Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 1326-1331
Received: 03-03-2018
Accepted: 07-04-2018

Asha Nabi
Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

MD Shah
Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

BA Padder
Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

MS Dar
Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Varsha Bharti
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India

Mushtaq Ahmad
Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Shakeela Sofi
Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Correspondence
Asha Nabi
Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Morphological characterisation and media preferences in *Wilsonomyces carpophilus*, the causal agent of shot hole disease of stone fruits in Kashmir

Asha Nabi, MD Shah, BA Padder, MS Dar, Varsha Bharti, Mushtaq Ahmad and Shakeela Sofi

Abstract
The study was carried out to ascertain the diversity in *Wilsonomyces carpophilus* on the basis morpho-cultural characteristics. Twenty five isolates of *W. carpophilus* collected from different stone fruits (peach, plum, apricot, almond and cherry) grown in three districts (Srinagar, Ganderbal and Baramulla) in the year 2011-2012 were maintained. All the isolates sampled were found pathogenic when inoculated on respective hosts. Significant variations were also observed in growth characteristics (texture, margins and colour), colony diameter and growth rates of different isolates. PDA (self-prepared) was the best medium as compared to other media tested. On PDA (self-prepared), the maximum colony diameter was recorded in isolates WcS8. On Asthana and Hawker's medium WcS5 showed maximum colony diameter and isolate WcS5 on PDA (Hi-Media). The overall growth rate increased upto sixth day of incubation and then started decreasing afterwards. Thus, *W. carpophilus* is highly diverse pathogen in terms of morphological characters and nutritional requirements and has a potential to overcome management strategies very quickly.

Keywords: morphological characterisation, *Wilsonomyces carpophilus*, stone fruits, shot hole

Introduction
Stone fruits are prone to various fungal, bacterial and viral diseases which is the major constraint in their production. Shot hole blight or Coryneum blight caused by *Wilsonomyces carpophilus* infects peach, nectarine, apricots, almonds, plums and cherries and causes shot hole of leaves and blight of apical twigs, blossoms and un-opened buds. The disease is most important and is a major threat to stone fruit industry in Kashmir valley. The disease is reported to cause 30 to 90 per cent losses to the crop under favourable climatic conditions (Dar and Teng, 1979) [4].

Earth is considered as an ideal home for microorganisms and their diversity has significant impact on environment (Malik, 2000) [7]. In 1990, the fungal diversity magnitude was estimated at 1.5 million species (Hawksworth, 2001) [6]. Pathogen populations from different geographical locations exhibit remarkable differences in cultural, morphological and biochemical characteristics (Thakur, 1999) [15]. Knowledge of variability of the fungal population associated with an infection improves the disease management strategies (Walker *et al.*, 2001) [19]. It is important to study variability within the population of pathogenic fungi in a geographical region to document the changes occurring in it (Sarma *et al.*, 2002) [11].

Therefore, an attempt to carry out nutritional studies and morphological characterisation was made

Materials and Methods
Collection, isolation, purification, identification and maintenance of pathogen
The infected leaves and fruits showing typical shot hole symptoms on different stone fruits such as peach, plum, apricot, cherry and almond, collected from three districts *viz.*, Srinagar, Ganderbal and Baramulla were brought to the laboratory for isolation of the pathogen. The diseased samples were thoroughly washed with running tap water and dried on blotting paper. Small bits of diseased tissue along with some healthy portion were surface sterilized in 0.1 per cent mercuric chloride solution for 20-30 seconds followed by washing in sterilized water thrice to remove traces of mercuric chloride. The bits were dried with sterilized blotting papers, transferred to Petri plates containing PDA medium under aseptic conditions and incubated at 24±1ºC.

Twenty five isolates of the pathogen collected from different stone fruits grown in different areas of three districts were purified using single spore technique (Tuite, 1969) [18]. The pathogen was identified on the basis of the cultural and morphological characters. The different isolates of shot hole pathogen were maintained on slants and Petri plates containing Asthana and Hawker's/PDA media at 24±1ºC until adequate sporulation. In all, 25 isolates were maintained having different geographical and host origin for further studies (Table 1).

Table 1: *Wilsonomyces carpophilus* isolates obtained from different geographical regions and hosts

District	Location	Isolate Number	Host
Srinagar	Shalimar	WcS1	Apricot
	Zakoora	WcS2	
	Shalimar	WcS3	Peach
	Boelvard	WcS4	
	Boelvard	WcS5	
	Shalimar	WcS6	Plum
	Boelvard	WcS7	
	Harwan	WcS8	
	Shalimar	WcS9	Almond
	Shalimar	WcS10	
	Boelvard	WcS11	
	Boelvard	WcS12	
Ganderbal	Repora	WcG1	Apricot
	Lar	WcG2	
	Dangerpora	WcG3	
	Haran	WcG4	Plum
	Haran	WcG5	Peach
	Dangerpora	WcG6	Almond
Baramulla	Pattan	WcB1	Apricot
	Pattan	WcB2	
	Pattan	WcB3	Peach
	Tangmarg	WcB4	
	Wadura	WcB5	Plum
	Pattan	WcB6	
	Pattan	WcB7	Cherry

