

Original Research Article

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Survival of *Didymella bryoniae* Incitant of Ridge Gourd Blight Under Temperate Conditions

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ABSTRACT

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Studies on role of infected seed and crop debris in the survival of *Didymella bryoniae* incitant of blight in ridge gourd were conducted under temperate conditions of Kashmir valley. The studies revealed that the pathogen perpetuated in the form of spores on seeds and plant debris stored indoors under ambient laboratory conditions as these produced viable spores throughout the observation period of twelve months. The pathogen was also observed to survive in the form of spores on plant debris stored indoors under ambient laboratory conditions, however, perpetuated as a dormant mycelium on plant debris left on the soil surface in open and was unable to survive on plant debris buried 7.5 cm deep in soil.

Introduction

Didymella blight caused by *Didymella bryoniae* (Aures.) Rehm. (anamorph: *Phoma cucurbitacearum* (Foutry) Sacardo) is an important disease of all the cucurbitaceous crops and reduces their yield as a result of defoliation, fruit infection and subsequent decay (Schenck, 1968). The disease is widely distributed in tropical countries and also reported frequently in areas having temperate climate (Sitterly, 1969). The disease was first reported from France in 1891 on cucumber (Roumequere, 1891) and since then it has been reported from many countries namely, USA, Britain, Germany, Japan, New Zealand,

Brunei, Mexico, Netherlands, Republic of Ireland, Salvador (Puninthalingam and Holliday, 1972). In India, the disease was first reported from Mysore on leaves of *Scheum edule* (Sohi and Prakash, 1972) and then subsequently on *Cucumis sativus* (Kumar and Khan, 1984), *Mamordicha charanta* (Kulwant and Shetty, 1996), *Benincasa hispida* (Pandey and Pandey, 2003) and *Cucumis melo* (Sudisha *et al.*, 2004). Yield losses due to this disease on watermelon have been estimated up to 100 per cent in Trinidad (Bala and Hosein, 1986), 43 per cent in USA (Kienath and Duthie, 1998), 30 per cent on muskmelon in Australia (Mc Grath *et al.*, 1993) and 35 per cent on cucumber in Poland (Leski, 1984).

The disease has emerged in the recent past as one of the major constraints in the successful cultivation of the crop in Kashmir valley and has been responsible for causing a marked reduction in both quality and quantity of the fruit yield. For an effective management of the disease mode of perpetuation of the pathogen during the crop-less off-season is imperative.

The present study was therefore, conducted under temperate conditions of Kashmir valley to find out the role of infected crop debris and seeds in perpetuation of the pathogen.

Materials and Methods

Perpetuation in/on seed

The seeds collected at harvest from severely infected ridge gourd plants were stored in cotton cloth bags in laboratory and assessed for presence of the pathogen through blotter and agar plate methods at monthly intervals.

Blotter method

Random sample of sixty seeds were taken at monthly intervals from harvest and surface sterilized by immersing in 0.1% mercuric chloride for one minute followed by three subsequent rinses with sterilized distilled water. The seeds were placed in sterilized Petri dishes on three folds of sterilized blotter papers moistened with sterilized distilled water. Twenty seeds were placed aseptically in each Petri dish maintaining three replications for each plate. The plates were incubated at $24\pm 1^{\circ}\text{C}$.

The blotter paper in Petri dishes was kept moist by carefully pouring a few drops of sterilized distilled water in the plates as and when required. The plates were examined from 4th up to 15th day after incubation for the appearance of fungal colonies.

Agar plate method

Sixty seeds were taken from the seed lot and surface sterilized. Twenty surface sterilized seeds were aseptically placed on 2 per cent water agar in Petri dishes and incubated at $24\pm 1^{\circ}\text{C}$. The plates were examined from 4th to 10th day after incubation for recording the percentage of seeds showing *D. bryoniae* growth.

