Detection, Seedborne Nature, Disease Transmission and Eradication of Seedborne Infection by *Rhizoctonia bataticola* (Taub) Butler in Groundnut

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Abstract

A study was conducted to determine the seedborne nature, disease transmission and eradication of seedborne infection by Rhizoctonia bataticola *in groundnut. In case of 10 groundnut accessions, infection percentage of* R. bataticola, *ranged between 13.3 to 73.3. Component-plating method indicated that the pathogen is located mainly on the seed coat and rarely in the endosperm and embryo. Infection in embryo and endosperm was always associated with seed coat infection. All accessions showed pre-emergence damping-off in the form of seed rot. Post- emergence damping-off was noticed in three accessions. The per cent infection ranged between 9.5 to 42.9 in case of pre-emergence damping-off, while it was 4.8 to 19.1 for post-emergence damping-off. The latent infection of* R. bataticola *from healthy seedlings was proved using Potato carrot agar medium. Infected seeds of 10 groundnut accessions were grown and no apparent symptoms were observed on the plants for one season, but seeds harvested from such plants resulted in seed infection of 6.1 – 33.3 % indicating the seed transmission nature of the pathogen from one generation to the next. Carbendazim + thiram @ 2g a.i / kg seed can be used as a routine treatment in quarantine laboratories to eliminate* R. bataticola *from groundnut seeds.*

Keywords: Groundnut, *Rhizoctonia bataticola*, seedborne infection, eradication

Introduction

Groundnut (*Arachis hypogaea* L) occupies the first place among oil seed crops grown in India with an area of 8.0 million ha and contributes 7.5 million tonnes to oil seed basket (Anonymous, 2004). Among the fungal pathogens that attack groundnut, *Rhizoctonia bataticola* (Taub.) Butler, the causal agent of dry root rot is important particularly in semi-arid regions. The fungus is both seed and soilborne (Mridha and Fakir, 1978). It causes both pre-emergence and post-emergence damping-off symptoms (Bhatia *et al*., 1998). Infected seedlings show brown discolouration at collar region. Lower leaves show yellowing and drooping and sudden death occurs in patches. The bark in collar region shows shredding. Minute dark sclerotia are seen in the shredded bark and root tissue. The pathogen can survive and multiply even at – 18 ^{o} C (Singh *et al*., 2003).

During export certification, *R. bataticola* was intercepted in 279 of the 46,749 samples of groundnut processed for seed health testing.(Girish *et al.*, 2001). During processing of exotic groundnut samples, *R. bataticola* was intercepted from Malawi and Niger (Chakrabarty *et al*., 2004). Seed borne infection of *R. bataticola* can be detected by incubation test wherein the fungus can be seen as black sclerotia scattered throughout the seed surface with or without aerial mycelium.

Though the seed transmission studies on *Macrophomina phaseolina*, the teleomorph of *R. bataticola*, were reported on sunflower (Raut, 1983), beans (Songa and Hillocks, 1998) and okra (Agrawal and Singh, 2000), related work on *R. bataticola* is very meagre. Hence, the present investigation was carried out to study the seed transmission nature of *R. bataticola* in groundnut seed, location of seedborne infection and eradication of the pathogen from seed.

Materials and methods

Seeds of ten *R. bataticola* infected groundnut accessions (Table 1) were selected from the rejected lot of export samples. These were kept in paper envelopes and stored at

Table 1. Infection of *Rhizoctonia bataticola* on sterilized and unsterilized seeds of ten groundnut accessions

Accession	*R. bataticola* infection (%)	
	Sterilized	Unsterilized
ICG 92160	13.3 (17.7)	13.3(17.7)
ICG 92029	53.3 (46.9)	20.0(26.6)
ICG 92034	20.0 (21.9)	13.3(17.7)
ICG 94039	33.3(35.1)	33.3(35.1)
ICG 94016	53.3(46.9)	40.0(38.8)
ICG 94062	46.7(43.1)	40.0(38.8)
ICG 94042	73.3(63.8)	66.7(54.9)
ICG 93115	66.7(54.9)	46.7(43.1)
ICG 94037	73.3(59.2)	40.0(38.8)
ICG 93139	53.3(46.9)	53.3(46.9)
LSD at 5% level	24.7	20.2

* Figures in parentheses are angular transformed values

20 ^{O} C for use in the study.

