Bhai R, Prameela TP, Vincy K, Biju CN, Srinivasan V & Nirmal Babu K 2019 Soil solarization and amelioration with calcium chloride or *Bacillus licheniformis* - an effective integrated strategy for the management of bacterial wilt of ginger incited by *Ralstonia pseudosolanacearum*. Eur. J. Plant Pathol. 154: 903–917. https:// doi.org/10.1007/s10658-019-01709-y.

Phyllosticta leaf spot

The leaf spot incited by *Phyllosticta zingiberi* is a major foliar disease of ginger which was reported for the first time in Godavari and Malabar regions of India (Ramakrishnan, 1942). This disease has been reported as the most destructive and the most common in the ginger-growing regions of India (Sohi et al., 1964; Sood and Dohroo, 2005; Singh, 2015) occurring either in mild or severe forms (Sampritha et al., 2023). Generally the disease appears towards the end of June with the onset of monsoon. The pathogen survives in the infected plant parts (leaf debris) and soil and spread through rain splashes as well as wind. The disease development is favoured by temperature (23-30°C), relative humidity (83-90%) and intermittent rainfall. Six to seven months old plants are more vulnerable and two weeks old leaves are most susceptible to the disease. Losses of 48.3 per cent in mother rhizome and 65.9 per cent in fresh rhizome were recorded at 58.3 per cent disease severity (Sood and Dohroo, 2005).

Symptomatology

Initially small, spindle to oval or elongated spots with white papery center and dark brown margins surrounded by yellow halo appear on younger leaves. The spots later expand and coalesce to form larger spots which eventually reduce the effective photosynthetic area. The infected areas often dry at the center forming shot-holes and subsequently, the entire leaf dries. In the advanced stage, the crop develops a greyish disheveled appearance. The spots are usually isolated but may coalesce into large patches leading to drying of entire leaves. Symptoms also occur on pseudostems and leaf sheaths. The spots are irregular, measuring 11.2-13.1 mm and lack the brown margin and yellow halo. They coalesce into bigger spots that also bear pycnidia.



Initial symptoms



Spots bearing pycnidia

Screening for disease resistance under field conditions Procedure:

A location with a known history of disease incidence or conducive environmental conditions should be chosen (warm and humid). The trial should be laid out in randomized block design (RBD) and the duration would be three years. The area under the experiment should be maintained under uniform shade level. Each test genotype(s)/ variety(s) should be sown in paired row (Row length of minimum 3m to maximum 5m based on availability of land) maintaining a spacing of 30 x 20cm. Recommended fungicide application meant for foliar disease management should be avoided to ensure disease development.

Recommended package of practices should be adopted to raise the crop which should be uniformly adopted in all the centers. The test entries shortlisted along with known/ reported susceptible and resistant checks [five replications each; one replication consists of three beds] should be used to screen for disease resistance. The test entries and the check varieties should be planted in 3 X 1 m^2 size beds with a spacing 20 X 30cm (rhizome to rhizome X row to row) to comprise a minimum of 30 plants per bed. The location/field should be endemic/conducive to natural infection of *Phyllosticta* leaf spot wherein recurring infection is recorded/observed during the period of experiments and previous years. Timely sprays/soil application of recommended plant protection chemicals should be adopted to manage insect pests and diseases other than *Phyllosticta* leaf spot.

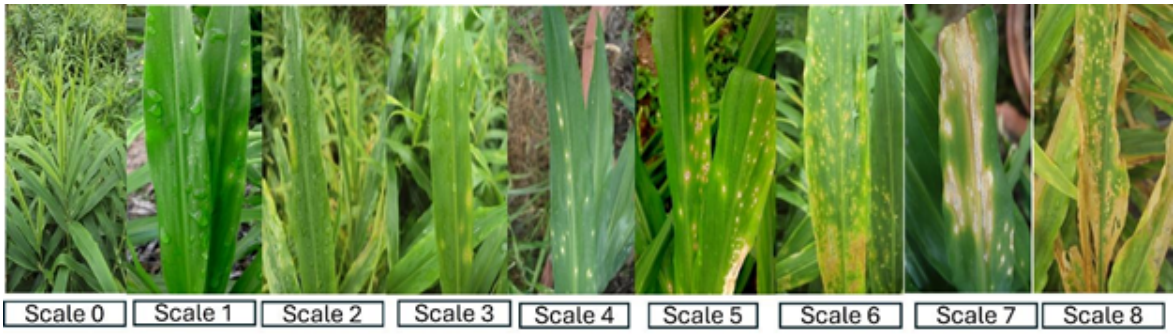
Disease-free healthy rhizomes weighing about 20-25g should be planted during second fortnight of April and the rhizomes should be treated with recommended fungicides/insecticides before planting. Three beds would constitute single replication and five replications (total of 15 beds per entry/check) should be maintained for each entries and checks. Natural incidence of *Phyllosticta* leaf spot should be recorded during September and October employing 0-8 scale as described. For disease scoring, observations from all the leaves of 15 randomly selected plants per bed should be recorded. Disease index (DI) should be calculated for each replication for each month separately and average DI can be calculated by combining data of other replications (average of two month data would represent the disease level of a particular entry/check for a particular year of screening). Based on average DI during a particular year, the entries can be delineated into different categories of susceptibility or resistance which needs to be validated based on the observations recorded during subsequent two years.