The number of seeds showing *D. bryoniae* growth in blotter and agar plate method were recorded and per cent infected seeds estimated by using the formula:

$$\text{Per cent infected seeds} = \frac{\text{Number of seed infected}}{\text{Total number of seeds examined}} \times 100$$

Spore viability

Twenty ridge gourd seeds were taken randomly at monthly intervals from harvest and 1 cm² hilum portion from each seed carved out and crushed in a pestle and mortar in 40 ml of distilled water. The crushed material was strained through a double layer of cheese cloth. Twenty milliliter of the filtrate was centrifuged at 6000 rpm for 15 minutes. After centrifugation, supernatant was discarded and pellet was made 5 milliliter by adding sterilized water (Filajdic and Sutton, 1995). Number of pycnidiospores/ ascospores was counted with the help of haemocytometer and the average spore load of three replications was estimated. To estimate the pycnidiospore/ ascospore germination one drop of 50 μl from processed sample was placed on a glass slide and incubated in a moist chamber at $24\pm 1^{\circ}\text{C}$. After 24 hour incubation, the slide was viewed under binocular microscope to record spore germination index of spore viability. The spore viability was recorded using the formula:

$$\text{Spore viability} = \frac{\text{Number of spores germinated}}{\text{Total number of spores viewed}} \times 100$$

Perpetuation through plant debris

The infected plant debris including fruit husk and leaves were collected at harvest from the diseased crop and kept separately in nylon mesh bags. The bags were divided into three sets of 12 bags each. One set, each of leaves and fruit husk, was placed on the soil surface in vacated ridge gourd fields. The other set was buried 7.5 cm deep in such soil and third set was stored under ambient laboratory conditions. Each set was replicated three times. One month after placement of the bags at appropriate positions, twenty bits of 1 cm² area were randomly taken from one randomly selected bag of each leaf and fruit husk and examined for the presence of pycnidia/pseudothecia. These leaf and fruit discs were then crushed separately in 40 ml of sterilized distilled water and for spore suspension preparation and estimation of per

cent viability of pycnidiospores/ascospores the method as described above for spore viability test of infected seeds was adopted.

Results and Discussion

Survival on seed

The seeds were found infected with *D. bryoniae* and the infection was found to persist from harvest up to 12 months of storage under ambient room conditions by using blotter and agar plate methods (Table 1). Similarly, the seeds were found to harbour viable spores of *D. bryoniae* throughout the observation period of twelve months starting from one month after harvest (in November during both the crop seasons of 2004 and 2005). The extent of viability constantly decreased with the advancement of storage period such that after 12 months of storage seeds harboured 18.6 and 20.3 per cent viable conidia in 2004 and 2005, respectively (Table 1).

Table.1 Survival of *Didymella bryoniae* in/on ridge gourd seeds observed at monthly intervals after harvest in 2004 and 2005

Month	Spore viability (%)		Seeds showing <i>D. bryoniae</i> growth (%)			
			Blotter method		Agar plate method	
	2004	2005	2004	2005	2004	2005
November 2004	93.9 (76.0)	93.7 (75.9)	45.0 (42.1)	48.3 (44.0)	41.6 (40.1)	45.0 (42.1)
December	95.4 (77.8)	92.9 (74.7)	41.6 (40.1)	45.0 (42.1)	38.3 (38.2)	43.3 (41.1)
January 2005	81.2 (64.3)	86.9 (68.9)	38.3 (38.2)	41.6 (40.1)	36.6 (37.2)	38.3 (38.2)
February	72.0 (58.0)	80.8 (64.0)	33.3 (35.2)	38.3 (38.2)	33.3 (35.1)	35.0 (36.2)
March	61.3 (51.5)	78.0 (62.0)	31.6 (34.2)	35.0 (36.2)	31.6 (34.2)	28.3 (32.1)
April	55.9 (48.4)	73.2 (58.8)	30.0 (33.1)	31.6 (34.2)	28.3 (32.0)	25.0 (29.9)
May	32.5 (34.7)	60.8 (51.2)	28.3 (32.1)	30.0 (33.1)	25.0 (29.9)	23.3 (28.8)
June	29.8 (33.0)	46.6 (43.0)	26.6 (30.9)	26.6 (30.9)	21.6 (27.6)	21.6 (27.5)
July	28.0 (31.9)	45.9 (42.6)	23.3 (28.8)	25.0 (29.9)	20.0 (26.4)	21.6 (27.5)
August	28.35 (32.0)	32.6 (34.8)	20.0 (26.4)	21.6 (27.5)	16.6 (24.0)	20.0 (26.5)
September	23.3 (28.8)	24.1 (29.4)	20.0 (26.5)	20.0 (26.4)	11.6 (19.8)	15.0 (22.5)
October	18.6 (25.5)	20.3 (26.7)	16.6 (24.0)	18.3 (25.2)	8.3 (16.5)	11.6 (19.8)
CD (P=0.05)	4.69	3.88	4.32	4.43	4.92	4.58