Determination of infection levels

To test the initial infection levels, infected seeds from each accession were tested in three replications @ 10 seeds / replication. Seeds were surface sterilized with 2.6 % sodium hypochlorite solution for 2 min and plated on potato carrot agar (PCA) medium containing potato (100g), carrot (100g), and Agar agar (20g). Plates were incubated at 22^{O} C in alternating cycles of near ultra-violet light (NUV) and darkness. The per cent infection of *R. bataticola* was recorded seven days after incubation.

Location of fungus in seed

Individual seed from each accession was dissected aseptically using sterile needle and forceps. Seed coat, endosperm and embryo, thus separated from a seed, were surface sterilized separately in sodium hypochlorite solution (2.6%) for 2 min and then incubated on PCA medium in the sterilized Petri plates. Enough care was taken in preventing cross contamination among the seed components. Presence of the fungus in each seed component was recorded seven days after incubation.

Seed transmission

From each accession surface sterilized seeds were sown in plastic pots (7 seeds/pot) containing sterilized soil and replicated thrice. Observations on pre- and post-emergence damping-off and germination were recorded upto 30 days at 5-day intervals.

Detection of latent infection

Fifteen seeds from each of the five accessions were sown in three plastic pots (5 seeds/pot) containing sterilized soil. Healthy looking seedling of one month old was selected from each pot and their stem bits were plated on PCA medium after surface sterilization. Observations on the presence of fungus were recorded seven days after incubation at 22^{0}C.

Seed to seed transmission

Fifteen seeds of each accession were grown in three plastic pots containing sterilized soil and grown until maturity in greenhouse. Pods were harvested and seeds were surface sterilized, placed on PCA medium and incubated for seven days at 22^{0}C. Seed mycoflora was recorded on the eighth day.

Eradication of *R. bataticola*

Three accessions, ICG 94016, ICG 94039 and ICG 93139 were used in the study. Seeds were treated with fungicides (Table 6) @ 2 g a.i./kg seed. The required quantity of each fungicide was uniformly coated on seed surface by gently shaking the seed in a Petri plate. One set of seed samples was plated on PCA medium in the laboratory and another set was sown in pots using sterilized soil in greenhouse. Observations on the presence of the fungus were recorded seven days after incubation in the laboratory, while the per cent pre- and post-emergence damping-off and germination were recorded at 5-days interval in the greenhouse.

Results and discussion

Determination of infection levels

Seed infection ranged between 13.3 to 73.3% among the accessions tested (Table 1). The infection was maximum in surface sterilized seeds of ICG 94037 and ICG 94042 (73.3%) followed by ICG 93115 (66.7%). ICG 92160 had the lowest infection of 13.3 % both under sterilized and unsterilized conditions. Sclerotia were found scattered through out the seed surface with less aerial mycelium. However, under unsterilized conditions, all accessions except three, recorded less infection (13.3 to 66.7%) compared to those under sterilized conditions. This may be due to the presence of other seed borne fungi like *Aspergillus* spp and *Rhizopus* spp.

