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RESEARCH ARTICLE

STUDIES ON COLLAR -ROT IN TUBEROSE

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ABSTRACT

Collar rot is a serious disease of tube rose (Polianthes tuberose L.) caused by Sclerotium rolfsii Sacc. reported in Odisha. The malady is rapidly gaining momentum inflicting heavy damage to this commercial ornamental crop. To study about this disease, with a chain of operations like planting material collection, isolation of pathogens, sterilisation of glassware, preparation of different media and inoculation in the media were conducted. Out of seven inoculation methods compared, artificial inoculation was highly successful, when inoculums mixed in top 2-3 cm soil and covered with leaf debris of tuberose. Ground nut shell and Sorghum grain media supported maximum Sclerotia and mycelia growth of the fungus.

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INTRODUCTION

Tuberose (*Polianthes tuberose* L.) has gained considerable importance among bulobous ornaments for the prettiness, elegance and sweet fragrance of its flowers. It has tremendous economy potential in cut –flower trade and perfumary industry. The flower remain fresh for pretty long time and stand long distance transportation. The flowers are used for making garlands, floral ornamental, bouquets and button holes. The long flowering spikes are excellent for table decoration arranged in bowls and vases. Variegated type is very attractive and suitable for the garden for beautification. Like any other crops, tuberose also suffers from several diseases. Of these Collar-rot is one. The disease often known as stem rot, sclerotial-rot or sclerotial wilt. This destructive disease tuberose was for the first time described from West Bengal of India (Das, 1961).The causal fungus was reported to be *Sclerotium rolfsii* Sacc. Its occurrence was also recorded from Ranchi area of Bihar (Dutta, 1975). In Orissa, the malady is rapidly gaining momentum inflicting heavy damage to this valuable commercial; ornamental crop in recent years. The disease causes rotting of leaves, bulbs and flowering shoot leading to mortality of entire clump.

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With the increase of area and intensive cultivation of susceptible varieties, the disease is likely to be accentuated in near future. Hence a necessary study is likely to be accentuated in near future.

MATERIALS AND METHODS

Different operations were conducted to study about the pathogen causing collar - rot in tube rose.

Collection of planting materials

The plant samples were collected from farmer's field. Each sample was labelled properly and taken into laboratory for examination of incidence of collar rot caused by *Sclerotium rolfsii.*

Isolation of Pathogens

With the moist blotter method recommended by ISIA (1953,1961), the diseased plant sample collected were washed and diseased collar parts were cut into pieces which were then washed and diseased collar parts were cut into pieces which were then disinfected with 1:1000 (0.1%) mercuric chloride solution. These were transferred to PDA slants after several washing in sterile water and incubated at 28^0C+1^0C. The

culture were maintained by sub-culturing time to time PDA slants.

The pure culture was obtained by transferring a young immature white Sclerotium from culture tube to a fresh PDA slant and incubated for 9-10 days. From this culture a young white Sclerotium was again transferred to sterilised PDA slant. Thus a pure culture was obtained and maintained by sub culturing.

Preparation different Media

Potato dextrose medium was prepared by boiling 200g of peeled sliced potato in 500ml of distilled water. 20 g of agar ager was added in another 500 ml of water an stirred properly then added 20 g dextrose and the final volume was adjusted to 1 lit by adding potato decanded solution.

Different natural media like sorghum grain medium(SGM),wheat grain medium (RSM),rice straw medium (RSM) and sand maize meal medium SMM) were prepared by taking 100 g grains ,shell or straw , soaked in water for about 6 hrs. They were sterilized separately in 250 ml Erienmeyer flask at 121^0C for 45 minutes. Sand maize meal medium was prepared as above taking 90 g of fine sand,10g of maize meal in which 20 ml of water was added.

Inoculation in the media

Each flask was seeded with a mycelia disc (5mm) from a 7 days old culture of S.rolfsii grown in PDA and incubated at $28+1^0$C for 3 weeks. The flask was used for each medium and each flask was considered as one replication.

Pathogenicity

To prove the pathogenicity of the fungus seven inoculation methods were tested such as (1) sclerotia mixed in entire soil, (2) sclerotia mixed in top 2-3 cm soil, (3) sclerotia mixed in 2-3 cm soil and covered with leaf debris, (4) sclerotia spread on surface soil, (5) sclerotia spread on surface soil and covered with leaf debris. (6) Mycelia propagules placed in the plant collar region and (7) mycelia propagules placed in plant collar region and covered with leaf debris, an compared with suitable check without any inoculation. The plants were raised in 15 cm pots filled with sterised soil autoclaved with at 1210C for 45 minutes for consecutive two days. Five bulbs were planted in each pot and two pots were taken as one replicate.

