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INCIDENCE AND HISTOPATHOLOGY OF *RHIZOCTONIA BATATICOLA* ON BLACK GRAM GROWN IN RAJASTHAN

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Vigna is one of the important genus among the pulses which form a major part of Indian dietary. Three field surveys covering all the major crop growing in districts of Rajasthan state were carried out. 123 seed samples were collected. Seven diseases were recorded in different fields. Various abnormalities were found on dry seed examination. A total of 39 fungal species were recorded in standard Blotter Method. During histopathological studies of infected seeds component plating, cleared whole mount preparations and microtome sections were used to determine the location of seed-borne pathogen. Symptomatology and histopathology of pod parts was also carried out.

Keywords : Dry seed examination; Field survey; Histopathology; Seed- borne pathogen.

Standing crop of Urdbean was inspected during the Kharif seasons in the districts of Banswara, Bhilwara, Chittorgarh, Dungarpur, Jhalawar, Kota, Sawai Madhopur, Tonk and Udaipur. For assessing infection in field a quadrat ($1m^2$) was used and number of diseased, healthy plants and weeds were counted and the seed samples were collected at the time of harvesting of the crop. A total of 123 samples from the above mentioned districts were collected. Screening of seed samples for seed-borne mycoflora, seed health testing procedures as described by the International Seed Testing Association (ISTA) were followed[1].

Dry seed examination - All the 123 seed samples collected were subjected to dry seed examination. 400 seeds per sample were drawn at random and examined by naked eye as well as under stereobinocular microscope (10-40x) for the presence of bold normal looking seeds, various discolourations, disorders, inert-matter, weed seeds and broken and insect damaged seeds.

Incubation tests

Standard Blotter Method (SBM)-All the seed samples were studied. 400 seeds per sample, 200 untreated and 200 pretreated with aqueous solution of sodium hypochlorite were tested. 25 seeds were spaced per Petriplate (9 cm diameter) containing 3 well moistened blotters and incubated at 26± 2°C under 12 hrs of alternating cycles of artificial light and darkness for seven days. Percentage of seed germination, seed-borne mycoflora, symptoms on seedling and other abnormalities were recorded on 8th day of incubation under stereobinocular microscope.

Histopathology of infected seeds- Histopathological study of naturally field infected seeds was carried out. Seeds were categorised into (i) asymptomatic seeds (Healthy looking) and (ii) symptomatic seeds. Symptomatic seeds were further categorised into (i) weakly and (ii) heavily infected on the basis of severity of seed symptoms. Each category was handled separately. The following methods were applied.

Component Plating- 50 seeds per category per sample were washed individually (1 seed/ test tube) 4-5 times with sterile distilled water, soaked for 3 hrs and dissected aseptically with the help of sterilized forceps and a pair of needles under stereobinocular microscope to separate various seed components viz seed coat, cotyledons and embryonal axis. Each component was surface sterilized in aqueous solution of NaOCl (1% available chlorine) and tested by standard blotter method.

Cleared and Wholemount Preparations- 20 seeds per category per sample were boiled individually in distilled water for 5-10 min, cooled and dissected to separate the seed components. To clear the seed components, they were than boiled in 10% aqueous solution of Potassium hydroxide for 10 min. and washed in hot distilled water. The cleared components were stained in cotton blue for 20-30 min and mounted in Polyvinyl alcohol (PVA). The component was pressed gently under the cover slip till the cells spread uniformly and kept at 60 °C in an oven for 48-72 hrs for drying[2].

Microtome Sectioning-5 seeds per category for each sample were boiled in distilled water for 10-15 min, fixed in 70% alcohol for 48 hrs, dehydrated through tertiary

butyl alcohol (TBA) series, infiltered and embedded in paraffin wax. The embedded material was cut at 10-13 μ thickness. The slides were stained with safranin and light green combination and mounted in DPX.

Results and Discussion

Dry Seed Examination-Black gram seed samples revealed seeds with purple discolouration, sclerotial seeds, shrivelled seeds, seeds with white mycelial growth and broken and insect damaged seeds besides the normal symptomless seeds.