Disease rating scale for Phyllosticta leaf spot (0-8 scale)	
Scale	**Symptoms on leaves**
0	No symptoms/disease
1	1 to 2 spots per leaf
2	3 to 5 spots per leaf
3	6 to 9 spots per leaf
4	10 to 15 spots per leaf
5	20 to 35% leaf area covered
6	36 to 60% leaf area covered
7	61 to 80% leaf area covered
8	Leaves completely dried
	(Senapati et al., 2012)

The Disease Index (DI) should be calculated at monthly intervals adopting the disease rating scale and averaged.

Disease Index (DI) =0 x number of leaves falling in the scale + 1 x number of leaves falling in the scale + 2 x number of leaves falling in the scale + 3 x number of leaves falling in the scale + 4 x number of leaves falling in the scale + 5 x number of leaves falling in the scale + 6 x number of leaves falling in the scale + 7 x number of leaves falling in the scale + 8 x number of leaves falling in the scale

Total number of leaves observed



Representative figures for different scales

DI calculated for one replication consisting of 15 randomly selected plants (similarly DI should be calculated for other four replications separately).

Categorization based on reaction towards Phyllosticta leaf spot	
Category	**Disease Index (DI)**
Highly resistant	Less than 0.4619
Moderately resistant	0.4619 to 1.0403
Tolerant	1.0403 to 1.6187
Moderately tolerant	1.6187 to 2.1971
Susceptible	2.1971 to 2.7755
Highly susceptible	More than 2.7755
	Senapati et al. (2012)

Selected references:

- Ramakrishnan TS 1942 A leaf spot disease of ginger caused by *Phyllosticta zingiberi* sp. n. Proc. Indian Acad. Sci. 15: 167–171.
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Root knot nematode

Ginger is the rhizome or underground stem of *Zingiber officinale*, an herbaceous perennial belonging to the family Zingiberaceae. Plant parasitic nematodes belonging to 17 genera have been reported on ginger and the most important are *Meloidogyne* spp., *Radopholus similis* and *Pratylenchus coffeae* (Eapen and Pandey, 2018). The root knot nematode (RKN) is a serious pest infecting ginger in almost all ginger growing regions of India such as Kerala, Karnataka, Odisha, Madhya Pradesh, Himachal Pradesh and West Bengal (Eapen and Pandey, 2018; Nair et al., 2019). The RKN is also reported to be involved in disease complexes with other major pathogens of ginger (Eapen and Pandey, 2018). The severity of rhizome rot caused by *Pythium* spp. is reported to be aggravated with *M. incognita* infection.

Symptomatology

The root knot nematodes cause galling and rotting of roots and underground rhizomes. The galls are formed on fibrous roots. Abnormal xylem and hyperplastic parenchyma are observed on the infected tissue except rhizome meristems. Extensive internal lesions are formed in the fleshy roots and rhizomes. Yellowing of leaves and prematurely senile and thin rhizomes are other associated symptoms. The affected rhizomes have brown, water-soaked areas on the outer tissues, particularly in the angles between shoots. Heavily infected plants remain stunted with poor tillering and have chlorotic leaves with marginal necrosis. The affected plants mature early, dry faster and die sooner than the healthy plants, leaving a poor crop stand at harvest.



