Volume-5, Issue-1, January -2016 • ISSN No 2277 - 8160

Research Paper Botany

Rhizoctonia Bataticola (Taub.) Butler. is a Serious Pathogen Causing Root Rot Disease in Smooth Gourd

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ABSTRACT

120 seed samples of smooth gourd collected from seven areas namely Bagoan, Dadhevi, Ratkankara, Kalatalo, Mandana, Nanta and Ranpur of Kota district of Rajasthan were studied. In dry seed examination occurrence of variously discolored seeds, brown discoloured seeds, black discoloured seeds, white discoloured seeds, small seeds, shriveled seeds and insect damaged seeds. 08 Seed samples from Bargoan (15.25%) and Mandana 38 (32.75%) carried higher frequency of Rhizoctonia bataticola causing root rot disease in smooth gourd. Component planting, whole-mount preparations and microtome sections confirmed its presence in different parts of the seed. Infection was confined only in seed coat region of asymptomatic seeds whereas in symptomatic seeds infection was dense and deep-seated and showed thick and knotty mycelium and microsclerotia in various parts of spermoderm (seed coat), aleurone layer, cotyledons and embryonal axis. Heavily infected seeds showed varying degree of withering and loosening of palisade layer. Heavily infected seeds did not germinate. Dry seed inspection, incubation tests and histopathological studies clearly showed the seed borne nature of Rhizoctonia bataticola. Control of Rhizoctonia bataticola caused root rot begins by planting certified seeds and avoiding overhead irrigation and watering through drip lines.

KEYWORDS : Irrigation, Rhizoctonia bataticola, Smooth gourd, Root rot,Withering.

Introduction:

Rhizoctonia bataticola [(Taub.) Butler] a well known root rot pathogen has been reported seed-borne in gourds crops. Suryanarayan (1963) was the first to observe *Rhizoctonia solani* causing root rot of bottle gourd belongs to cucurbitaceae. It causes destructive root rot disease in smooth gourd (*Luffa acutangula*) during warm, wet growing season. Cucurbit is the largest group of summer vegetable crops. All cucurbit crops are grown for the ripe or unripe fruit and generally thrive in hot weather and have similar culture requirement. Most cucurbits grown in desert are annuals except tumba, which is perennial. *Luffa cylindrica* (L.) Roem. commonly called smooth gourd, widely distributed in tropics and subtropics, as a cultivated and naturalized plant. Luffa is derived from the cucumber and marrow family and originates from America (Mazali and Alves, 2005; Sultana and Ghaffar, 2009**;** Shakoor et al., 2011). There is no much information on seed borne nature of *Rhizoctonia bataticola* in smooth gourd. During the survey the fungus was found to the invariably associated with smooth gourd seeds of Bargoan and Mandana areas of Kota districts and the study was concentrated on its incidence, penetration and host parasite relationship of host parasite in seeds and above ground plant parts of smooth gourd.

Materials and methods:

Survey was done in seven areas of Kota districts in Rajasthan namely Bargoan, Dadhevi, Ratkankara, Kalatalo, Mandana, Nanta and Ranpur to find out the areas of production of smooth gourd. 120 seed samples and plant parts were collected and screened using ISTA methods. Dry seed examination of the seed samples was done and they were categorised as symptomless and symptomatic carrying infection of brown discoloured seeds. Samples of infected plant material were also collected and subjected to dry seed examination, SBM and PDA tests. In standard blotter test 10 seeds per plate were spaced in sterilized petriplate containing three well moistened blotters and incubated at 26 ± 2^{0}C under 12 h of alternating cycles of artificial light from Phillips fluorescent tube fitted at a distance of 60cm apart and darkness for seven days (Anon, 1985). Percentage of seed germination, seed borne mycoflora, its percent incidence, pattern of fungal growth, its effect on germination, seedling symptoms and other abnormalities in seedlings were recorded on eight day of incubation. A few cases incubation was prolonged and observations were made on 14th days. While in PDA forty three seed samples belonging to all seven areas of Kota districts of Rajasthan were studied. Sterilized seeds and plant parts were aseptically plated on petriplates (10 seeds/ petriplate) containing 15-20 ml of PDA media and incubated for 7 days at 26 ± 2^{0}C in 12h/12h of alternating cycles of artificial day light and darkness. Percent incidence of mycoflora, seed germination and abnormality of seedlings were examined by naked eyes as well as under stereo binocular microscope on eight day of incubation.