Seeds with Purple Discolouration : 37 seed samples showed purple discoloration in the range of 5-7.75%. These affected samples belonged to Banswara (8), Bhilwara (11), Chittorgarh (9), Kota (4), Tonk (2) and Udaipur (3). The discoloured seeds generally yielded *Aspergillus niger* and rarely *Rhizoctonia bataticola.*

Sclerotial Seeds : 17 seeds samples carried 2-10% black, pin head like microsclerotia on hilar region and on seed surface. On incubation such seeds yielded *R. bataticola.* The infected samples came from Banswara (9), Bhilwara (1), Dungarpur (5), Kota (1) and Sawai Madhopur (1).

Shrivelled Seeds : 47 seed samples were found to carry shrivelled seeds (0.5-12.75%). The samples were from Banswara (12), Bhilwara (11), Chittore (9), Dungarpur (2), Jhalawar (3), Kota (2), Sawai Madhopur (2), Tonk (3) and Udaipur (3). On incubation such seeds revealed *Fusarium oxysporum.*

Seeds with White Mycelial Growth : Only 5 samples carried seeds with whitish growth of hyphae. The incidence of such seeds varied from 0.25-3% and always yielded *F. oxysporum.* The samples belonged to Banswara (2), Bhilwara (2) and Kota (1).

Broken and Insect Damaged Seeds : Seeds included in this category were with broken seed coat, splitted and insect damaged seeds. Such seeds were observed in 9 samples from Banswara (4), Bhilwara (2), Chilttore (1) and Udaipur (2). No specific fungi were recorded on such seeds.

Incubation Tests-123 and 34 seeds samples of black gram were tested by Standard Blotter Method (SBM). A total of 39 saprophytic as well as parasitic fungal species were encountered. The fungal species recorded in SBM were *Actinomyces* sp., *Alternaria alternate* (Fr.) Keissler, *A.brassicicola* (Schw.) Wilts., *A.tenuissima (Fr.)* Wiltsh, *Aspergillus candidus Link ex. Fries, A.flavus Link ex.* Fries, *A.fumigatus* Fresen, *A.nidulans* (eidam) Winter, *A.niger* van *Tiegh, A.ochraceous* Wilchem. *A.sulphureous* (Fres.) Tom & Church, *Chaetomium globossum* Kunze *ex.* Fr., *C. indicum* Corda, *Cladosporium cladosporoides* (Fres.) de vries, *Colletotrichum lindemuthianum, Curvularia lunata* (Wakker) Boedijin, *C.pallescens* Boedijin, *C.robusta* Kilpatrick & Luttrell, *Doratomyces stemonitis* (Pers ex. Fr.) Morton & Smith, *Drechslera halodes* (Drechsler) Subram. & Jain, *D. hawaiiensis* (Bungicourt) Subram. & Jain ex Ellis, *D.tetramera* (Mckinney) Subram. & Jain, *Fusarium culmorum* (Cooke) Wr. *F.moniliforme* Sheldon, *F.nivale* (Fries) Cesati, *F.oxysporum* Schelecht. *Ex.* Fr., *F.semitectum* Berk. & Rav., *Macrophomina phaseolina (Tassi)* Goid, *Melanospora zamiae* Corda, *Memnoniella echinata* (Rivolta) Galloway, *Myrothecium roridum* Tode ex Fr., *Paecilomyces fusisporus* (Saksena), *Penicillium* spp. *Periconia* sp., *Phoma* sp., *Rhizoctonia bataticola* (Taub). Butler, *Rhizopus nigricans* Ehrenb., *Stemphylium vesicarium* (Wallr.) Simmons and *Trichothecium roseum* Link ex. Fr.

Component Plating (Table 2)-The various part of black gram seed *viz,* seed coat, cotyledons and embryonal axis could be easily separated in both asymptomatic and symptomatic seeds. In asymptomatic seeds infection was observed in seed coat and cotyledons. In the two samples the infection in seed coat and cotyledons of asymptomatic seeds varied from 16-38% and 5-0%, respectively. Embryonal axis remained free from infection.

In symptomatic seeds, the incidence of infection (due to *R. bataticola)* was 100% in seed coat, 18-25% in cotyledons and 0-18% in HR axis of weakly infected seeds. Heavily infected seeds revealed cent per cent infection in all the components. The growth of the fungus was dense and the number of sclerotia were higher on cotyledons as compared to seed coat and hypocotyl shoot root axis. The components of the heavily infected seeds showed rotting.