Poor crop stand caused by root-knot nematode infection



Severely infected ginger rhizome (along with healthy rhizome)

Screening for nematode resistance under artificial conditions (pot culture)
Establishment and maintenance of root knot nematode culture

The root knot nematode infected ginger plants should be collected and *Meloidogyne* spp. should be extracted and inoculated onto healthy, susceptible brinjal or coleus plants grown in pots or growbags. One week after transplanting, each plant should be inoculated with approximately 100 freshly hatched second-stage juveniles (J2) from single eggmass. The plants should be maintained under screen-house conditions for nematode multiplication. After three months, a fresh batch of susceptible host plants should be planted and inoculated to maintain and further multiply the culture. For routine maintenance, infected roots should be processed to extract egg masses and the juveniles should be obtained using modified Baermann funnel method. Freshly extracted J2 should be used for artificial inoculation. If not used immediately, the suspension may be stored at 4°C for 3-5 days and subjected to confirmation of juvenile viability before use.

Screening procedure

Healthy, uniform seed rhizomes of ginger (approximately 25 g each) should be selected, with a minimum of 10 rhizomes per replication and six replications per entry. Each rhizome should be planted individually in a polythene bag (15 X 30 cm) containing sterilized potting mixture consisting soil, sand and farmyard manure in 2:1:1 ratio. The experiment should be laid out in completely randomized design (CRD). One month after germination, each plant should be inoculated with 1000 freshly hatched second-stage juveniles (J2) by making three equidistant holes around the rhizosphere, which should be subsequently covered with soil. IISR Varada and IISR Mahima will serve as susceptible and tolerant checks. Two months after inoculation, the plants should be carefully uprooted and the roots should be washed gently to remove adhering soil. Root galling and egg mass production should be assessed. Egg masses should be visualized by staining the roots with Phloxine B following Holbrook et al. (1983), followed by keeping the roots in glycerol for 24 hours to facilitate observation. Root galling should be scored using the 0-5 root-galling index (Taylor and Sasser, 1978). The final nematode population (Pf) should be estimated separately from soil using Cobb's sieving and decanting method followed by the Baermann funnel method and from roots using acid fuchsin-acetic acid staining followed by root maceration. The reproduction factor (RF) should be calculated as: RF =Pf/Pi, where (Pf) is the total final nematode population recovered from soil and roots and (Pi) is the initial inoculum of 1000 J2 per plant. Germplasm accessions will be classified according to their root-galling index (RKI), egg mass index (EMI) and RF in comparison with the respective checks. Accessions exhibiting an EMI/RKI below 2 will be shortlisted for further evaluation during the second season.

Rating scale for root-knot nematode presence on roots (0-5)	
Number of egg masses	**Egg mass index (EMI)**
0	0
1-2	1
3-10	2
11-30	3
31-100	4
>100	5
	Taylor and Sasser (1978)

Categorization based on reaction towards nematode infection		
Gall index	**R factor**	**Categories**
≤2	≤ 1	Resistant
≤2	> 1	Tolerant
≥2	≤ 1	Moderately tolerant
≥2	> 1	Susceptible
		Sasser *et al.* (1984)

Screening for nematode resistance under natural conditions

Establishment of sick plot for root knot nematode

A separate corner of the field measuring approximately 1 m × 1 m will be selected for establishing the sick plot, with suitable precautions to prevent nematode-contaminated soil or drainage water from reaching adjacent fields. The soil will be prepared properly for planting. Brinjal or coleus, which are suitable hosts for *M. incognita*, will be used for nematode multiplication after confirming host suitability. A pure culture of *M. incognita* will be obtained from the screen house and used to inoculate each plant with approximately 100 freshly hatched second-stage juveniles (J2) extracted from single eggmass, one week after transplanting. The plants will be allowed to grow and support nematode multiplication. After sufficient multiplication, the plants will be destroyed, and the soil will be thoroughly mixed by ploughing. A second crop of the susceptible host will then be planted to further increase the nematode population. Soil samples will be collected to assess the nematode density, and the sick plot will be used for germplasm resistance screening once a reasonably uniform population of at least 1 J2 per cc of soil is established and confirmed.

Screening procedure

Plant the germplasm accessions or cultivars in the sick plot alongside appropriate check varieties, including IISR Mahima as a moderately resistant check against *M. incognita* . Conduct the experiment using a randomized block design (RBD) with a minimum of four replications and 12 plants per accession per replication in a block measuring approximately 1.0 m × 1.0 m, totally 5 blocks including control without nematode.

