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Perpetuation of Rice Sheath Blight Pathogen (*Thanatephorus cucumeris*) Under Temperate Conditions of Kashmir, India

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A B S T R A C T

The studies conducted on mode of perpetuation of sheath blight pathogen (*Thanatephorus cucumeris*) of rice (*Oryza sativa* L.) in/on rice seeds, plant debris and through sclerotia was studied at different placement conditions *viz*., on soil surface, at 5 cm soil depth and in-door under roof cover after crop harvest. The rice seeds were found to harbour externally viable pathogen up to five months from the harvest. The pathogen survived the crop less off-season as mycelium in infected straw and as sclerotia when placed either on soil surface, or 5 cm deep in soil or in-doors under roof cover. However, the viability decreased at all the placements with increase in storage period. After seven months, the maximum straw bits (68.66%) harboured viable *T. cucumeris* when placed in-doors under roof cover followed by placement at 5 cm soil depth (45.50%) and on soil surface (34.33%). In case of sclerotia, maximum viable sclerotia (91.04%) were found in-doors under roof cover, followed by placement at 5 cm soil depth (74.71%) and on soil surface (64.66%).

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop of India. It is staple food crop of Jammu and Kashmir where it occupies 261.35 thousand hectare area with an annual production of 5001 thousand tones (Kaloo *et al.*, 2014). The crop is attacked by a number of fungal, bacterial and viral diseases, which inflict heavy yield losses every year. Sheath blight of rice has attained the status of a major disease in the recent past from what was described as a minor disease by Ramakrishna (1971). The disease has wide geographic distribution and now occurs throughout the temperate and tropical rice production areas, being most prominent where rice is grown under intense, high fertility production system (Eizenga *et al.*, 2002). Sheath blight of rice was first reported in India by Paracer and Chahal (1963), while Mir (1986) reported it from Kashmir. The sheath blight of rice caused by *Thanatephorus cucumeris* [Anamorph: *Rhizoctonia solani* Kuhn] is one

of the important biological constraints in achieving the stable rice production. The disease can result in yield losses ranging from 20 to 50 per cent (Rajan, 1987). However, under conditions of heavy severity, yield loss of more than 70 per cent has been reported from Chennai, India (Baby, 1992), and even complete crop failure has also been reported in Vietnam (Ou, 1992).

The perpetuation of the pathogen in any crop eco-system forms an important component of recurrence of any endemic or epidemic disease. *T. cucumeris* is known to survive between seasons as dormant mycelium in infected rice straw, in/on rice seeds and sclerotia (Singh, 1998; Acharya *et al.*, 2004) and weeds/crops plants (Acharya and SenGupta, 1998). Survival of the pathogen during the off season may provide potential source of primary inoculum of *T. cucumeris* in ensuing cropping season. It is considered essential for adopting judicious control measures as the disease is prevalent in entire rice growing areas of Kashmir valley and therefore, studies on this aspect were conducted.

Materials and Methods

The perpetuation of pathogen was studied through seeds, infected straw and sclerotia.

Perpetuation in/on seeds

The seeds collected at harvest from severely infected rice plants (cv. Jhelum) were stored in cotton cloth bags in laboratory and assessed for presence of the pathogen at monthly intervals. The study was conducted using two separate methods *viz*., agar plate and paper towel method.

Agar plate method

Three hundred seeds were taken at monthly intervals from the diseased seed samples to study external and/or internal nature of perpetuation of the pathogen. Half of the seeds were surface sterilized with 0.1 per cent mercuric chloride for 30 seconds followed by three subsequent rinses in sterilized distilled water and placed on 2 per cent water agar medium in sterilized Petri plates (90 mm) under aseptic conditions of laminar air flow. Ten seeds were placed aseptically in each Petri dish and incubated at 28 ± 2^{0}C for 48 hours. Five such Petri dishes were maintained and observed for viability of pathogen every month. Rest half of seeds were inoculated on 2 per cent sterilized water agar medium and incubated without surface sterilization to study the possibility of external seed borne nature of the pathogen. The per cent seeds exhibiting *T. cucumeris* growth was recorded as an index of pathogen perpetuation through seeds.

