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Seed infection and Transmission of *Rhizoctonia bataticola* in *Lagenaria siceraria* (mol.) Standl.

Dr. Prenita Gupta
Dept of Botany, Govt. Science College, Sikar
E-mail : guptaprenita@gmail.com

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Abstract: Naturally infected seeds of bottle gourd [*Lagenaria siceraria* (mol.) Standl] with *Rhizoctonia bataticola* bear brown to black pin head like microsclerotia and show seed rot, root rot and charcoal fruit rot disease. Incidence of seeds with microsclerotia in seed samples of Rajasthan varied from 2.25-18.25%. On incubation the detection of *R. bataticola* ranged from 1-70% in untreated and 1-68% in 2% chlorine pretreated seeds. Asymptomatic seeds also carried the infection but it was confined to seed coat only (extra- embryonal) but in symptomatic microsclerotial seeds, the infection was intra- embryonal. Severity of symptoms on seed was directly associated with degree of seed infection and seed tissue disintegration.

Key words : Bottle gourd, *Rhizoctonia bataticola*, seed-borne, transmission.

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Introduction:

Macrophomina phaseolina (Tassi) Goid = [*Rhizoctonia bataticola* (Taub.) Butler] is a common pathogen and produce two on more distinct types of symptoms (Dhingra and Sinclair, 1973). It is soil as well as seed-borne and causes seed rot, root rot and charcoal fruit rot.

Macrophomina root rot of bottle gourd was first reported by Maholay and Sohi (1983). Gaffar and Akhtar (1968) observed infection on ridge gourd and reported, sclerotia on roots were almost unaffected by ten months dry storage in the laboratory or two months under dry to moderately moist soil conditions. The present investigation has been carried out to study the extent of *Rhizoctonia bataticola* infection in seeds of bottle gourd and its role in disease development der laboratory and field condition.

Materials and Method

Eighty two seed samples of bottle gourd were collected separately in paper bags from ten districts during 2004, 2005 and 2006. The seed samples were stored under natural conditions and then subjected to dry seed examination for disease symptoms under stereobinocular microscope. All the seed samples were screened by Standard Blotter Method (ISTA, 1999).

Two seed samples from Jaipur districts carrying infection of *R. bataticola* were used for location and transmission studies. For histopathology both asymptomatic and symptomatic seeds were used. Location of pathogen was studied by component plating, cleared wholemount preparation of seed coat, aleurone layer, cotyledons and embryonal axis and microtome sections of whole seed in two selected samples.

For component plating 20 seeds of each category were soaked in water for 6hr. Seed coat, aluer one layer, cotyledon and embryonal axis dissected aseptically and plated on moistened blotter for 7 days to isolate the pathogen. In wholemount preparation, 10 seeds of each category were boiled individually i in distilled water for 10-15 min., cooled and dissected to separate seed components. The seed components were boiled separately in 10% aqueous solution of KOH for 5-20 min. depending on the softening of the tissue, washed thoroughly in warm water and boiled in 5 ml of lectophenol containing 2 ml of cotton blue for 3-5 min. The stained softened seed components were kept on a slide under a coverslip in a drop of polyvenyl alcohol and pressed gently to spread the tissue uniformly (Singh et al., 1977). For microtome sectioning 10 seeds of each category were boiled in water for 10 min., fixed in 70% ethanol for 48hr, dehydrated through Tertiary Butyle Alcohol series and embedded in paraffin wax. One or two transverse incision were made with a blade into each seed to ensure proper dehydration, infiltration and embedding. The paraffin blocks were cut to expose the tissues and immersed in 1% aqueous solution of lauryl sulphate for 12hr, washed thoroughly in distilled water and transferred to a mixture of glycerine and acetic acid (1:1 v/v) for 7 days for further softening. Serial microtome sections were cut (20-25 micron thick) and stained with safranine and light green combination. All stained sections were mounted in DPX (Johnson, 1940).

Disease transmission of seed-borne inoculum was determined by using: blotter method (10

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seeds/plate) and water agar seedling symptom test (1 seed/tube) using two replicates of 100 seeds/category/sample for each test Khare *et al.(*1977). The seeds were pretreated with 2% sodium hypochlorite for 2-3 min before sowing.

