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Methods to study soft rot of turmeric caused by *Pythium myriotylum* Drech.

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Turmeric is one of the valuable spice crop. It is cultivated for various purpose. During cultivation, different fungi infect turmeric. Among these, soft rot is major one. Hence, the present investigation has been undertaken to study the soft rot. For this study the different methods used were dry seed examination, washing method, blotter paper method, agar method, rolled towel method, cut rhizome method, bore method, slice method, sand method and soil method. Among these methods, bore method and slice method is most suitable for the investigations of soft rot as well as other diseases.

Key words : Turmeric, Soft rot, *Pythium myriotylum*.

INTRODUCTION

Turmeric (*Curcuma longa* Linn.) is an important spice crop cultivated for its underground rhizome. The rhizome of the turmeric contains a colouring pigment known as “curcumin” and volatile oil “tumerol”. The rhizome is valued for its medicinal property and it’s usefulness as dyeing agent to cotton, silk, etc. (Appaji Rao and Sarmal, 1962). It is an important condiment of spice exported from India. Turmeric is a perennial herb and the crop is propogated vegetatively from the rhizome. The diseases of economic importance *viz.* responsible for yield losses are foliage spots caused either by *Taphrina maculans* Butler, *Colletotrichum capsici* (Syd.) Butler and Bisby and rhizome rot induced by various fungal pathogens *i.e., Pythium myriotylum* Drech., Sclerotium spp. and *Fusarium* spp.

The rhizome borne fungi are responsible for low germinability to reduce yield and deteriorate quality of rhizome. *Colletotrichum capsici* is known to be carried through scales of the rhizome (Rangaswami, 1972). However, more information needs to be explored especially about the fungal component involved in inducing different types of rots. The cut surface of rhizomes provides open court for infection and especially the mode of storage of sets to be used for planting, which aggravates the fungal infection. The high water content of the propagative materials or rhizome in comparison with true seeds adds to their vulnerability to infection by soil borne mycotlora. By keeping this in view, the present investigation has been carried to find out suitable method to study soft rot of turmeric.

MATERIALS AND METHODS

The turmeric rhizomes were subjected for detection of mycoflora by using the standard technique as recommended by ISTA (1966). The following methods were adopted to study thy soft rot of turmeric:-

- Dry seed examination
- Washing test
- Blotter paper method
- Agar method
- Rolled towel method
- Cut rhizome method
- Bore method
- Slice method
- Sand method
- Soil method

First method is dry seed examination. In this method, dry or infected rhizome sets of turmeric to ascertain the association impurities are classified as inert matter as per ISTA rules inclusive of discolouration, association of plant parts, spore masses, sclerotial bodies, etc. Examination was done with the help of low power binocular microscope.

In the second method *i.e.* washing test, the small pieces of infected rhizome of turmeric were taken and soaked in sufficient amount of sterile distilled water for 5 to 10 minutes and shaked over in a shaker machine. The liquid obtained was centrifused in a centrifuse machine at 2500 to 3000 rpm for 10 to 15 minutes. Thus, the two layers obtained of which sediment was examined under a compound microscope for identification of fungi.

Third method is blotter paper method, in which three layered blotter papers of equivalent size of Petridish were

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soaked in sterile distilled water and kept in the Petridishes. The rhizome of turmeric size between 1 cm x 1 cm were placed in, Petridishes, The Petridishes were incubated at room temperature ($28^0C \pm 1^0C$). Observation were recorded on 5th day for the development of fungi on the rhizomes. Distilled water added whenever required for moistening in the blotter paper.

In the agar method, sterile potato dextrose agar (PDA) was used. The sterile PDA was poured in sterile Petridishes. Pieces of infected rhizome of 2 cm x 1 cm size were selected for isolation work. The sample was surface sterilized with 0.1% $HgCl_2$ solution for 2 minutes and then passed through three changes of sterile distilled water, After this, the pieces were transferred on PDA in the Petridishes. The Petridishes were incubated at room temperature for 5 days and observations were recorded on 5th day of incubation.

For rolled towel method, the two towel papers were used of size 42 cm x 30 cm. The pieces of infected or healthy rhizomes of turmeric were placed on towel paper which was moistened with the sterile distilled water. On this towel paper, the infected and healthy rhizomes were kept and covered with another towel paper and both were rolled. These rolled towel papers were incubated at room temperature for five for observations. The towel papers were covered by polythene bag for retention of moisture and water was added to moisten the paper.

The sixth method is cut rhizome method in which the rhizomes are cut into the pieces of size 2 cm x 1 cm and put it on the blotter paper in the Petridishes and inoculated with the pathogen in the disc of 5 mm in the centre. Sterile distilled water was added for moisten blotter paper.