Cleared Wholemount Preparations (Table 2)-Thick, dark brown to black, septate, branched intercellular mycelium and microsclerotia were observed in wholemount preparations of seed coat, aleurone layer, cotyledons and hypocotyl-shoot-root axis. In asymptomatic seeds infection was observed in seed coat (10-26%), rarely in cotyledons (10%-nil) and was not observed in embryo. Weakly to heavily infected seeds revealed heavy inoculum in seed coat, less in cotyledons and no infection in embryo in case of weakly infected seeds. But the seeds with heavy infection showed dense aggregation of mycelium and sclerotia in all the components. The cells of cotyledons showed necrosis and depletion of cell contents.

Microtome Sectioning-Microtome sections revealed the exact distribution and expanse of fungal mycelium and microsclerotia in different seed components of categorized seeds (Fig.1).

The descriptions for location of pathogen in

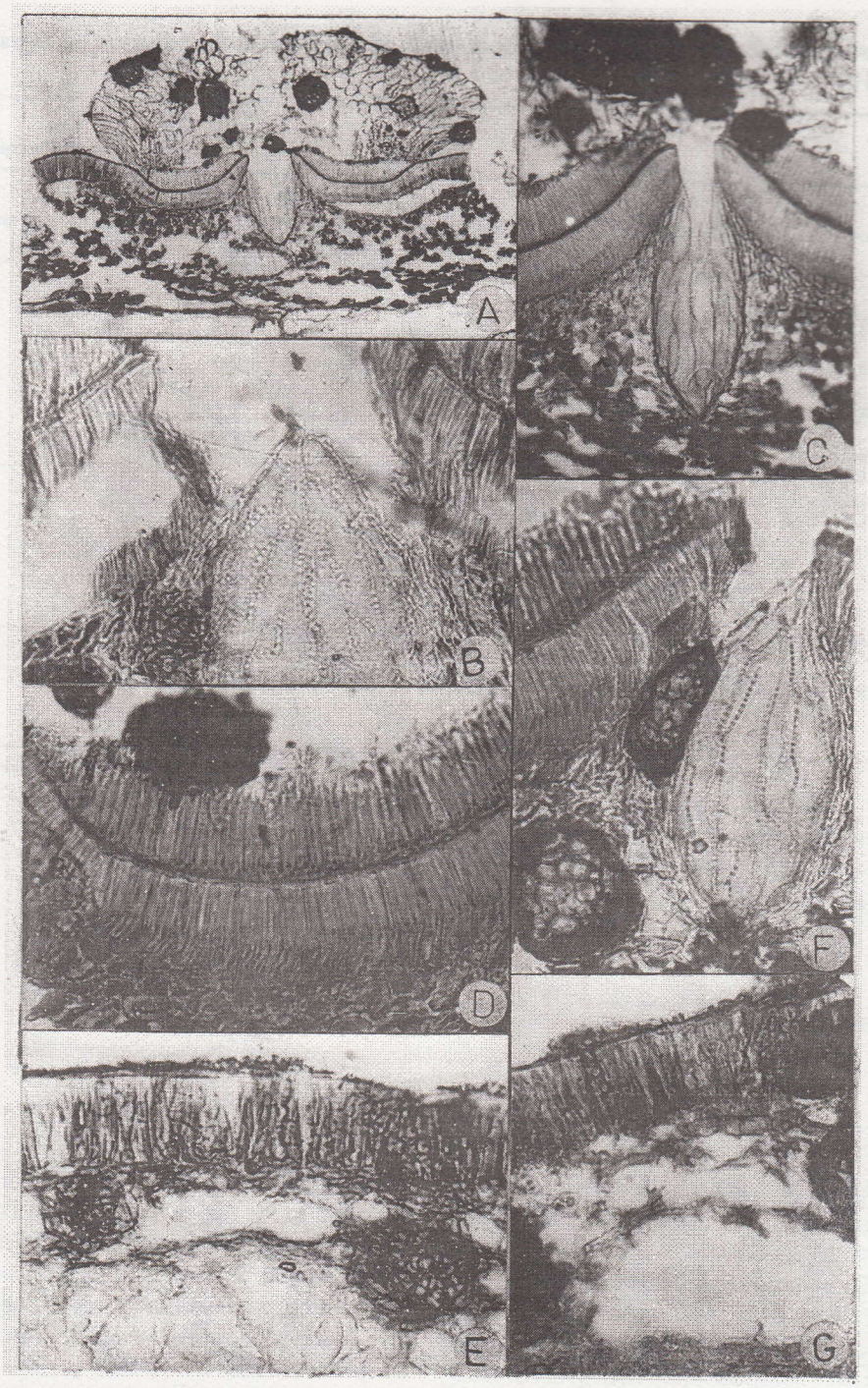

Fig.1. Photomicrographs of microtome sections of seeds infected with *R. bataticola.*
A & B - T.S. through hilar region in soft parenchymatous tissues of funiculus (A) and on counter palisade cells (B). C - As above showing microsclerotia and dark-brown mycelium in counter palisade layers, stellate parenchyma and hilar trachieds. D - Microsclerotia on counter palisade. E - Microsclerotia formed around trachiedal bar and below the counter palisade. F & G - T.S. of seed coat showing abundant mycelium in palisade cells and microsclerotia in parenchyma layer.

Table 1. Fungal diseases observed on urdbean in farmer fields of 6 villages in four districts of Rajasthan during the Kharif seasons of 1994 and 1995.

			DISTRICTS					
			Banswara		Bhilwara		Dungarpur	Udaipur
S.No.	Symptoms	Causal organisms	VILLAGES Naugama (%)	Chhinch (%)	Shahpura (%)	Jethana (%)	Sagwara (%)	Maoli JN. (%)
1.	Leaf spots	*Curvularia lunata*	–	–	5	–	–	–
2.	Leaf spots	*Macrophomina phaseolina*	10	10	–	10	5	–
3.	Leaf blight	*Macrophomina phaseolina*	10	–	–	5	15	–
4.	Leaf blight	*Alternaria alternata*	–	–	5	5	–	–
5.	Powdery mildew	*Erysiphe polygoni*	–	–	2	–	–	–
6.	Root rot	*Macrophomina phaseolina*	5	15	–	2	–	–