Results ang Discussion:

Forty four seed samples out of 82 samples, carried, seeds with pin head like, black, minute spots or. microsclerotia of *R. bataticola,* on seed surface . Sclerotia were distributed throughout the seed surface and on micropylar region. Some cracked seeds, also carried microsclerotia in inner surface of seed coat. Incidence of symptomatic seeds varied from 2,25-18. 25%. Such seeds on incubation revealed 1- 70% infection of *R. bataticola* in untreated and 1-68% in chlorine pretreated seeds. .Sultana and Gaffar (2007-2009) isolated *R.solani* from seeds of Bitter gourd. Varma and Kumar (2016) reported that *R.bataticola* is a serious pathogen causing root rot disease of smooth gourd

In component plating the asymptomatic seeds carried extra-embryonal infection 5%, in seed coat whereas in symptomatic (weakly infected) seeds, extra-embryonal infection ranged from 40-55% (50-55% in seed coat, 40-45% in aleurone layer) and intra-embryonal from 15-35% (30-35% in cotyledon and 15-25% in embryonal axis). The seeds with heavy infection showed 75-85% infection in seed coat 65-70% in aleurone layer 55-60% in cotyledons and 40-45% in embryonal axis.(Fig. A-D) Sultana *et al.*(1994) was also isolated *Macrophomina phaseolina* from seed coat, tegmen, cotyledons and embryo of Pumpkin. Infected seed give rise to infected seedling that transmit the pathogen in to the fruits.

Cleared components of seed revealed thick, knotty brown colored – septate mycelium and black Sclerotia. Only 5% infection occur in seed coat of asymptomatic category. In weakly symptomatic category 45-50% infection occur in seed Coat, 35 - 40% in aleurone layer, 25-30% in cotyledons and 15-25% in embryonal axis. In heavily infected Category, seed coat carried 70-80% (Fig. 1E) infection, aleurone layer 60-65% (Fig. 1F), cotyledons 50-60% (Fig. 1G) and embryonal axis 25-30% infection (Table-1).

Histological observations

In seed coat of asymptomatic seeds, mycelium and microsclerotia were restricted to outer layer of seed coat only. Whereas in weakly symptomatic category inter-and intracellular mycelium and sclerotia were distributed to outer prismatic palisade layer, sclerenchymatous cells, seed coat parenchyma, aleurone layer and cotyledonary cells and intercellular hyphae in sclerotic layer of seed coat. Hyphal infection was not distinct in embryonal region. |

Heavily infected seeds showed various degree of withered, and loose palisade layer (Fig, 2A, E) and completely deformed palisade cells of hilar region showing direct penetration through the seed coat. Hyphae invaded the sclerenchymatous layer(Fig.2B). Cells with heavy infection showed disintegration and lysis of cells .

Spongy parenchymatous cells were full of mycelium and microsclerotia. Hyphal bits and sclerotia were seen in aleurone layer, cotyledons (Fig. 2C,D) and space between two cotyledons.(Fig. 2F) Necrosis, cell disintegration and lysis occurred in seed coat parenchyma, sclerechyma, aleurone layer and cotyledonary cells due to aggregation of mycelium and sclerotia.

Disease Transmission

Seed-borne infection of *M. Phaseolina* resulted in failure of radicle emergence, seed rot, symptomatic seedling and their mortality in blotter method and water agar test. It caused browning of radicle and brown discoloration of root-shoot transition zone which progressed toward hypocotyl and downwards to radicle on 4th day followed by dark necrotic cotyledonary leaves. On 10th day apical part of a few seedlings killed and complete mortality was observed on 15th days of sowing.

Asymptomatic seeds from infected seed lots with 5-10% extra-embryonal infection resulted in 92-96% germination, 0% seedling infection and 3-5% total loss. Symptomatic (weakly) seeds with 40-55% extra-embryonal infection, 15-35% intra-embryonal infection resulted in 65-72% germination, about 11% Seedling infection and 34-44% total loss. Heavily infected seeds with 65-85% extra-embryonal infection and 40-60% intra-embryonal infection resulted in 4,4% germination with 100% total loss in petriplate method.

The inoculum present in superficial layers of seed coat was transmitted to seedling and plant. Seed with intra-embryonal infection (deep seated inoculum) rotted prior to germination or sometimes showed post emergence mortality. Similar effects of *M. phaseolina* was also noted by Sharda and Shetty

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(1987) in black gram and Bhatia *et al*. (1998) in guar seeds.