For component plating seeds were soaked for 6-12hrs in sterilized water individually, and seed components viz. seed coat, aleurone layer, cotyledons and embryonal axis were dissected aseptically with the help of sterilized forceps and a pair of needles under stereobinocular microscope and inoculated at 26 ± 2^{0}C.

For wholemount preparations seeds were boiled in 10% KOH for 15 minutes to clear the tissue, washed thoroughly and seed coat, aleurone layer, cotyledons and embryonal axis were separated. Each component was boiled separately in 10ml of lacto phenol containing 2ml of trypan blue/ cotton blue (1:1 v/v) for about 15 min and mounted in polyvinyl alcohol (Singh, Mathur and Neergaard, 1977).

For microtomy usual microtome techniques were followed (Johansen, 1940). The wax embedded seeds were chopped from one side and emerged in 1% aqueous solution 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 seven days for further softening. Blocks were cut through microtome at 15-20 µ thickness, deparaffinised, stained with safranine and light green combination and mounted in DPX. For further studies two samples were selected, one form the Bargoan (Sg-47) and other from Mandana (Sg-86) of Kota districts in Rajasthan.

Results & Discussion:

120 seed samples were collected from various smooth gourd growing areas of Kota districts in Rajasthan. Dry seed examinations of seed samples were done. Besides normal seeds of Bargoan (Sg-47) and Mandana (Sg-86) showed various discoloration viz. brown discoloured seeds 13(3.0-15.25%); 54(2.25-35.25%), black discoloured seeds 10(11.75-18.75%); 32(2.25-20.75%), white discoloured seeds 6(2.25-10.75%); 25(4.75-16.25%), small seeds 14(0.5-9.50%); 52(0.5-8.75%), shrivelled seeds 12(0.5-9.50%); 48(0.5-10.5%) and insect damaged seeds 7(0.25-2.50%); 32(0.25-4.75%).

Brown discoloured seeds showed black pin head like minute spots (microsclerotia) of *Rhizoctonia bataticola* covered the seed surface of about 70 samples out of 120 seed samples of seven areas of Kota districts in Rajasthan viz. Bargoan, Dadhevi, Ratkankara, Kalatalo, Mandana, Nanta and Ranpur. Incubation of these seeds symptomatic seeds yielded pure growth of the pathogen. The pathogen was recovered in 8(3.0-15.25%) and 38(4.25-32.75%) seeds in dry seed examination of both the seeds samples of Bargoan and Mandana where as in untreated and pretreated (standard blotter test) and potato dextrose agar it was 7(13-30%), 36(1-70%); 6(6-38%), 32(3-68%)

and 5(8-40%), 18(1,70%) in both the samples respectively.

Observations of the collected infected samples lead to the following conclusions. In dry seed inspection Bargoan and Mandana disease occurrence was found to be maximum 15.25% and 32.75% in comparison to other areas. It was found to be minimum in Ranpur 4(4.0-7.75%) and in Nanta 5(2.25-10.25%). It was found to be minimum in PDA methods. The smooth gourd is commonly susceptible to *Rhizoctonia bataticola* causing root rot diseases during summer to rainy season when the conditions are favorable for the growth of fungi (Kamal and Khan 1967).