In bore method, the cork borer of ½ cm diameter was sterilized and inserted in rhizome upto 1.5 cm deep and was taken out with a bit of its flesh. The inoculum was added in the bore with 1 ml suspension of 5 mm diameter pathogen culture. Kept this inoculated pathogen on the blotter paper in the Petridish and noted the observation after five days.

While in the slice method, rhizome was cut in the slice of thickness 0.2 cm placed on blotter paper. In the centre of slice, a 5 mm disc of pathogen was inoculated. Observations were recorded after 5 days. Added sterile distilled water on the blotter paper for moisten.

In sand and soil method both sand and soil were sterilized in the oven. Kept these sand and soil in the sterilized Petridishes, the rhizome of size 2 cm x 1 cm was kept on this Petridishes. Added sufficient amount of water to moist the sand and soil. These Petridishes were incubated at room temperature and observation was taken after five days.

RESULTS AND DISCUSSION

Samples of infected turmeric. were analysed for the presence of rhizome borne mycoflora by employing different techniques.

– In dry rhizome examination, the turmeric showed two types of rots, soft rot and dry rots. In dry rot and soft rot, rhizomes gots discolouration, presence of mycelia, sporulation and mustard like bodies were noticed.

– The examination of rhizome washing revealed presence of condial masses of following fungal genera- *Aspergillus*, *Rhizopus*, *Fusarium*, *Helminthosporium*, *Colletotrichim*, whitish mycelium etc.

– Blotter paper method :

The given data presented in below table revealed that the percentage of infection was significant in infected rhizome rather than healthy rhizome. In this method, the germination of turmeric was less as compared to percentage of infection. The mycoflora which was found in the blotter method were *Mucor* spp., *Rhizopus* spp., *Helminthosporium* spp. and *Pythium* spp.

Incubation period (Days)	% of infection		% of germination		% of mycoflora	
	US	S	US	S	US	S
5	5	-	-	-	3	-
6	10	5	2	-	5	-
7	15	12	5	2	7	5
8	25	20	10	7	14	7
9	35	25	15	10	18	12
10	50	40	20	15	25	18

– Agar plate method :

In the agar method, the percentage of infection was more as compared to other methods, as there was increase in incubation period, the percentage of infection also increased. The percentage of mycoflora was also high.

Incubation period (Days)	% of infection		% of mycoflora		% of germination	
	US	S	US	S	US	S
5	10	5	5	2	-	-
6	20	10	12	5	-	-
7	35	20	20	10	-	-
8	50	35	30	18	5	5
9	75	50	45	25	5	7
10	80	60	50	30	7	10

The mycoflora found in this agar methods were *Aspergillus* spp. *Pythium myriotylum*, *Sclerotium* spp., *Fusarium* spp. and *Alternaria* spp.

– *Rolled towel method :*

In rolled towel method the percentage of infection was not more than 40% at 9th day of incubation. The turmeric got germinated 75% on 9th day of incubation period. The percentage of infection was less than the percentage of germination of turmeric. Hence, the rolled towel method was supposed to be the best germinability method because the per centage of germination was more as compared to other methods.

Incubation period (Days)	% of infection	% of mycoflora	% of germination
5	5	2	10
6	15	10	20
7	20	12	35
8	35	18	50
9	40	25	75

– *Cut rhizome method :*

In cut rhizome method, the growth of pathogen was significantly growing fast. The growth of pathogen increased with the increase in incubation period.

Incubation period (Days)	Linear growth (mm)
4	5
5	6
6	6.5
7	8
8	8.5

– *Cork borer:*

In the cork borer, the percentage of rotten rhizome is less as it was inoculated with the *Pythium myriotylum*.

– *Slice method :*

From the given data, there was slow growth of pathogen as compared to cut rhizome method. As in slice method there was less storage food material the fungi did not grow as fast in cut rhizome method. The slice method was not significant in the growth of pathogen.

Incubation period (Days)	Linear growth (mm)
4	5
5	5.2
6	5.5
7	6
8	6.5

– *Sand method :*

From the given data the percentage of infection increased as there was increase in incubation period. The percentage of germination was less.

Incubation period (Days)	% of infection	% of mycoflora	% of germination
4	15	7	5
5	20	10	5
6	25	17	7
7	30	20	10
8	40	30	15

– *Soil method :*

As given in the data, the percentage of infection was more as compared to Sand method. The percentage of germination was also more.

Incubation period (Days)	% of infection	% of mycoflora	% of germination
4	15	5	-
5	20	10	-
6	20	15	5
7	25	20	8
8	30	22	10

Hande (1983) also studied the rhizome borne fungi of turmeric. He has used these different methods to study rhizome rot. He is also of the opinion that Cork borer method and Cut rhizome method are suitable for the investigation. The experimental results are similar to the Hande (1983). Cut rhizome method is more suitable as it gave more growth in less incubation period, therefore it is of practical importance to study the rhizome borne diseases and their control.

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