Follow standard recommended agronomic practices throughout the experiment. Two months after planting, carefully uproot the plants and wash the roots gently to remove adhering soil. Stain the roots with Phloxine B to visualize egg masses and score egg-mass production using the 0–5 egg-mass index (EMI) of Taylor and Sasser (1978).Estimate the final nematode population (Pf) separately from roots using acid fuchsin–acetic acid staining followed by root maceration, and from soil using Cobb's sieving and decanting method followed by the Baermann funnel method. Calculate the nematode reproduction factor (RF) using the formula: RF = Pf/Pi, where (Pf) is the total final nematode population recovered from soil and roots, and (Pi) is the initial nematode population. Determine the host reaction of each accession based on the root-knot index (RKI), EMI and RF. Shortlist accessions exhibiting an RKI/EMI ≤ 2 and Rf ≤ 1 subjected for further resistance evaluation under controlled conditions.

Rating scale for root-knot nematode presence on roots (0-5)	
Number of galls/ egg masses	**Root knot index (RKI)/ Egg mass index (EMI)**
0	0
1-2	1
3-10	2
11-30	3
31-100	4
>100	5
	Taylor and Sasser (1978)

Designation of host status based on the degree of resistance		
Gall index/Egg mass	**R factor**	**Degree of resistance designation**
≤2	≤ 1	Resistant
≤2	> 1	Tolerant
≥2	≤ 1	Moderately tolerant
≥2	> 1	Susceptible
		Sasser *et al.* (1984)

Selected references:

- Eapen SJ & Pandey R 2018 Nematode parasites of spices and medicinal plants . In: Sikora RA, Coyne D, Hallmann J & Timper P (eds) Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI, UK, pp. 755–794.
- Hajihassani A, Ye W & Hampton BB 2019 First report of *Meloidogyne javanica* on ginger and turmeric in the United States. J. Nematol. 51: 1–3. https://doi.org/10.21307/jofnem-2019-006.
- Prasath D, Eapen SJ & Sasikumar B 2016 Performance of turmeric (*Curcuma longa*) genotypes for yield and root-knot nematode resistance. Indian J. Agric. Sci. 86(9): 1189–1192. https://doi.org/10.56093/ijas.v86i9.61516.
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- Taylor AL & Sasser JN 1978 Biology, Identification and Control of Root-Knot Nematodes (*Meloidogyne* spp.). Cooperative Publication, Department of Plant Pathology, North Carolina State University and US Agency for International Development, Raleigh, North Carolina, USA, 111 p.

Turmeric

Rhizome rot

Rhizome rot pertains to the economic aspects of turmeric cultivation, due to its severe damage on the consumable part of the crop, leading to much pecuniary distress for the farmer. It is the most damaging of all the diseases affecting turmeric and is reported as a major soil-borne problem in all turmeric growing tracts. The disease was reported to be caused by different species of Pythium namely, *Pythium myriotylum, Pythium graminicolum* and *Pythium aphanidermatum*. Yield losses due to rhizome rot and root rot up to 95% in Tamil Nadu, 50 to 60% and 0-80% in Andhra Pradesh and 50% in North Telangana region of Andhra Pradesh.

Symptomatology

The disease initially appears as water-soaked lesions at the base of the pseudostem, followed by leaf yellowing. The infected roots become brown and rotten with infection progressing to the rhizomes, which soften, decay and change from bright orange to brown. The infection spreads to the fingers and mother rhizome, eventually causing plant death. Splitting affected rhizomes reveals brown to dark-brown fibro-vascular tissues, while leaves gradually dry from the margins and eventually wither completely (Rao, 1995). Symptom expression depends on the time of infection and extent of rhizome damage; infections restricted to roots or secondary and tertiary fingers may produce few visible symptoms. Rhizome rot is most prevalent during the southwest monsoon, particularly during active crop growth phase, when prolonged rainfall and waterlogging favour disease development (Sankaraiah et al., 1991).

Screening for disease resistance Screening of turmeric germplasm under artificial conditions

Earthen pots measuring 30 cm in diameter will be filled with sterile soil thoroughly mixed with fungal mass culture. The pots will be covered with black polythene sheets and incubated for seven days. After incubation, turmeric rhizomes will be planted in the pots. Following sprout emergence, the inoculum grown on a sand-maize meal medium or mycelial suspension will be introduced into the potted plants. Watering will be carried out regularly to maintain adequate soil moisture. The plants will be monitored closely for 45 days after sprout emergence. Healthy and susceptible checks will be maintained and three replications will be maintained for each germplasm. The percentage of disease incidence will be recorded.

Inoculum preparation

The mycelial suspension of *Pythium aphanidermatum* should be prepared by harvesting mycelial mats from seven-day-old culture grown in potato dextrose broth (PDB). The harvested mycelial mats should be homogenized in a blender for 1 minute and strained through a double-layered muslin cloth. The resulting suspension should be diluted with sterile distilled water to obtain 15-20 mycelial bits per microscopic field. The inoculum should then be applied at a concentration of 10%.