Paper towel method

Fifty surface sterilized and unsterilized seeds were separately placed in the grooves of sterilized seed germination papers rolled after sprinkling distilled water and incubated at 28 ± 2^{0}C. In all, 150 seeds of each category were assessed for *T. cucumeris* growth and per cent seeds harbouring *T. cucumeris* was calculated.

Perpetuation through plant debris (diseased stem) and sclerotia

The paddy straw infected with *T. cucumeris* was cut into 3-5 cm long pieces, put in nylon mesh bags and placed separately under different placement conditions *viz*., in-doors/under-roof conditions, on soil surface and buried 5 cm deep in soil. The samples from the bags containing infected straw bits were randomly drawn from all the placement conditions separately at monthly intervals. The infected straw bits were thoroughly

washed with tap water, surface sterilized with sodium hypochloride (1%) for 30 seconds, and rinsed thrice with distilled sterilized water to remove the traces of sodium hypochloride. These bits were then dried on sterilized blotter paper to remove the excess of water, aseptically placed on Petri plates containing sterilizes PDA amended with streptomycin sulphate (200 µg ml^{-1}) and incubated at 28±2°C. Observations on number of straw bits exhibiting *T. cucumeris* growth were recorded after two days of incubation and expressed as per cent infected straw bits exhibiting mycelial growth of *T. cucumeris* by using the following formula:

Per cent infected straw bits exhibiting *T.cucumeris* growth = (Total no. of infected straw bits exhibiting *T. cucumeris* growth / Total no. infected straw bits examined) ×100

Perpetuation through sclerotia

Sclerotia harvested from 20 days old culture of *T. cucumeris* were put in double layer of 1.7 mm diameter nylon mesh bags at the rate of 150 sclerotia per bag. These bags were placed on soil surface and buried 5 cm deep in soil on well-marked areas. Besides, sclerotia were also placed in Khadi bags in laboratory at room temperatures and assessed for their viability at monthly intervals. The sclerotial samples placed at soil surface and 5 cm soil depth were randomly drawn at monthly interval and collected in 250 ml flask containing 150 ml water and placed on a wrist-action shaker to remove the adhering soil particles (Roy, 1986).

The sclerotia placed at indoor under laboratory conditions in Khadi bags were only thoroughly washed with sterilized distilled water. The sclerotia were then surface sterilized with sodium hypochloride (1%) for 1.5 min and placed in Petri plates containing sterilized PDA amended with 200 µg ml^{-1} streptomycin sulphate and incubated at 28±2°C. The sclerotial viability was recorded as the per cent sclerotia germinated using following formula:

$$\text{Per cent sclerotial viability} = \frac{\text{No. of sclerotia germinated}}{\text{Total No. of sclerotia examined}} \times 100$$

Results and Discussion

Perpetuation through seeds

Random seed samples collected from diseased rice plants after harvest, were examined for the presence of *T. cucumeris* growth using standard agar plate and paper towel methods, separately for surface sterilized and unsterilised seeds.

Using surface sterilized seeds

The results (Table 1) obtained, indicated that none of the surface sterilized seeds exhibited growth of the *T. cucumeris* either on agar plate or on germination paper, indicating that the pathogen was not internally seed borne.

Using unsterilized seeds

Persual of the data (Table 1) revealed that highest percentage of seeds (6.17%) harbouring viable *T. cucumeris* was recorded immediately after crop harvest (November), followed by that in December (4.00). A decrease in per cent seeds possessing viable *T. cucumeris* was observed as the storage period advanced such that no seed was found to harbour viable *T. cucumeris* after five months.

The agar plate and paper towel methods differed significantly in assessing the viable *T. cucumeris* from seed samples. On an average, agar plate method resulted in determining the higher percentage (2.46%) of

seeds harbouring viable *T. cucumeris* than paper towel method (1.67). Using agar plate method, the highest percentage (7.67%) of seeds were found to harbor the viable *T. cucumeris* immediately after harvest (November) which decreased gradually as storage period advanced such that only 0.67 per cent seeds exhibited viable *T. cucumeris* four months after harvest (March), while no seed exhibited *T. cucumeris* growth five months after harvest (April) and onwards.

