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Research Article

INFECTION OF *RHIZOCTONIA SOLANI* IN SEEDS OF SPONGE GOURD (*LUFFA CYLINDRICA* L. ROEM)

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ABSTRACT
Luffa cylindrica (L. Roem) seeds naturally infected with *R. solani* showed black pin head like spots (microsclerotia) on 99(2.25-35.25) out of 120 seed samples. These seeds on incubation yielded pure growth of pathogen. On the basis of severity of infection seeds were characterized as asymptomatic (control) and symptomatic seed. Components of heavily infected seeds showed the heavy aggregation of mycelium and sclerotia. Cleared whole mount preparations of these seeds showed the presence of exact location of pathogen in seed components. In asymptomatic it is restricted to the layers of seed coat (10%-5%). In symptomatic (weakly and heavily infected) seeds pathogen invades into the aleurone layer, cotyledons and embryonal axis. In microtome cut sections of symptomatic seeds showed septate, branched, dark coloured inter as well as intra cellular mycelium and sclerotia in the epidermal, hypodermal, palisade layer, parenchymatous cells and inner epidermis. The pathogen is both externally and internally seed borne. The internal inoculum affects seed germination.

Keywords: *Luffa Cylindrica, Microsclerotia, Pathogen, Rhizoctonia Solani, Symptomatic Seeds*

INTRODUCTION
Smooth gourd (*Luffa cylindrical* L. Roem) is an important vine crop extensively grown in Rajasthan. It is a multipurpose crop having paramount importance and is widely accepted for its nutrients. According to Seebold (2010) common fungal disease affecting cucurbit fruit crops are belly rot (*Rhizoctonia solani*), Choanephora rot (*Choanephora cucurbitarum*), cottony leak (*Pythium sps.*), Fusarium fruit rot (*Fusarium sps.*) and Southern blight (*Sclerotium rolfsii*). Shakir *et al.,* (1995) recorded 11 seed borne fungi associated with sponge gourd seed. Shakoor *et al.,* (2011) isolated 6 fungal species from seeds of bitter gourd. During survey of seed borne mycoflora *R. solani* was recorded as an important seed borne pathogen.

MATERIALS AND METHODS
120 seed samples of sponge gourd were collected from 7 selected areas namely Arjunpura, Arandkhera, Giridharpura, Kethun,Nayanohra, Raipura and Dhakarkheri of Kota district of Rajasthan. For screening of samples for seed borne mycoflora ISTA (International seed health testing association) procedures were followed (Anon, 1985). It included dry seed examination and incubation test (SBM and PDA).
Histopathological studies were carried out for the location of *R. solani* in *Luffa cylindrica* L. Roem seeds. The seed samples of sponge gourd ac. no. Sg 7 (Arjunpura) and Sg-56 (Kethun) were selected. The seeds of each sample were catagorised as Asymptomatic (bold healthy looking) and Symptomatic seeds. The symptomatic seeds were further classified as weakly and heavily infected on the basis of severity of seed symptoms. The methods employed for histopathology are component plating, cleared wholemount preparation of seed components and microtome sectioning (Johansen, 1940).
In Component plating each sample was washed and soaked in distilled water for 6-12 h. different seed components viz. seed coat, aleurone layer, cotyledons and embyonal axis were dissected aseptically under stereobinocular microscope with the help of sterlized forceps and needle. Each component was surface sterlized with 2% available chlorine and tested by standard Blotter Method and Potato Dextrose Agar Method. Observations were taken after 7 days of incubation.
In Cleared Wholemount Preparation seed sample infected with *R. solani* were boiled individually in aqueous solution of 10% KOH for 15 minutes to clear the tissue. Aleurone layer and cotyledons were

boiled for 15 min in lactophenol containing cotton blue (1:1, v/v) and mounted in poly vinyl alcohol (Omar, Bolland and Heather, 1979). All the seed components were press gently under cover slip till the cells spread uniformly (Singh *et al.,* 1977). The slides were then kept in an oven at 60º C for drying.