Figures in parentheses are arc sine transformed values

Table.2 Fructification and pycnidiospore/ ascospore production and viability on infected ridge gourd leaves kept under different conditions after harvest in 2004 and 2005

Month	*No. of pycnidia/ pseudothecia cm ⁻² fruit husk area						*No. of pycnidiospores/ ascospores cm ⁻² fruit husk area						*Pycnidiospore/ ascospore viability (%)					
	Ambient conditions		Leaf burial at soil depth (cm)				Ambient conditions		Leaf burial at soil depth (cm)				Ambient conditions		Leaf burial at soil depth			
			0		7.5				0		7.5				0		7.5	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
November 2004	47.6	49.8	45.6	49.8	17.2	23.6	5166	4643	4100	3956	0.0	0.0	90.4 (72.1)	87.6(69.3)	92.2	90.1	NA	
December	40.1	46.9	34.2	39.5	0.0	0.0	3646	3916	3453	3126	0.0	0.0	76.5 (61.0)	73.2(58.8)	81.2	86.9	NA	
January 2005	38.0	44.9	21.2	18.0	0.0	0.0	3166	2926	0	0	0.0	0.0	64.0 (53.1)	58.6(49.9)	NA	NA	NA	
February	31.2	39.9	13.2	10.2	0.0	0.0	2643	2336	0	0	0.0	0.0	48.4(44.1)	49.0(44.4)	NA	NA	NA	
March	23.5	33.0	19.4	20.7	-	-	1833	2173	1846	2100	-	-	30.3 (33.5)	38.4(38.3)	85.2	82.6	NA	
April	20.6	29.0	29.6	20.0	-	-	1413	1856	2533	2836	-	-	23.3 (28.8)	35.0(36.2)	94.4	80.2	-	
May	18.5	21.3	24.5	17.4	-	-	1046	1643	2013	2126	-	-	20.3 (26.7)	32.7(34.9)	84.8	80.0	-	
June	12.1	16.4	19.5	11.6	-	-	1026	1393	1226	1640	-	-	20.0 (26.5)	28.9(32.5)	78.6	73.4	-	
July	10.9	15.1	-	-	-	-	956	876	-	-	-	-	16.4 (23.9)	24.2(29.4)	-	-	-	
August	12.5	12.6	-	-	-	-	646	630	-	-	-	-	10.4 (18.8)	19.0(25.9)	-	-	-	
September	8.5	9.5	-	-	-	-	576	646	-	-	-	-	9.6 (18.0)	13.7(21.7)	-	-	-	
October	6.5	8.3	-	-	-	-	416	343	-	-	-	-	8.8(17.2)	7.2(15.6)	-	-	-	
CD(P=0.05)	2.90	3.07	3.28	2.54			2.95	3.23	2.59	3.18			2.60	3.29				

Figures in parentheses are arc sine transformed values

- Material perished; NA Spores not available

Table.3 Fructification and pycnidiospore/ ascospore production and viability on infected ridge gourd fruit husk kept under different conditions after harvest in 2004 and 2005