Rhizoctonia bataticola was recorded on incubated components of asymptomatic as well as symptomatic seeds (Fig.:-1A-E). In asymptomatic seeds mycelium and microsclerotia were observed 14, 2% on seed coat; 14, 2% on cotyledon and 6, 2% on HSR in both the seed samples respectively whereas in symptomatic weakly to heavily infected seed samples the growth of the fungus was observed in seed coat (88, 46%; 92, 86%; 100, 96%), cotyledon (76, 40%; 86, 76%; 100, 90%) and hypocotyls shoot root axis (54, 40%; 86, 80%; 100, 90%) in both the seed samples respectively (Table-1). These components rotted completely on 6th day of incubation due to infection.

The seed coat, cotyledons and hypocotyls shoot root axis could be easily separated in asymptomatic seeds but it was difficult to do so in heavily infected seeds.

Anatomically smooth gourd seed consists of many layered spermoderm (seed coat), thin perisperm, endosperm and an embryo consisting of two large leaf like flat cotyledons. The seed coat is multilayered which consists of outer epidermis, hypodermis, palisade parenchyma, ridge parenchyma and the innermost inner epidermis. The endosperm is represented by the aleuronic layer consist of two or three layer of very thick walled polygonal cells containing oil and protein granules.

In cleared whole mount preparations of the seed component showed thick, knotty, branched, septate, brown coloured inter and intracellular mycelium and microsclerotia. Mycelium and microsclerotia were restricted to the seed coat only in asymptomatic seeds while it was observed in all parts of weakly and heavily infected seeds. Mycelium and microsclerotia was dense in all the components of heavily infected seeds of both the seed samples.

In microtome sectioning weakly infected symptomatic seeds showed presence of branched, septate, dark coloured mycelium and microsclerotia in soft hilar tissue. Dark coloured septate mycelium was both inter and intracellular in seed coat parenchyma. Mycelium and microsclerotia also appeared in between epidermal eruptions on seed coat. Aggregation of microsclerotia leads to disintegration and distortion of cells. Presence of mycelium and microsclerotia in epidermis, subepidermis, palisade layer, parenchymatous cells and inner epidermis caused weakening and deformation of cells. Heavy infection results in distortion of hypodermal sclerenchymatized cells. Components of cell remain unaffected.

In heavily infected seeds the dense aggregation of thick, septate and knotty mycelium and microsclerotia was observed in all the seed components. Heavily infected seeds showed varying degree of withering and loosening of palisade layer and complete deformation of hilar cells due to direct penetration through the seed coat (Fig.1F&G). Heavy incidence of the pathogen leads partial to complete disintegration of parenchymatous cells. Due to the pressure of dense mycelium and microsclerotia stressed stellate parenchymatous either disintegrate or their lysis occurs.

A thick and knotty mycelium and sclerotia was also found in thick walled cells of aleurone layer, cotyledons, plumule and radical axis of the embryo (Fig. 1I&J). Aggregation of mycelium causes lossening of polygonal cells. Due to sclerotia the cells get disintegrated and forms lytic cavities filled with scattered aleurone grains. Depletion of cytoplasm and food content occurs in the cells. Uneven thickening of the cells of abaxial side of the cotyledons were also seen (Fig: 1H). Heavy infection penetrated the storage tissue of the cotyledonary region and lead to deformities in the cotyledons. Cell components are deficient and cell appears empty due to disintegration of starch grains and proteinoplast.

Rhizoctonia bataticola has been separated to be seed-borne in smooth gourd (*Luffa cylindrica*) seeds. The infected seeds may be asymptomatic or symptomatic. The symptomless infected seeds show infection in inoculcation tests and produce thick and knotty mycelium and sclerotia. Similar symptoms were reported in sponge gourd (Sadda and Varma, 2014), in okra (Sharma, Jain and Sharma, 2013) and in cluster bean (Pareek and Varma, 2013).