Using Paper towel method, the maximum number of the seeds exhibiting *T. cucumeris* growth were only 4.67 per cent immediately after harvest (November). The number of infected seeds showed a gradual decrease to 1.33 two months (January) after the harvest and 0.00 per cent four months (March) after the harvest.

Perpetuation in/on soil

The possibility of perpetuation of pathogen in/on soil through diseased straw (plant debris) and sclerotia were investigated

Table.1 Viability of *Thanatephorus cucumeris* in infected rice straw kept under different conditions after crop harvest

Year/Month	Mycelial viability (%) in infected rice straw			
	On soil	**At 5 cm soil depth**	**In-doors under roof cover**	**Mean**
Ist Year				
November	74.67 (59.55)	83. 33 (65.90)	100.00 (90.00)	**86.00 (71.89)**
December	69.33 (56.37)	72.00 (58.05)	95.33 (77.51)	**78.88 (63.97)**
2nd Year				
January	53.33 (46.90)	68.67 (55.96)	87.33 (69.14)	**69.77 (57.33)**
February	36.00 (36.86)	60.67 (51.16)	77.33 (61.56)	**58.00 (49.86)**
March	22.67 (28.43)	41.33 (40.00)	66.67 (54.73)	**43.55 (41.05)**
April	13.33 (21.41)	23.33 (28.88)	53.33 (46.90)	**29.99 (32.39)**
May	5.33 (13.34)	12.67 (20.85)	40.00 (39.23)	**19.33 (24.47)**
June	0.00 (0.33)	2.00 (8.13)	29.33 (32.79)	**10.44 (18.85)**
Mean	**34.33 (32.92)**	**45.50 (41.11)**	**68.66 (58.98)**	

CD (P=0.05)
Months = 1.62
Placement = 0.71
Months x placement = 2.20

***** Mean of three observations (50 straw bits / observation); Figures within parentheses are angular transformed values

Table.2 Perpetuation of *Thanatephorus cucumeris* in/on rice seeds collected from infected tillers in autumn and assessed at monthly intervals during storage

Month	Per cent seeds exhibiting *R. solani* growth					
	Surface sterilized seed			Unsterilized seeds		
	Paper towel method	Agar plate method	Mean	Agar plate method	Paper towel method	Mean
Ist Year						
November	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**	7.67 (2.85)	4.67 (2.27)	**6.17 (2.56)**
December	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**	5.37 (2.41)	2.67 (1.78)	**4.00 (2.10)**
2nd Year						
January	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**	4.67 (2.27)	1.33 (1.35)	**3.00 (1.81)**
February	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**	1.33 (1.35)	0.67 (1.08)	**1.00(1.21)**
March	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**	0.67 (1.08)	0.00 (0.70)	**0.33 (0.89)**
April	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**
May	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**
June	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**	0.00 (0.70)	0.00 (0.70)	**0.00 (0.70)**
Mean	**0.00 (0.70)**	**0.00 (0.70)**	**0.00 (0.70)**	**2.46 (1.50)**	**1.67 (1.16)**	
CD(P=0.05)						
Month :	**-**		**0.41**			
Method :	**-**		**0.20**			
Months x Method :	**-**		**0.58**			

* Mean of observation of 150 seeds (50 seeds/replication) ; Figures within parentheses are square root transformed values

Table.3 Viability of sclerotia of rice sheath blight pathogen (*Thanatephorus cucumeris*) under different conditions after crop harvest

Year/Month	***Viable sclerotia (%)****			***Mean***
	On soil	***At 5 cm soil depth***	***Under roof cover***	
Ist year				
November	100.00 (90.00)	98.67 (83.37)	100.00 (90.0)	**99.55 (87.79)**
December	98.67 (83.37)	96.67 (79.59)	100.00 (90.0)	**98.44 (84.28)**
2nd year				
January	86.67 (68.58)	90.00 (71.56)	98.67 (84.58)	**87.88 (74.50)**
February	72.33 (58.28)	87.67 (69.44)	96.33 (79.59)	**85.44(68.88)**
March	54.67 (47.67)	77.33 (61.56)	94.67 (76.70)	**75.55 (61.96)**
April	44.33 (41.74)	61.33 (51.55)	89.33 (70.95)	**64.99 (54.73)**
May	35.33(36.46)	50.67(45.38)	78.67 (62.49)	**54.89 (48.11)**
June	25.33(30.21)	35.33 (36.46)	70.67 (57.21)	**44.89 (41.95)**
Mean	**64.66 (57.03)**	**74.71 (62.35)**	**91.04 (76.19)**	
CD (P=0.05)				
Months : 1.61				
Method : 0.93				
Months x Method : 2.79				