Month	*No. of pycnidia/ pseudothecia cm ⁻² fruit husk area						*No. of pycnidiospores/ ascospores cm ⁻² fruit husk area						*Pycnidiospore/ ascospore viability (%)					
	Ambient conditions		Leaf burial at soil depth (cm)				Ambient conditions		Leaf burial at soil depth (cm)				Ambient conditions		Leaf burial at soil depth (cm)			
			0		7.5				0		7.5				0	7.5		
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
November 2004	78.1	71.8	76.6	71.8	13.4	24.6	6926	6576	6126	4936	0.0	0.0	94.2 (66.5)	93.2 (74.9)	92.05	91.1	NA	NA
December	70.8	69.2	63.2	53.6	0.00	0.0	6200	5963	4533	3863	0.0	0.0	84.6 (59.7)	89.4 (71.0)	87.33	88.7	NA	NA
January 2005	62.4	65.7	36.7	30.6	0.00	0.0	5036	5376	0	0	0.0	0.0	72.5 (52.2)	77.1 (61.4)	NA	NA	NA	NA
February	54.4	57.8	22.2	10.5	0.00	0.0	4520	4656	0	0	0.0	0.0	51.2 (45.7)	53.2 (46.8)	NA	NA	NA	NA
March	43.2	52.1	24.2	19.4	0.00	-	3865	4226	2946	1453	-	-	43.2 (41.1)	45.2 (42.2)	89.55	90.6	-	-
April	37.1	47.1	41.0	37.2	-	-	3353	3623	3756	2100	-	-	41.5 (40.1)	33.2 (35.2)	88.05	82.4	-	-
May	30.2	37.2	25.1	18.2	-	-	2850	3150	2253	1353	-	-	38.4 (38.2)	29.8 (33.0)	85.16	80.2	-	-
June	24.1	31.4	18.4	12.5	-	-	2630	2413	1676	1000	-	-	32.0 (34.4)	23.4 (28.9)	80.38	75.1	-	-
July	20.2	21.5	13.2	9.2	-	-	1746	1853	1240	813	-	-	28.4 (32.2)	24.2 (29.4)	78.25	-	-	-
August	14.1	19.6	-	-	-	-	1573	1356	-	-	-	-	22.5 (28.3)	21.0 (27.3)	-	-	-	-
September	10.1	13.5	-	-	-	-	1256	923	-	-	-	-	18.5 (25.5)	16.2 (23.7)	-	-	-	-
October	8.2	10.5	-	-	-	-	983	780	-	-	-	-	13.2 (21.3)	11.8 (20.1)	-	-	-	-
CD(P=0.05)	2.27	2.83	3.25	3.10			3.20	3.29	3.64	2.41			0.91	1.57				

Figures in parentheses are arc sine transformed values

- Material perished; NA Spores not available

These findings provide sufficient grounds to infer that the pathogen *D. bryoniae* perpetuates in/on the seeds as spores and the pathogen inoculum reaches the cropping fields along with seeds. Similar observations have also been made by several workers. Rankin (1954) found the invasion of *D. bryoniae* in the epidermis and sclerenchyma layers and isolated the fungus from cotyledons and embryo. Chen and Bao (1990) noted the survival of the fungus both in and on the seeds of infected fruits up to 21 months of storage at room temperature. Sudisha *et al.*, (2006) also reported the seed borne nature of *D. bryoniae* and noted mean incidence of 31 per cent in seed coat, 11 per cent in cotyledons and 4 per cent in embryo while evaluating the different components of infected seeds.

Survival on plant debris

The periodic examination of the infected debris placed indoors, on soil surface and buried under soil indicated the presence of fruiting bodies as well as viable spores throughout the observation period of twelve months when placed indoors under roof protection and only up to four to five months when left on soil surface as debris perished beyond this period (Table 2 and 3). However, only empty fruiting bodies were noticed during January and February when left on the ground surface, suggesting the survival of *D. bryoniae* as dormant mycelium. The survival of the pathogen lasted for only four to five months when buried in soil, beyond this period debris was decomposed. The lower recovery rate of *D. bryoniae* from the infected plant debris buried in soil or placed on soil surface could be because of the fact that the hibernating pathogen was exposed to chilly winters, and had to compete with other soil inhabiting microorganisms for food and niche, a situation quite different than the one when the trash is stored under roof protection.

Kienath (2002) could not recover *D. bryoniae* beyond 6 to 7 months of storage in buried infected watermelon debris which he attributed to the antagonistic activities of saprophytic soil microorganisms that seemed to have eliminated the pathogen resident in the watermelon debris. Van Steekelenburg (1983) observed old pseudothecia with ascospores and pycnidia with some pycnidiospores, even after storage of diseased plant debris for 18 months, whereas on debris kept on soil surface in open only empty fruiting bodies were observed during winter months when average monthly temperature was below 5°C. Similarly, Chiu and Walker (1949) found empty pycnidia and pseudothecia in the overwintered crop debris but could isolate the fungus readily and suggested the overwintering of fungus in winter as dormant mycelium.

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