7.	Wilting	*Fusarium oxysporum* and *M. phaseolina*	10	–	2	5	–	2

Table 2. Percent infection of *Rhizoctonia bataticola* in different parts of seeds of black gram.

Components	S.No. 9218			S.No. 9233		
	Asymptomatic	Weakly	Heavily	Asymptomatic	Weakly	Heavily
Component plating						
Seed Coat	16	100	100	38	100	100
Cotyledons	5	18	100	–	25	100
Embryonal axis	–	–	100	–	18	100
Cleared wholemount preparations						
Seed Coat	26	100	100	10	100	100
Cotyledons	10	–	88	–	–	98
Embryonal axis	–	–	60	–	–	56

different categories of seeds are provided separately.

Asymptomatic seeds : No infection was observed in the sections of asymptomatic seeds.

Symptomatic weakly infected seeds: Branched, septate and dark coloured mycelium and microsclerotia were observed at hilar region and within as well as below the palisade layer or in the region of hourglass and parenchyma of seed coat. Microsclerotia formation was most frequent below the palisade layer. The hyphae also ramified in all the layers of seed coat causing slight loosening and deformation of cells. Hyphal mat occurred in the parenchymatous region. In hilar region, the mycelium and microsclerotia mostly colonized the stellate parenchyma. Hyphae were also found penetrating directly through hilar trachieds or from hilar tissues. Inter and intra cellular mycelium were observed in the upper few layers of cotyledonary cells. Mycelium mostly spreaded through the intercellular spaces along the cell wall. Cell contents were found lower as compared to asymptomatic seeds. Hyphal infection was also observed in radicular region of embryo.

Symptomatic heavily infected seeds : Heavily infected seeds showed withered and loose palisade layer, completely deformed hourglass cells and thick mycelial mats occupying parenchymatous cells. In the seed coat hyphae and microsclerotia were inter-as well as intracellular in palisade layer and hour glass cells. Microsclerotia occurred in all parts of seed. The microsclerotia developed at and within the hilar pore and

Table 3. Distribution of *Macrophomina phaseolina* infection on symptomatic pod clusters collected from 12 black gram plants.