Location of pathogen in seed

Component-plating method indicated that the pathogen was located mainly on the seed coat on all the accessions tested and rarely in the endosperm (2 accs) and embryo (3 accs) (Table 2). Infection varied from 17-67% in the seed coat, 8% in the endosperm and 8-17% in the embryo. Infection in

Table 2. Seedborne infection of *Rhizoctonia bataticola* detected in three components of groundnut seed using component plating technique

Accession	*R. bataticola* infection (%)		
	Seed coat	Endosperm	Embryo
ICG 92160	17.0	0.0	0.0
ICG 94039	67.0	0.0	0.0
ICG 94016	58.0	0.0	8.0
ICG 94062	42.0	8.0	0.0
ICG 94042	50.0	0.0	0.0
ICG 93115	67.0	0.0	8.0
ICG 94037	67.0	8.0	17.0
ICG 93139	58.0	0.0	0.0

embryo and endosperm was always associated with seed coat infection. High seed coat infection of *R. bataticola* was also noticed by Bhatia *et al.,* (1998) during their studies on guar.

Seed transmission

The per cent infection ranged between 9.5 to 42.9 in case of pre-emergence damping-off, while it was 4.8 to 19.1 for post-emergence damping-off (Table 3). Results indicated that all accessions showed pre-emergence damping-off in the form of seed rot. Post- emergence damping-off was noticed in three accessions, indicated by the weak and succulent radicle after five days of seedling emergence and continued up to 10 days. Ultimately seedlings wilted and died. Similar studies conducted by Raut (1983) and Agrawal and Singh (2000) proved the seed transmission of *M. phaseolina,* in the form of pre- and post-emergence mortality in sunflower and okra, respectively.

Table 3. Germination, pre- and post-emergence damping-off of surface sterilized groundnut seeds due to *Rhizoctonia bataticola* in the greenhouse experiment

Accession	Germinated healthy seedlings (%)	*R. bataticola* infection (%)	
		Pre-emergence damping-off	Post-emergence damping-off
ICG 92160	42.8 (56.8)	38.1(37.9)	19.1(25.6)
ICG 92029	80.9 (68.5)	14.3 (18.2)	4.8 (12.7)
ICG 92034	61. 9 (52.1)	38.1 (37.9)	0.0
ICG 94039	80. 9 (68.5)	19.1 (25.5)	0.0
ICG 94016	57.1 (49.2)	42.9 (40.9)	0.0
ICG 94062	85.7 (71.8)	9.5 (14.8)	4.8 (12.7)
ICG 94042	80. 9 (68.5)	19.1 (25.6)	0.0
ICG 93115	85.7 (67.8)	14.3 (18.2)	0.0
ICG 94037	85.7 (67.8)	14.3 (18.2)	0.0
ICG 93139	57.1 (49.1)	42.9 (40.9)	0.0
LSD at 5%	23.0	22.4	10.2

* Figures in parentheses are angular transformed values

Detection of latent infection

Detection of *R. bataticola* on stem pieces of three symptemless groundnut plants plated on PCA medium revealed the presence of the fungus in either one (ICG 92034, ICG 94039 and ICG 94016) or two (ICG 94037 and ICG 93139) plants of the accessions tested. This indicates that *R. bataticola* can survive as symptomless carrier in groundnut. It appears that infection moved from the seed coat to the seedling and subsequently to the stems thus playing an important role in the disease transmission. Songa and Hillocks (1998) also found that bean seeds harvested from healthy looking plants showed 2.5% infection by *M. phaseolina.*

Seed to seed transmission

Seeds harvested from apparently healthy plants grown from infected seeds of ten groundnut accessions showed infection range of 6.1 – 33.3% (Table 4). ICG-94042 recorded maximum infection (33.3%) followed by ICG-94039 (27.3%). This indicated the seed transmission of the pathogen from one generation to the next.

Eradication of *R. bataticola*

In the laboratory test, carbendazim, carbendazim + thiram

Table 4. Infection of *Rhizoctionia bataticola* on seeds harvested from apparently healthy groundnut plants grown from infected seeds

Accession	Seeds tested (no)	Seeds infected (no.)	Infection percentage
ICG-92160	33	2	6.1
ICG-92029	20	0	0.0
ICG-92034	26	0	0.0
ICG-94039	22	6	27.3
ICG-94016	17	3	17.6
ICG-94062	9	0	0.0
ICG-94042	3	1	33.3
ICG-93115	11	0	0.0
ICG-94037	17	4	23.5
ICG-93139	9	2	22.2

and benomyl + thiram could eliminate *R. bataticola* from all the three accessions (Table 5). In benomyl treatment the fungus could not be eliminated from ICG-93139. Thiram was least effective as the seed infection ranged between 12.5 to 47.5% compared to 37.5 to 50% in control.