The sand: maize meal medium (SMM) at the ratio of 9:1 should be prepared by adding 90 g of riverbed sand, 10 g of maize meal and 20 mL of distilled water. The medium should be autoclaved at 15 lb pressure for 20 minutes. After cooling, 10 bits of pathogen culture along with potato dextrose agar (PDA) should be aseptically transferred into the medium and sealed airtight. The inoculated medium should be incubated for 2 weeks to allow multiplication of the pathogen. Subsequently, the SMM will be thoroughly mixed with sterile soil at 10% (w/w) and kept for one week before use.

Field screening turmeric germplasm against rhizome rot

The turmeric lines will be sown during the kharif season. No plant protection measures will be undertaken during the crop growth period to facilitate natural disease development. The trial will be laid out in an augmented block design using different lines, including the highly susceptible variety. Border rows will be planted with highly susceptible variety to ensure uniform natural disease infection. Uniform-sized fingers (40 fingers, each measuring 5–6 cm in length and weighing approximately 20–25 g) will be planted in plots measuring 3.0 × 1.0 m, with a spacing of 30 × 15 cm in four rows, accommodating 40 rhizomes per plot. Farmyard manure will be applied at 20 tonnes per hectare, along with inorganic fertilizers supplying nitrogen, phosphorus and potassium kg per hectare, in the form of urea, single superphosphate and muriate of potash, as per the recommendations of SAUs. The experimental plots will be irrigated by flood irrigation at intervals of 10–15 days. Weeding will be carried out at 20, 40, 80 and 120 days after planting. Rhizome rot incidence will be recorded at harvest by directly counting the number of affected plants out of the total number of plants planted.

Disease incidence will be calculated using the following formula and categorized according to the five-point scale suggested by Reddi Kumar and Anandam (2006).

Both mother and finger rhizomes of all plants will be examined for rhizome rot infection, and the affected plants will be recorded. The disease rating scale used for screening turmeric germplasm against rhizome rot incidence will be presented in Table.

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \text{ X } 100$$

Disease scale for screening of rhizome rot

Scale	PDI	Disease reaction
1	0 -10 %	Resistant (R)
2	11-20%	Moderately Resistant (MR)
3	21-30%	Moderately Susceptible (MS)
4	31-40%	Susceptible (S)
5	Above 41%%	Highly Susceptible (HS)
		Reddi Kumar and Anandam, 2006

Selected references:

- Lakshmi Naga Nandini M, Ruth CH & Gopal K 2017 Screening of turmeric germplasm for resistance to rhizome rot. Int. J. Curr. Microbiol. Appl. Sci. 6(11): 2518–2526. https://doi.org/10.20546/ijcmas.2017.611.296.
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- Shahiba AM, Jayalekshmy VG, Sajeena A & Arun Chacko 2022 Morpho-molecular identification and pathogenicity of *Fusarium oxysporum* pathogens associated with vascular wilt in cowpea (*Vigna unguiculata* L.). Int. J. Plant Soil Sci. 34: 937–948.

Leaf blotch

The leaf blotch disease is caused by *Taphrina maculans*, an ascomycetous fungus. Generally the disease appears and proliferates during September to November. The pathogen survives in the infected leaf debris and spreads through wind. The disease development under field conditions is favoured by relative humidity (80%) and temperature (21-23°C).The disease has been observed to cause considerable damage in Gujarat, Tamil Nadu, Andhra Pradesh and Odisha.

Symptomatology

The disease is characterized by the appearance of spots in large numbers on both surfaces of the leaves, generally being more numerous on the upper surface. It first appears as pale yellow discolouration not sharply defined from the rest of the tissue, soon becoming dirty yellow and then deepening to the colour of bay shade.. The spots are usually small, 1-2 mm and coalesce freely. The infected leaves are not distorted, but they have a reddish-brown appearance and become yellow. In case of severe infection, numerous spots appear on the leaves. But the leaves retain their vitality for a long period in spite of heavy infection and the plants are not killed.



Leaf spot

The leaf spot of turmeric is caused by *Colletotrichum capsici*, a hemibiotrophic pathogen that causes significant losses due to the destruction of active photosynthetic areas. Generally the disease appears and proliferates during August to November. The pathogen survives in the infected planting material (rhizomes), leaf debris as well as collateral hosts and spreads through wind. The disease development under field conditions is favoured by high relative humidity and wet weather.