Parts	Early maturing pods																Late maturing pods							
	1A		3A		4A		5A		6A		7A		10A		11A		4B		5B		10B		12B	
	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H	W	H
1. No. of pods (2)	1	1	(2) –	2	(4) 3	1	(2) –	2	(3) 2	–	(3) 1	–	(4) 3	–	(3) –	3	(2) 1	–	(1) –	–	(1) –	–	(2) –	1
2. Peduncle		+		+*		+		+		–		–		–		+	+*	–	–	–	–	–	–	–
3. Pedicel	+	+	–	2	3	1	–	2	–	–	–	–	–	–	–	3	1	–	+	–	+	–	–	–
4. Proximal end																								
(a) outer surface	+	+	–	2	2	1	–	2	1	–	–	–	2	–	–	3	2	–	–	–	–	–	–	+
(b) inner surface	+	+	–	2	3	1	–	2	1	–	–	–	2	–	–	3	2	–	+	–	–	–	–	+
(c) seeds	+	+	–	2	3	1	–	2	–	–	–	–	1	–	–	3	2	–	+	–	–	–	–	+
5. Middle																								
(a) outer surface	–	+	–	2	2	1	–	2	2	–	1	–	2	–	–	3	–	–	–	–	–	–	–	+
(b) inner surface	+	+	–	2	2	1	–	2	2	–	1	–	3	–	–	3	2	–	+	–	–	–	–	+
(c) seeds	+	+	–	2	2	1	–	2	2	–	1	–	3	–	–	3	2	–	+	–	–	–	–	+
6. Distal end																								
(a) outer surface	–	+	–	2	1	1	–	2	2	–	–	–	1	–	–	3	1	–	–	–	–	–	–	+
(b) inner surface	+	+	–	2	1	1	–	2	2	–	–	–	1	–	–	3	2	–	–	–	+	–	–	+
(c) seeds	+	+	–	2	1	1	–	2	2	–	–	–	2	–	–	3	2	–	–	–	+	–	–	+

\+ Presence
– Absence
* Cleared wholemount praparation revealed pathogen
Note Ommitted pods showed no infection.

from there hyphae traversed the tracheidal bar. The microsclerotia and mycelium were also observed in the counter palisade layer, hilar parenchyma and remnants of funicle region.

The cells of cotyledons and plumule radicle axis were heavily colonized by the hyphae and microsclerotia. The epidermis of cotyledons disintegrated and could not be differentiated. The hyphae spread all along the cell from abaxial surface to region of cotyledons. The cells were vacuolated and contain very little cell contents. The microsclerotia mostly developed in spaces between seed coat and cotyledons, seed coat and radicle axis, in between two cotyledons, within the cotyledons, plumule and radicle. The pathogen ramified all along the cell wall in cotyledons and the cell wall was replaced by dark brown coloured thick hyphae. Hyphae even invaded the cell and was seen all along the starch grains. The plumule in heavily infected seeds showed maximum damage and appeared as deformed mass tissues. In seeds the seed coat, cotyledons and hypocotyl shoot root axis carried densely found mycelium and sclerotia. Cavities due to disintegration of cells were common in embryo. No infection was observed in vascular elements of cotyledons.

Symptoms observed during field surveys- During field survey in Rajasthan, leaf spot, leaf blight, powdery mildew, dry root rot and wilting diseases were observed in the standing crop of black gram (Table 1).

Leaf Spots : Small to large, irregular, pale reddish spots were observed on leaves of mature plants. Majority of the plants, with such symptoms were systemically infected and similar symptoms were also observed on stems and pods. Incubation tests and cleared wholemount preparations of the affected parts suggested the presence of characteristic mycelium and sclerotia of *Rhizoctonia bataticola.* It also produced pycnidia. The disease was recorded from the districts of Banswara (10%) and Dungarpur (5%). The affected mature plants showed infection on pods also.

Leaf Blight : Black gram fields in Banswara and Dungarpur districts revealed occurrence of 20% and 5% leaf blight disease respectively. The symptoms were twisting and distortion of leaves. Initially the veins on the abaxial surface of top leaves turned reddish. The interveinal areas also turned reddish and later becomes dark black. Finally the whole leaf appeared blighted. Incubation of such leaves yielded pure growth of *R. bataticola.*

Some fields in these districts also showed leaf blight caused by *Alternaria alternate.* The blighting started as small brown to dark brown patches of discolouration. The patches gradually increased in size and number. The whole leaf later turned dark brown and finally blighted.