In Microtome sectioning sample were soaked overnight in sterlized distilled water in an oven at 60º C until they soften. The seeds were fixed in 70% alcohol for 48 h in vials. For proper infiltrations the seeds were given 2-3 fine incisions and dehydrated through tertiary butyl alcohol (TBA) series, infiltrated and embedded in paraffin wax (BDH). The embedded materials were cut into the blocks. Wax blocks were cut to expose the tissue and emerged in 1% aqueous solutions of sodium lauryl sulphate for 12 hrs (1:1, v/v) and then transferred to acetoglycerine (mixture of acetic acid + glycerol in 1:1 ratio) for 7 days for further softening. Blocks were cut through microtome at 15-20µ thickness, deparaffinised, stained with safranine and light green combinations and mounted in DPX (Johansen, 1940).

RESULTS

In dry seed examination black pin head like minute spots (microsclerotia) of *Rhizoctonia solani* covered the seed surface of about 99 samples out of 120 seed samples of sponge gourd belonging to 7 areas of Kota district viz. Arjunpura, Arandkhera, Giridharpura, Kethun, Raipura, Nayanohra and Dhakarkheri of Rajasthan (Table 1). Incubation of these symptomatic seeds yielded pure growth of the pathogen. The percent range of fungi associated with such seeds varies between (2.25-35.25%). Weakly symptomatic seeds carried 1-10% sclerotia whereas heavily infected seeds were sparsely to fully cover with sclerotia (Table 2).

Table 1: Number of infected seed samples and percent range of *Rhizoctonia solani* in dry seed examination, SBM & PDA in different areas of Kota district

Areas	No. of seed samples	Dry seed examination	SBM		PDA
			Untreated	Pretreated	
Arjunpura	15	13(3.0-15.25)	12(8-50)	10(6-40)	8 (9-51)
Arandkhera	10	8(12.0-18.25)	7(13-30)	7 (6-38)	5 ((8-40)
Giridharpura	12	10(3.75-16.25)	8 (6-40)	8 (6-40)	6 (9-51)
Kethun	60	54(2.25-35.25)	50 (4-78)	48 (7-59)	25 (8-68)
Raipura	8	4 (5.75-13.75)	2 (6.25-11)	-	-
Nayanohra	7	4 (6.25-15.00)	2(6-11)	-	-
Dhakarkheri	8	6(3.75-14.25)	5 (6-21)	4 (3-20)	2 (2-7)
Total	120	99(2.25-35.25)	86(4-78)	77 (3-59)	46 (2-68)

Table 2: Percent infection of *Rhizoctonia solani* in different parts of asymptomatic and symptomatic (weakly and heavily infected) seeds of smooth guard in component plating and cleared whole mount preparation (SC-Seed Coat, AL-Aleurone Layer, COT-Cotyledon, EA-Embryonal Axis)

Component Plating												
Ac. No.	**Asymptomatic**				**Symptomatic**							
					Weakly				**Heavily**			
	SC	AL	COT	EA	SC	AL	COT	EA	SC	AL	COT	EA
Sg-7	15	5	-	-	55	40	30	25	85	65	50	35
Sg-56	10	-	-	-	40	35	25	20	70	50	40	20
B- Cleared Wholemount Preparation												
Ac. No.	**Asymptomatic**				**Symptomatic**							
					Weakly				**Heavily**			
	SC	AL	COT	EA	SC	AL	COT	EA	SC	AL	COT	EA
Sg-7	10	-	-	-	40	35	25	15	70	55	40	25
Sg-56	5	-	-	-	35	30	20	10	65	50	35	15

Samples of all the selected areas of Kota districts viz. Arjunpura, Arandkhera, Giridharpura, Kethun, Raipura and Dhakarkheri of Rajasthan carried *Rhizoctonia solani* infected seeds but higher incidence of fungi were recorded in samples from Kethun, Arjunpura, Nayanohra and Dhakarkheri.