Three types of seed discolourations such as necrotic lesions, fungal growth and pigmentation on seed surface have been described by Neergaard (1977). *Rhizoctonia bataticola* is found to be associated with brown sclerotial seeds. The fungi are reported from the seeds of bitter gourd (Manthachitra, 1971; Nair, 1982; Maholay, 1986; Mathur, 1990; Sultana and Ghaffar, 2007) and bottle gourd (Manthachitra, 1971; Maholay & Sohi, 1976; Richardson, 1979; Maholay, 1989; Shakir and Mirza, 1992; Sultana and Ghaffar, 2009). It has also reported many other crops such as soybean (Mathur, 1992; Sharma, 1992), cowpea, moth bean (Varma, Singh and Singh, 1992), black gram (Singh, 1997) and sesame (Dubey, 2000).

Sultana and Ghaffar (2007, 2009) isolated *Rhizoctonia solani* from seeds of bitter gourd and reported seed borne nature of the pathogen in bottle gourd. Pande, Rao and Sharma (2007) observed *F. oxysporum* on chick pea seeds. Singh (2010) reported *Macrophomina* root-rot losses in cluster bean. In present study brown discoloured seeds show the presence of *Rhizoctonia bataticola*. Gupta, Dubey and Singh (2011) revealed either dark brown discolouration or seeds covered with white mycelial crust showed the infection of *Fusarium semitectum* in seeds and causing wilt disease in *Dalbergia sisso*. Zaidi and Pathak (2013) reported various seed deformities due to fungal infection in chick pea seeds besides normal looking viz. wrinkled big seeds, wrinkled small seeds and damaged seeds.

In present study once the infection reaches the seed at appropriate stage and time of development the infection spreads quickly. This may account for lack of distinction between weakly and heavily infected seeds.

The present study clearly indicates that *R. bataticola* is not only seed borne but deep seated. The heavy infection causes lysis and disintegration of host cell. Control of *Rhizoctonia bataticola* root rot begins by planting certified and disease free seeds, no seed saving from infected plants and use of disease free seeds of variety resistant to root rot disease. Other measures include planting in well drained soil free from surface runoff water, practicing crop rotation, avoiding overhead irrigation and watering through drip lines or tapes or haoses (Singh and Singh, 1978). Varma, Singh and Singh (1989) reported infection of *Rhizoctonia solani* localized in seed coat and hilar region in symptomless seeds of moth bean while it was observe deep seated and showed microsclerotia and inter and intracellular mycelium in all components of symptomatic seeds viz. seed coat, cotyledon and hypocotyl radicle axis. Weakening and depletion of cell contents were also observed in severe infection of pathogen. Similar results were also reported due to infection of *Rhizoctonia bataticola* in *Vigna aconiitifolia* (Varma et al., 1992).

Acknowledgement
One of the author Varma R wishes to thank University Grants Commission, New Delhi for financial assistance.

Table-1 : Percent infection of *Rhizoctonia bataticola* in different parts of Asymptomatic and Symptomatic (weakly to heavily infected) seeds of smooth gourd in component plating and cleared wholemount preparation.

COMPONENT PLANTING

SAMPLE NO.	ASYMPTOMATIC			SYMPTOMATIC								
		Weak			Moderately			Heavy				
	SC.	COTY	HSR	SC.	COTY	HSR	SC.	COTY	HSR	SC.	COTY	HSR
Sg. 47	14	14	6	88	76	54	92	86	86	100	100	100
Sg. 86	2	2	2	46	40	40	86	76	80	96	90	90

CLEARED WHOLEMOUNT PREPARATIONS

SAMPLE NO.	ASYMPTOMATIC			SYMPTOMATIC								
		Weak			Moderately			Heavy				
	SC.	COTY	HSR	SC.	COTY	HSR	SC.	COTY	HSR	SC.	COTY	HSR
Sg. 47	18	12	2	90	84	84	94	86	86	100	100	100
Sg. 86	8	6	2	40	34	30	74	60	60	98	92	90

SC = Seed Coat **COT** = Cotyledon **HSR** =HYpocotyl-Shoot-Root Axis

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