RESULTS AND DISCUSSION

Morphology and cultural characterstics of the fungus was described in detail. Seven methods of inoculation were tried to prove the pathogenicity of the fungus and see their effectiveness. All the methods of inoculation tested successfully caused infection and expressed disease symptoms on inoculated host plants. However, no disease symptoms were seen in un-inoculated control plants until end of the experiment. Maximum infection of 100% was achieved where sclerotia were mixed in top 2-3 cm soil and covered with leaf debris of tube rose (Table 1). Mycelial propagules placed in plant collar region and covered with leaf debris of tube rose and sclerotia mixed in top 2-3 cm layer of soil also proved successful in creating high disease incidence. All other methods of inoculation produced satisfactory infection except the method where sclerotia mixed in entire soil and sclerotia spread on surface soil. High incidence and rapid symptom development in this method of inoculation may be ascribed due to easy availability of

Table 1. Effect of different methods of inoculation of Sclerotium rolfsii in causing collar-rot in tube rose

Methods of inoculation	Plant infection (%)	Incubation period (days)
Sclerotia mixed in entire soil	26.7 (30.99)	17
Sclerotia mixed in top 2-3 cm soil	83.3 (70.07)	9
Sclerotia mixed in top 2-3 cm soil and covered with leaf debris of tuberose	100.0 (90)	7
Sclerotia spread on surface soil	36.7 (37.22)	15
Sclerotia spread on surface soil and covered with leaf debris of tuberose	70.0 (57.00)	11
Mycelia propagules placed in plant collar region	72.0 (58.05)	11
Mycelia propagules placed in collar region and covered with leaf debris of tuberose	84.0 (70.07)	9
Control	0 (0)	0
SE(m) +_	(5.88)	
C.D. (0.05)	(18.11)	

Figures in prenthes represent log transformed value

Table 2. Growth of *Sclerotium rolfsii* in different culture medias

Culture media	Number of Sclerotia	Mycelial growth
Sorhum grain medium (SGM)	656.6696.48)	+++
Wheat grain medium(WGM)	210.00(5.34)	+++
Ground nut Shell medium(GSM)	2044.00(7.62)	++
Rice Straw medium(RSM)	403.33 (5.99)	+
Sand- Maize meal medium (SMM)	1367.33 (7.21)	+
Potato dextrose medium (PDA)	746.66 (6.61)	+++
SE (m) +	(0.18)	
C.D. (0.05)	(0.12)	

Figures in prenthes represent log transformed value

senescent plant tissue and a warm moist environment created near the base of the plant due to moisture conservation by mulching of leaf debris. It was from the table 2 that, the fungus was successfully isolated on potato dextrose agar from the infected leaf and stem.

The morphological characters of the fungus in diseased sample and in pure culture as regards to its mycelia growth was radiating with much aerial hyphae and aggregated to form rhizomorph. Hyphae were hyaline, septate and thinned walled. Clamp connection were formed frequently. Branching were at an acute angle. Sclerotia were almost round and dark coloured and found embded in host tissues. Similar morphological characters of *S. rolfsii* was described by earlier workers (Das, 1961, Das, 2002, Mukhopadhyay, 1971; Saccardo, 1911) very well matched with the present finding.

Shew and Beute (1984) opined similar view while conducting experiment on *S. rolfsii* in groundnut. Further a thin layer covering might have fascinated aeration and moisture conservation providing congenial condition for high infection. This method may be useful in testing varietal resistance in large scale in field condition. High infection was also recorded where mycelia propagules were placed in the collar region and covered with plant debris or scelortia mixed in the top 2-3 cm soil. Placing propagules of *S. rolfsii* is a cumberson and laborious process. This method may not be adopted for mass screening of variety for above reason Pandey *et al.* (1994) achieved success in infecting ground nut employing above method. Chiranjeebi *et al.* (1981) and Kondey *et al*.(2008) also reported the effectiveness of mixing of scelorotia of S. Rolfsii in top soil creating artificial epipaytotics of stem- nut of Groundnut.

Out of six media compared for sclerotia production and mycellial are growth of *S. rolfsii*, ground nut shell medium (GSM) supported maximum scleorotial production followed by sand maize meal medium (SMM). Sorghum (SGM) and wheat grain medium (WGM) and encouraged good mycellial growth than scleorotial production. The finding corroborates with those of Pandey *et al.* (1994, Dutta, 2002) who claimed higher production of scelorotia of *S. rolfsii* in GSM and good mycellial growth in Sorghums grain medium (SGM).

The ability of G.nut shell medium (SGM) to support sclerotial production compared to sorghum or wheat grain medium may be attributed to the lower weight (Volume to weight ratio) of substrate as compared to Sorghums grain as observed by Boyle (1961).

So it was concluded that out of seven methods of inoculation, maximum infection occurred where sclerotia were mixed in top 2-3 cm soil and covered with leaf debris.Out of six culture media highest mycelia growth was observed in sorghum grain medid followed by wheat grain media.

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