120 and 59 samples of sponge gourd were studied by blotter method and PDA plate method respectively. 86, 77 and 46 samples isolated *Rhizoctonia solani* in untreated, pretreated seeds in SBM and PDA respectively. Except Raipura, all the selected areas of Kota districts recorded the pathogen in untreated and pretereated seeds (SBM). The incidence of fungus was 4-78% in untreated, 3-59% in pretreated and 2-68% in PDA test. On incubation, the seeds yielded dark brown, septate, branched, mycelium, microsclerotia of the pathogen.

In component plating in both asymptomatic as well as symptomatic weakly infected seeds we could easily separate various components of the sponge gourd seed viz. seed coat, aleurone layer, cotyledons and embryonal axis. While various components of heavily infected seeds could not be separated easily.

The components of asymptomatic seeds, mycelium and microsclerotia were observed (15, 10%) on seed coat (5, 0%) on aleuron layer of both the samples respectively. Scelrotial infection in weakly infected seed recorded (55, 40%) on inside and outside of seed coat, (aleurone layer (40, 35%), cotyledon (30, 25%) and embryonal axis (25, 20%) (Figure 1 B&C) of the two samples respectively. Microsclerotia were found on all the components of heavily infected seeds with higher incidence. Heavily infected seeds of both the samples recorded (85, 70%) on seed coat, (65, 50%) on aleurone layer, (50, 40%) on cotyledons and (35, 20%) microsclerotia in embryonal axis (Figure 1D) respectively. 6^{th} day of incubation showed complete rotting of the components (Table 2).

In cleared Wholemount Preparation the seed component showed microsclerotia with branched, septate, brown coloured, thick, knotty mycelium (Table 2, Figure 1E&F.). Microsclerotia and mycelium were restricted to the seed coat only (10, 5%) in asymptomatic seeds while it was observed in all parts of weakly infected seeds. In both the samples the infection was (40, 35%) in seed coat, (35, 30%) in aleurone layer (25, 20%) in cotyledons and about (15, 10%) in embryonal axis respectively. In heavily infected seeds it was (70, 65%) in seed coat, (55, 50%) in aleurone layer, (40, 35%) in cotyledon and (25, 15%) in embryonal axis of both the samples respectively.

The microtome section of two samples of asymptomatic seeds viz. ac. nos. Sg-7 and Sg-56 of sponge gourd carried mycelium in 4, 2 seeds respectively out of 10 seeds. Some outer layers (epidermis, subepidermis and scelerenchymatous palisade layers) of spermoderm were infected with the mycelium and microsclerotia while it was not observed in any other tissue. Sections of all the components of asymptomatic seeds had intact and well developed cells.

In weakly infected seeds spermoderm and tissue of hilar fissure showed branched, septate, dark coloured mycelium and microsclerotia. Dark coloured septate mycelium was both inter and intra cellular in seed coat perenchyma. Cuticular eruptions of seed coat also show the presence of microscelrotia between them. Sclerotia lead to the disintigration and deformation of cells. Presence of mycelium and sclerotia in epidermis, subepidermis, palisade layer, parenchymatous cells and inner epidermis caused weakening and deformation of cells (Figure 1). Components of cell remain unaffected. Between aleurone layer and cotyledons and also between two coteledons hyphae ramification was observed.

Heavily infected seeds covered with dense microscelerotia all over the seed surface. All the components of heavily infected seed showed the presence of thick septate and knotty mycelium and sclerotia. Heavy incidence of the pathogen leads partial to complete disintegration of parenchymatous cells. Due to pressure of dense mycelium and microsclerotia stressed stellate parenchymatous either disintegrate or their lysis occurs. Loosening of palisade layer and varying degree of withering of seed coat layers also observed. Microsclerotia and mycelium were also observed in hilar parenchyma.

Heavy aggregation of mycelium along with many microsclerotia in thick walled cells of aleurone layer causes lysis and disintegration of their cell walls (Figure 1G&H).