Cleared preparation of infected seedling or plant showed inter-and intra cellular mycelium and microsclerotia of *Rhizoctonia bataticola* in cortical and vascular tissue of radicle , hypocotyls, cotyledon and stem, roots and leaves in mature plant as also observed by Varma, Singh and Singh (1992) in moth bean and Agrawal and Singh (2000) in okra. Jain and Sharma (2013) reported phytopathological effects of mycoflora associated with Okra seeds.

Fig A-D Component Plating of Bottle Gourd Seeds Infected With *R. bataticol*

A-Incubated Seeds With Pycnidia and Sclerotia
B- Seed coat Covered With Sclerot
C-Mycelium and Sclerotia in Micropylar Regi
D-Aleuron Layer of Seed With SclerotiaF

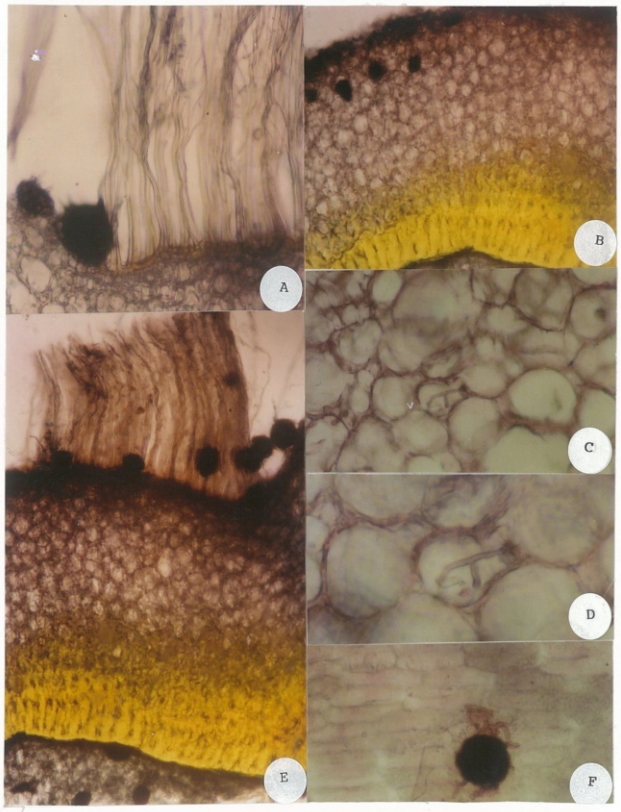

Fig. 2 A,E—Symptomatic Seed showing Mycelium and Sclerotia in Prismatic Palisade Layer
B—Hyphae invaded Sclerenchymatous Layer
C,D—Mycelium and Sclerotia in Parenchymatous Layer
F—Hyphae and Sclerotia present on Cotyledons

Table-1: Incidence of *R. bataticola* in component of seed and its effect on seed germination, seedling infection and disease transmission.

	Incidence in Different seed component										Disease transmission					
	Component plating				Cleared wholemount				Petriplate method				Water agar method			
Category	Extra emb.		Extra emb.		Extra emb.		Extra emb.		germi nation	S.S.W.S.	Seedling Mortality	Total loss	Germi nations	Seedling mortality	S.S.W.S.	Total loss
	SC	AL	COT	EA	SC	AL	COT	EA								
Sample- BG12 **Asymptomatic**	10	5	-	-	5	-	-	-	96	3	-	4	97	-	3	3
Symptomatic weekly	55	45	35	25	50	40	30	25	72	20	6	34	62	15	20	53
Symptomatic heavily	85	70	60	45	80	65	60	30	4	0	4	100	2	2	0	100
Sample-bg35 **Asymptomatic**	5	-	-	-	-	-	-	-	92	5	-	8	95	-	5	5
Symptomatic Weakly	50	40	30	15	45	35	25	15	65	20	9	44	70	13	20	43
Symptomatic heavily	75	65	55	40	70	60	50	25	4	6	4	100	5	5	0	100
Emb.=																

Emb. = Embryonal, SC =Seed Coat, AL = Aleurone Layer, COT = Cotyledon, EA = Embryonal Axis, S.S.W.S. = Surviving Seedling with Symptoms

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