Symptomatology

The disease appears usually in August and September, when there is high and continuous humidity in the atmosphere, but it has also been observed in comparatively dry weather in October and November in certain areas. The pathogen attacks only the leaves. Infection is usually confined to leaf blades and may occasionally be extended to leaf sheaths. Elliptic to oblong spots of variable size appear on both the surfaces, but more on the upper. These spots gradually increase in size and may eventually attain a length of 4-5 cm and breadth of 2-3 cm. A fully developed spot is greyish-white at the centre with a brown margin all around a yellowish well-defined halo around the infected area. The central region of the spot soon becomes papery and torn. The production of yellow halo is responsible for the production of toxin. Endotoxin produced by pathogen has been observed to play a definite role in symptom expression. Occasionally two or more spots may coalesce to form large necrotic patches, involving a major portion of the leaf blade. In case of very severe infection, most of the leaves may dry up, presenting a bare appearance in the field. In such cases the loss may be more than 50% (Ramakrishnan, 1954).



General recommendations for screening:

A location with a known history of disease incidence or conducive environmental conditions should be chosen (warm and humid). Well-drained, sandy loamy soil with neutral pH is ideal. Kharif growing season (Warm with high humidity) is the most favourable for natural disease development. Genotype(s)/ variety(s) to be screened should be sown in Randomized Block Design (RBD) or Augmented Block Design with 2-3 replications satisfying the degree of freedom. Each test genotype(s)/ variety(s) should be sown in paired row (Row length of minimum 3 to maximum 5m based on availability of land) maintaining a spacing of 30 x 20cm. Recommended fungicide application meant for

foliar disease management should be avoided to ensure disease development. Uniform irrigation and basic agronomic care should be adopted. In case of dry spell, at the time of irrigation, humid condition should be maintained in the crop canopy by spraying 2% urea (limited to 2 spray) or plain water to make plants more succulent during active crop growth period.

Screening for disease resistance under artificial condition:

To create sufficient disease pressure, every five test genotype(s)/variety(ies), two rows of susceptible genotype/variety as check should be sown. In absence of susceptible check, resistant check or released resistant variety can be used. Each block should start and end with one/ two rows of susceptible check. If available, a resistant check should be included in the set of test genotype(s)/ variety (s) meant for screening for comparison.

To create further more disease pressure, artificial inoculation by spraying with the spores/ ascospores water suspension of respective pathogens of the target foliar disease against which screening is to be done.

Source of inoculum : Last season leaves infected with foliar disease kept properly in plastic net bags serve as the source of inoculum.

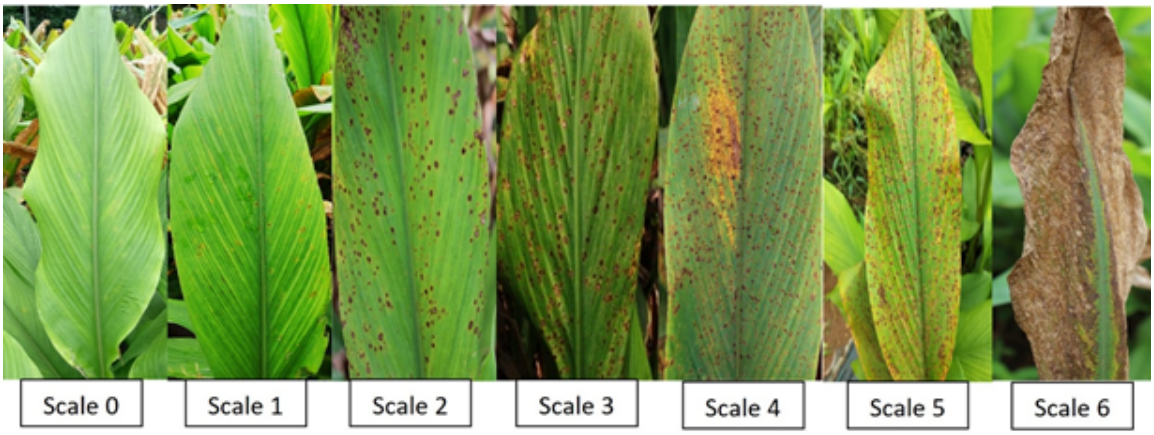
Method of field inoculation : 2-3 previous season infected leaves with either foliar disease (as per objective of disease screening) should be soaked in 5 lits of water (meant for inoculation in 100m2) for at least 48hrs followed by macerating it for enhancing the release of spore/ ascospore in the water. Before spraying the water having spores/ ascospores suspension should be filtered through masleen cloth to avoid clogging of sprayer nozzle while spraying. The water suspension containing spore/ ascospore is thus prepared is ready for inoculation on standing turmeric genotype(s)/ variety(s) under screening or in foliar disease management trial plots (usually of 3x1m plot). The spray volume can be prepared accordingly based on area to be covered.