In heavily infected seeds dense mycelium and sclerotia also cover cotyledons, plumule and radicle axis of the embryo. Presence of thick and knotty mycelium leads to disintegration and undifferentiation of outer epidermal cells of cotyledons and uneven thickening of the cells of abaxial side of the cotyledons. Dark coloured hyphal bits were also observed between the two cotyledons. Heavy infection penetrated the storage tissue of the cotyledonary region and lead to deformities in the cotyledons. Cell components are

deficient and cells appear empty due to disintegration of starch grains and proteinoplast. The embryonal axis and at the tip of plumule and radical also showed the presence of microsclerotia.

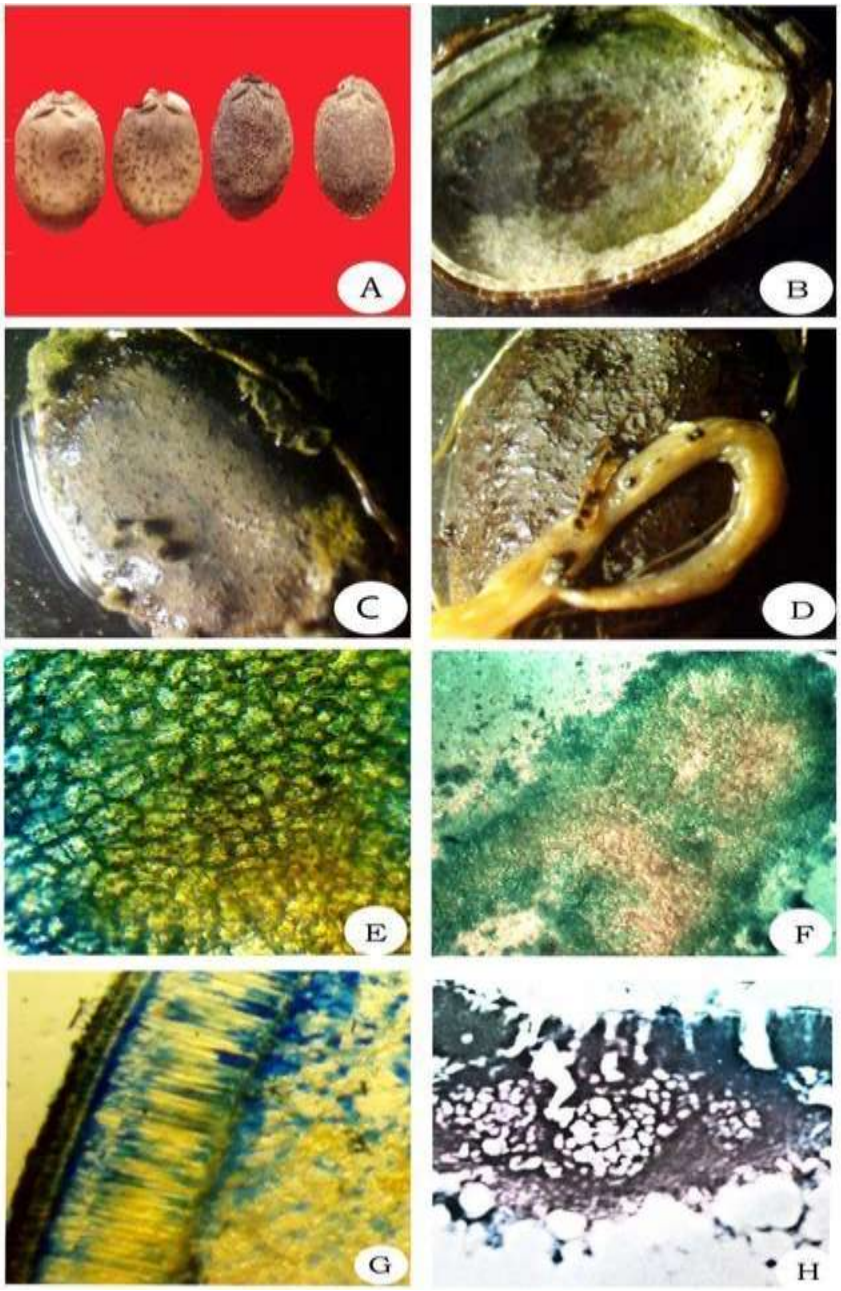

Figure 1 A-H: Histopathalogical studies of *Rhizaotonia solani* on infected seeds