Perpetuation through straw

The infected rice straw bits kept under different conditions revealed that irrespective of straw placements, highest per cent infected straw bits exhibited *T. cucumeris* growth (86.00%) immediately after crop harvest in November followed by December and January i.e., 77.88 and 69.77%, respectively. There was a gradual decrease in straw bits exhibiting *T. cucumeris* growth as the storage period advanced such that a minimum of 19.33 per cent straw bits exhibited *T. cucumeris* growth six months after harvest (May) followed by 10.44 per cent seven months after the harvest (June). On an average, infected straw bits placed in-door under roof cover harboured the maximum straw bits exhibiting *T. cucumeris* growth (68.66%) followed by the placement at 5 cm depth (45.50); placement in soil surface showed the minimum (34.33%) growth. There existed a significant interaction between the placement condition and the storage period. The straw bits placed on the soil surface exhibited 74.67 and 69.33 per cent *T. cucumeris* viability in November and December, respectively. The viability of the pathogen showed a gradual decline such that only 5.33 per cent straw bits exhibited *T. cucumeris* viability six months after harvest (May), which was completely lost (0.00%)seven months (June) after harvest of crop. The straw bits buried 5 cm depth in soil showed 83.33 and 72.00 per cent growth of the pathogen in November and December, respectively. Again there is gradual decline in pathogen viability that reaches 2.00 per cent seven months after crop harvest (June) (Table 2).

When placed in-doors under roof covers, viability of *T. cucumeris* was recorded 100.00 per cent immediately after harvest in November. However, the viability of the *T. cucumeris* was decreased to 95.33 per cent in December. A gradual decrease in viability of *T. cucumeris* with increase in storage period such that only 29.33 per cent straw bits exhibited viability of *T. cucumeris* seven months after the harvest (June).

Perpetuation through sclerotia

The samples of sclerotia of *T. cucumeris* kept either in/on soil or in-door under roof cover, were drawn at monthly intervals and assessed for their viability. The data (Table 3) revealed that the sclerotia remained viable in considerable proportions from crop harvest till next seed sowing under all the placement conditions studied. On an overall basis, the number of viable sclerotia was maximum (91.04%) when the sclerotia were placed under roof cover. At 5 cm soil depth placement, the sclerotial viability was 74.71 per cent whereas on soil surface least (64.66%) sclerotia remained viable. Irrespective of the condition of sclerotial placement, the maximum number of sclerotia (99.55%) was viable immediately after crop harvest in November. The viability gradually decreased as the placement period advanced such that only 64.99 per cent viable sclerotia were observed five months after crop harvest (April) and only 44.89 per cent seven months after crop harvest (June). There existed a significant interaction between the sclerotial placement condition and the storage period. The sclerotia placed on the soil surface exhibited 100.00 per cent viability immediately after crop harvest (November) and 98.67 per cent in December. The viability showed a gradual decline as the storage period advanced reaching a minimum of 25.33 per cent seven months after harvest (June). Similarly, the sclerotia buried in the soil showed 98.67 per cent viability immediately after crop harvest in November and 96.67 per cent in December. In this case also, the viability showed a gradual decline reaching a minimum of 35.33 per cent seven

months after crop harvest (June). When placed indoors under roof covers, the sclerotia retained full viability (100.00%) for the first two months i.e. November and December. In January also a high proportion of 98.67 per cent sclerotia exhibited viability. Thereafter a gradual decrease in sclerotial viability was observed reaching a minimum of 70.67 per cent seven months after harvest (June).

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