Time of inoculation : 1st spray with water suspension containing spores/ ascospores should be done at 90 to 120 DAS based on sowing done during 15th May to 15th June respectively to meet the conducive weather condition for disease development. 2-3 such spray of inoculum at weekly interval is sufficient to maintain the inoculum load on turmeric genotype(s)/ variety(s) under disease screening for expression of conspicuous disease symptom.

Data recording: To record the per cent disease index/ intensity (PDI) of leaf blotch and leaf spot, at least five and maximum ten plants should be selected randomly from each genotype (s)/ variety(s) or plots under foliar disease management trial.

In case of foliar disease management trials, plants in border rows/ outer row of the plot should be avoided for recording disease. One leaf each from the top, middle and bottom portion of the plants should be considered for recording the disease score for both leaf blotch and leaf spot disease following a 0–6 rating scale (Gohel et al., 2021).

Disease Rating Scale	Leaf area infected (%)
0	No symptoms on the leaf
1	0.1 to 10% necrotic leaf area
2	10.1 to 20% necrotic leaf area
3	20.1 to 30% necrotic leaf area
4	30.1 to 40% necrotic leaf area
5	40.1 to 50% necrotic leaf area
6	> 50% necrotic leaf area
	Palarpawar and Ghurde (1989)



Representative figures of different scales

Based on the above disease rating scale, PDI is to be calculated for genotype(s), variety(s) under disease screening and also for plot under different treatment in a foliar disease management trial using the following formula:

$$PDI = \frac{\sum \text{ of individual disease ratings}}{\text{Number of leaves observed} \times \text{Maximum disease rating scale}} \times 100$$

(Wheeler, 1969)

Based on thus calculated PDI (%), the genotype(s)/ variety(s) can be grouped in the different categories of resistance or susceptibility given by Datar and Mayee, 1981 and used recently by Basak et.al., 2026 with slight modification as given below:

PDI	Category/ Disease Reaction
Upto 5%	Highly Resistant
5.1-10%	Resistant
10.1-25%	Moderately Resistant
25.1-50%	Susceptible
>50.1%	Highly susceptible
	Datar and Mayee (1981)

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- Ramakrishnan TS 1954 Leaf spot disease of turmeric (*Curcuma longa* L.) caused by *Colletotrichum capsici* (Syd.) Butler & Bisby. Indian Phytopathol. 7: 111–117.
- Wheeler BEJ 1969 An Introduction to Plant Diseases and Fungi. John Wiley and Sons Ltd, London, 301 p.

Root knot nematode

Turmeric is an important rhizomatous spice affected by several plant parasitic nematodes. Sixteen genera of plant parasitic nematodes have been reported to be associated with turmeric, among which root knot nematodes (*Meloidogyne* spp.), lesion nematodes (*Pratylenchus* spp.), burrowing nematode (*Radopholus similis*) and reniform nematode (*Rotylenchulus reniformis*) are considered economically important (Eapen and Pandey, 2018). Among these, *M. incognita* is a major constraint to turmeric production in several growing regions of India. Both *M. incognita* and *M. javanica* have been reported to infect turmeric; however, most of the reports are focused on *M. incognita*. The infection of root knot nematodes results in gall formation on roots and may adversely affect root development, plant growth and rhizome yield.





Symptomatology

Root knot nematode (*M. incognita*) infection in turmeric causes characteristic gall formation on the roots, resulting in distorted root system and reduced root growth. The infected plants exhibit stunted growth, reduced tillering, yellowing of leaves, wilting during hot periods and premature leaf drying under severe conditions. Nematode damage impairs water and nutrient uptake, leading to poor plant vigour, reduced rhizome development and yield. The severity of symptoms depends on nematode population density and environmental conditions. Root galling and the presence of egg masses on infected roots can help identify infestation.