A. Normal looking seeds and black discoloured seeds with the infection of *R. Solani*
B. Growth of sclerotia on the seed coat region
C. Disrupted cotyledon due to fungal infection
D. Infected embryonal axis
E. Dense inter and intra cellular mycelium in seed coat cells
F. Cleared whole mounting of infected aleurone layer and cotyledon
G. Hand cut section of seed coat and cotyledon
H. T.S part of seed coat showing sclerotial aggregation and hyphal mat in cotyledon epidermis

DISCUSSION

In the present study *Rhizoctonia solani* is the major pathogen of the vine crop. 86 samples of the smooth gourd were infected with *Rhizoctonia solani* is showing 4-78% incidence. The study has proved the accurate determination of incidence and spread of inoculum in seed. Component plating and incubation were used for incidence but wholemount cleared preparation gave priliminary detection of pathogen. Detail study about location and distribution of fungal mycelium in seed can be possible only from microtome sectioning of naturally infected seed.

Hedgecock (1904) was the first to report microsclerotia of *R. solani* on seeds of bean. Seed borne nature of fungus has been reported by Sultana and Ghaffar, 2007 in bitter gourd and in 2009(a, b) in bottle gourd. They observed the infection of *R.solani* in different seed components of (*Luffa cylindrica* L.Roem). Shakir, Mirza and Ahmed (1995) observed the pathogen in different components of seeds of sponge gourd (*Luffa cylindrica* L. Roem)**.**

The present study has revealed that inoculum of *R.solani* occurs in asymptomatic as well as symptomatic seeds of sponge gourd. However the incidence was low and infection was confined to the seed coat in asymptomatic seeds. But in symptomatic (weakly and heavily) infected seeds, pathogen was present in all components of seed. The infection and distribution of the pathogen was correlated with degree of symptoms.

In the present study, symptomatic seeds showed septate, branched, dark coloured inter as well as intracellular mycelium and sclerotia in the epidermal, hypodermal, palisade layer, parenchymatous cells and inner epidermis. Immature to mature sclerotia in between the cells of parenchymatous hypodermis resulted disintegration, lysis and vacuolation of cells and tissue. This is beacause of the activity of pectolytic and cellulolytic enzymes produced by the pathogen as reported by Goel and Mehrotra (1974). *R.solani* is known to produce enzymes like pectin transeliminase, pectin metyl estarases and cellulolytic enzyme (Albersheim, Neukon and Deuel, 1960). Kumar *et al.*, 2009 reported *R.solani* isolates that produce extra cellular cellulase, pectolytic and protease enzymes. According to Kuc, 1962; Bateman and Basham, 1976 pathogen secretes carbohydrate, protein, lipid degrading enzyme which results in depletion of cell contents of endosperm and embryo. There was tissue disintegration, presence of necrotic cells due to pathogen in different parts viz. seed coat, aleurone layer and cotyledons. (Poromarto, 1998) reported in *R. solani* infected soyabean there was evidence of cuticle degradation at the point of penetration. Infected hyphae moved to adjacent epidermal cells by direct penetration of epidermal radial walls. There were epidermal and cortical cell necrosis, beginning with the fragmentation of the tonoplast and followed by the disintegration of cytoplasm, organales and plasma membranes.According to Singh, 1989 observed in *R. solani* infected cotton seed the cuticular layer disappears in the regions of close contact between the hyphae and the epidermal outer surface. During the pre- penetration stages the cell walls of the seedlings underwent a significant, visible degraded.

In heavily symptomatic seeds, cells were under stress and either lysed or disintegrated because of microsclerotia and mycelium. Hilar vascular bandles also filled with hyphal bits. Singh and sinclair (1985) observed that the penetration of *Cercospora sojinain* soyabean seeds takes palace through seed pores and hilar tracheids.

Thus the present study indicates that there is extra embryonal infection in asymptomatic seeds which is mostly confined to the outer seed coat layers. In symptomatic seeds the infection is intra embryonal and deep seated and distributed in all components of seed.

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