Leaf Spots : In district Bhilwara three out of five fields showed leaf spot caused by *Curvularia lunata.* The spots appeared in the form of concentric rings. The spots gradually increased in size and caused shot holes in leaves.

Powdery Mildew : Infection of powdery mildew was observed in black gram fields of Bhilwara district (2%). The leaves showed growth of mycelium, conidiophore and conidia. Cleistothecia were formed at maturity. The causal organism was identified as *Erysiphe polygoni.* The affected leaves dries after sometime and defoliation occurred.

Dry root rot : Frutifications of *R. bataticola (Macrophomina phaseolina)* were observed on roots of black gram in fields of Banswara (10%), Dungarpur (5%) and Tonk (5%). The splitted roots showed pathogen in cortical regions and vascular regions.

Wilt : This disease was observed in fields belonging to Banswara (10%), Bhilwara (2%), Dungarpur (5%) and Udaipur (5%). The tap roots were fairly affected leading to stunted growth of the plant and drying of leaves. Both *Fusarium oxysporum* and *M. phaseolina* were found to associated with the disease.

Symptomatology of infected pods (Table 3)- *Macrophomina phaseolina,* the charcoal rot pathogen produces symptoms on pods. The intensity of symptoms vary from pod to pod. Symptoms on green pods include irregular to spherical scattered dark reddish patches. Dried pods revealed light brown discolouration and were devoid of hairs. The severly infected pods frequently revealed pycnidia throughout of outer surface, ventral and dorsal sutures, pedicels and peduncles. Density of pycnidia varied with degree of infection.

Studies on splitted pods showed that the severly infected pods do not contain seeds whereas partially infected pods contained seeds of reduced size. The seeds were surrounded with sclerotia mostly on hilar region and on whole surface.

Cleared wholemount preparations of different part of pods suggested that *M. phaseolina* has both local as well as systematic infection.

Peduncle : Peduncle of systemically infected pods showed fungal mycelium in cortical and vascular region while that of pods with localised symptoms showed no infection.

Pedicel : Observations similar to peduncle were obtained on pedicel preparation.

Ventral Suture : It revealed inter as well as intracellular mycelium in epidermal cells.

Pericarp : In green pod mycelium penetrating through stomata was observed. The pycnidia observed were with ostiole on fully matured pods.

During the present study 123 samples of black gram from 9 districts of Rajasthan were colleted and studied in detail to obtain a comprehensive information regarding the seed-borne fungi of the crop. Incubation test has shown that seeds with microsclerotia carried infection of *Rhizoctonia bataticola*. Bhatia[3] found *Fusarium oxysporum* to cause white crust on guar seeds. During incubation studies 39 fungi belonging to 22 genera were found. This is the largest number of fungi recorded in any one study of the crop. Singh and Chohan[4], Saxena and Singh[5] and Reddy and Subbayya[6] recorded 6, 3 & 9 fungal species, respectively on black gram seeds. *R. bataticola* is the major pathogen of the crop in the state. 79 samples were infected with *R. bataticola* showing 46.5% incidence. The incidence of *R. bataticola* was high in samples belonging to Banswara, Dungarpur & Kota districts. The southern districts of Rajasthan receives maximum rainfall and have high temperature. High relative humidity and high temperature are most congenial for the pathogen survival[7].

Field surveys carried out in the districts of Banswara and Dungarpur have shown that *R. bataticola (Macrophomina phaseolina)* has wide host range, causing various diseases on different parts of the same plant. Disease free area as a control measure for growing seed samples has been suggested by Saxena and Gupta[8] in Agra region.

Seed-borne nature of *R. bataticola* has been reported in several crops[9]. Sinha and Khare[10] reported *M. phaseolina* in naturally infected cowpea seeds. Sharda and Shetty[11] studied the location of *M. phaseolina* in black gram seeds using component planting of the four seed components, the seed coat was found to be the main site of infection. In the present study a detailed and critical attempt is made to determine the exact expanse of seed-borne inoculum in various tissues of seeds of black gram. The microtome sections yielded precise information on location and distribution of fungal mycelium in seed.

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