Screening for nematode resistance under artificial conditions (pot culture)

Establishment and maintenance of root knot nematode culture

The root knot nematode infected ginger plants should be collected and *Meloidogyne* spp. should be extracted and inoculated onto healthy, susceptible brinjal or coleus plants grown in pots or growbags. One week after transplanting, each plant should be inoculated with approximately 100 freshly hatched second-stage juveniles (J2) from single eggmass. The plants should be maintained under screen-house conditions for nematode multiplication. After three months, a fresh batch of susceptible host plants should be planted and inoculated to maintain and further multiply the culture. For routine maintenance, infected roots should be processed to extract egg masses and the juveniles should be obtained using modified Baermann funnel method. Freshly extracted J2 should be used for artificial inoculation. If not used immediately, the suspension may be stored at 4°C for 3-5 days and subjected to confirmation of juvenile viability before use.

Screening procedure

Healthy, uniform seed rhizomes of ginger (approximately 25 g each) should be selected, with a minimum of 10 rhizomes per replication and six replications per entry. Each rhizome should be planted individually in a polythene bag (15 X 30 cm) containing sterilized potting mixture consisting soil, sand and farmyard manure in 2:1:1 ratio. The experiment should be laid out in completely randomized design (CRD). One month after germination, each plant should be inoculated with 1000 freshly hatched second-stage juveniles (J2) by making three equidistant holes around the rhizosphere, which should be subsequently covered with soil. IISR Varada and IISR Mahima will serve as susceptible and tolerant checks. Two months after inoculation, the plants should be carefully uprooted and the roots should be washed gently to remove adhering soil. Root galling and egg mass production should be assessed. Egg masses should be visualized by staining the roots with Phloxine B following Holbrook et al. (1983), followed by keeping the roots in glycerol for 24 hours to facilitate observation. Root galling should be scored using the 0-5 root-galling index (Taylor and Sasser, 1978).

The final nematode population (Pf) should be estimated separately from soil using Cobb's sieving and decanting method followed by the Baermann funnel method and from roots using acid fuchsin-acetic acid staining followed by root maceration. The reproduction factor (RF) should be calculated as: RF =Pf/Pi, where (Pf) is the total final nematode population recovered from soil and roots and (Pi) is the initial inoculum of 1000 J2 per plant. Germplasm accessions will be classified according to their root-galling index (RKI), egg mass index (EMI) and RF in comparison with the respective checks. Accessions exhibiting an EMI/RKI below 2 will be shortlisted for further evaluation during the second season.

Rating scale for root-knot nematode presence on roots (0-5)	
Number of egg masses	**Egg mass index (EMI)**
0	**0**
1-2	1
3-10	2
11-30	3
31-100	4
>100	5
Taylor and Sasser (1978)	

Categorization based on reaction towards nematode infection		
Gall index	R factor	Categories
≤2	≤ 1	Resistant
≤2	> 1	Tolerant
≥2	≤ 1	Moderately tolerant
≥2	> 1	Susceptible
Sasser *et al.* (1984)		

Screening for nematode resistance under natural conditions
Establishment of sick plot for root knot nematode

A separate corner of the field measuring approximately 1 m × 1 m will be selected for establishing the sick plot, with suitable precautions to prevent nematode-contaminated soil or drainage water from reaching adjacent fields. The soil will be prepared properly for planting. Brinjal or coleus, which are suitable hosts for *M. incognita*, will be used for nematode multiplication after confirming host suitability. A pure culture of *M. incognita* will be obtained from the screen house and used to inoculate each plant with approximately 100 freshly hatched second-stage juveniles (J2) extracted from single eggmass, one week after transplanting. The plants will be allowed to grow and support nematode multiplication. After sufficient multiplication, the plants will be destroyed, and the soil will be thoroughly mixed by ploughing. A second crop of the susceptible host will then be planted to further increase the nematode population.

Soil samples will be collected to assess the nematode density, and the sick plot will be used for germplasm resistance screening once a reasonably uniform population of at least 1 J2 per cc of soil is established and confirmed.

Rating scale for root-knot nematode presence on roots (0-5)	
Number of galls/ egg masses	**Root knot index (RKI)/ Egg mass index (EMI)**
0	0
1-2	1
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>100	5
Taylor and Sasser (1978)	

Designation of host status based on the degree of resistance		
Gall index/Egg mass	**R factor**	**Degree of resistance designation**
≤2	≤ 1	Resistant
≤2	> 1	Tolerant
≥2	≤ 1	Moderately tolerant
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Sasser *et al.* (1984)		

Selected references:

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Thank You



ICAR-Indian Institute of Spices Research
Post bag No. 1701, Marikunnu P. O.,
Kozhikode- 673 012, Keralam, India.
Phone: 0495- 2731410, Fax: 0495-2731187
e-mail: mail@spices.res.in, Web site: www.spices.res.in