In the greenhouse experiment, only carbendazim + thiram could eliminate *R. bataticola* from all the three groundnut accessions with significant increase in germination over control (Table 6). Benomyl + thiram was the second best where it could eliminate the fungus from two accessions. Ram Reddy *et al*., (1991) reported the effectiveness of carbendazim + thiram in controlling *R. bataticola* from groundnut in the laboratory using poisoned food technique. It was effective in increasing germination, shoot length, root length and total dry weight. It is evident from the results that the combination of systemic fungicide with non-systemic fungicide was more effective in controlling seed borne infection of *R. bataticola* from groundnut seeds than sole treatment. Carbendazim + thiram @ 2g a.i / kg seed can be used as a routine treatment in quarantine laboratories to eliminate *R. bataticola* from groundnut seeds. It is expected that the results of the studies would help in exporting seeds of groundnut even to those countries where *R. bataticola* is of quarantine concern.

Table 5. Effect of fungicides on seed infection by *Rhizoctonia bataticola* in laboratory

Fungicide/combination	Accession	Seed infection (%)
Benomyl	ICG-93139	5 (6.6)*
	ICG-94039	0.0
	ICG-94016	0.0
Carbendazim+Thiram	ICG-93139	0.0
	ICG-94039	0.0
	ICG-94016	0.0
Benomyl+Thiram	ICG-93139	0.0
	ICG-94039	0.0
	ICG-94016	0.0
Thiram	ICG-93139	47.5 (43.5)
	ICG-94039	12.5 (17.9)
	ICG-94016	20.0 (23.1)
Captan	ICG-93139	12.5 (17.9)
	ICG-94039	10.0 (13.3)
	ICG-94016	10.0 (13.3)
Carbendazim	ICG-93139	0.0
	ICG-94039	0.0
	ICG-94016	0.0
Control	ICG-93139	50.0 (45.0)
	ICG-94039	37.5 (37.6)
	ICG-94016	50.0 (45.0)
LSD at 5%		7.6

*Figures in parentheses are angular transformed values

Table 6. Effect of fungicides on seed infection by *Rhizoctonia bataticola* in the greenhouse

Fungicide/ combination	Accession No.	Damping-off (%)	Germination (%)
Benomyl	ICG-93139	10 (13.3)*	90 (76.7)
	ICG-94039	0	100 (90.0)
	ICG-94016	5 (6.6)	95 (83.3)
Carbendazim+ Thiram	ICG-93139	0	100 (90.0)
	ICG-94039	0	100 (90.0)
	ICG-94016	0	95 (83.3)
Benomyl+ Thiram	ICG-93139	0	100 (90.0)
	ICG-94039	0	100 (90.0)
	ICG-94016	10 (13.3)	85 (73.5)
Thiram	ICG-93139	10 (13.3)	85 (73.5)
	ICG-94039	10 (13.3)	85 (73.5)
	ICG-94016	15 (16.4)	85 (73.5)
Captan	ICG-93139	10 (13.3)	90 (76.7)
	ICG-94039	15 (16.4)	85 (73.5)
	ICG-94016	5 (6.6)	95 (83.3)
Carbendazim	ICG-93139	15 (16.4)	85 (73.5)
	ICG-94039	15 (16.4)	85 (73.5)
	ICG-94016	10 (13.3)	90 (76.7)
Control	ICG-93139	65 (53.9)	35 (36.1)
	ICG-94039	30 (29.1)	70 (60.8)
	ICG-94016	55 (48.2)	45 (41.8)
LSD at 5%		10.9	11.8

* Figures in parentheses are angular transformed values

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