Pathogenicity tests

Pathogenicity tests of isolates were carried out by inoculating them on respective hosts using detached leaf technique (Sukumar and Ramalingum, 1981) to prove the Koch's postulates. Healthy leaves nearly of same age and size were collected from different stone fruits and brought to the laboratory for pathogenicity tests. Leaves were thoroughly washed with tap water, surface sterilized with ethanol and immediately rinsed with sterilized water. Sterilized Petri plates of 120 x 10 mm poured with water agar were used as moist chambers. Leaves of different stone fruits/cultivars were placed on these water agar Petri plates and petioles of leaves were inserted into the water agar to maintain turgidity.

Cultural characteristics

Colony characteristics such as growth type, type of margins and colony colour of different isolates were assessed on potato dextrose agar medium (PDA). Growth type, colony colour and texture was also recorded after 10 days of incubation.

Colony diameter and growth rate on different media

Colony diameter and vegetative growth rate was recorded on three media *viz.*, PDA (self-prepared), PDA (HiMedia, BioSciences) and Asthana and Hawker's media. Approximately 20 ml of sterilized media were poured in Petri plates and then allowed to solidify. A 6 mm diameter disc cut with the help of sterilized cork borer from seven days old cultures of different isolates was inoculated at the center of each Petri plate and each treatment was replicated five times. The inoculated Petri plates were incubated at 24±1ºC and colony diameter was recorded after 10 days of incubation and growth rate was recorded each day.

Results and Discussion

Pathogenicity tests

All the isolates tested were found to be pathogenic when inoculated on their respective hosts with varied incubation periods (Plate 1). The maximum incubation period was found in case of cherry (7 days) and minimum in almond isolates (2 days).

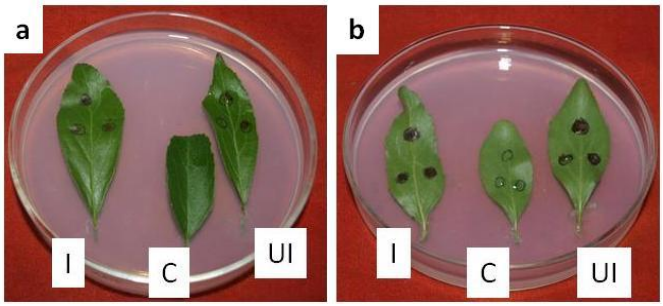

Plate 1: Pathogenicity test of plum isolate a) upper side and b) lower side of the leaves

Cultural characteristics

Different isolates of *Wilsonomyces carpophilus* cultured on potato dextrose agar medium showed significant variations in their colony characteristics (Table 2). Most of the isolates showed flat cottony to fluffy type of growth and few showed velvety type of growth Cottony type of growth was observed in 10 isolates (WcS1, WcS2, WcS9, WcS12, WcG2, WcG6, WcB1, WcB2, WcB6 and WcB7), fluffy type in 11 isolates (WcS4, WcS5, WCs6, WcS7, WcS8, WcS10, WcS11, WcG1, WcG5, WcB3 and WcB5) and velvetty in 4 isolates (WcS3, WcG3, WcG4 and WcB4) (Plate 2). Isolates WcB1, WcB3 and WcB7 obtained from Baramulla showed prominent zonations in their colonies. Colony margins of most of the isolates were regular except five (WcS1, WcS10, WcS11, WcS12 andWcB6) which showed irregular margins (Table 2). Colony colour also varied from whitish to dull white in 8 isolates (WcS3, WcS5, WcS8, WcS11, WcB1, WcB3, WcB4 and WcB6), greyish white to grey in 2 isolates (WcS1 and WcS10), dull white to greyish centre surrounded by blackish region in 3 isolates (WcS2, WcS7 and WcS12), whitish to dull white centre surrounded by olivaceous green region in 5 isolates (WcS4, WcS6, WcS9, WcG4, and WcB7), whitish to grey centre surrounded by greyish green region in 2 isolates (WcG1 and WcB2). Greyish white centre surrounded by olivaceous green region was observed in WcB5 and whitish centre with light green outer region was observed in WcG2. Greyish green colour was observed in WcG3 whereas dull white centre surrounded by light brown region in WcG6. Light greyish to olivaceous green colour was observed in WcG5 and greyish centre surrounded by greyish green region in WcG1. Sofi *et al.* (2013) [14] also reported variation in colony characteristics of *Alternaria mali* isolates. Similarly Torres-Calzada *et.al* (2013) [16] characterised isolates of *Colletotrichum gleosporoides* and *Colletotrichum capsici* into nine groups on the basis of colony characteristics.

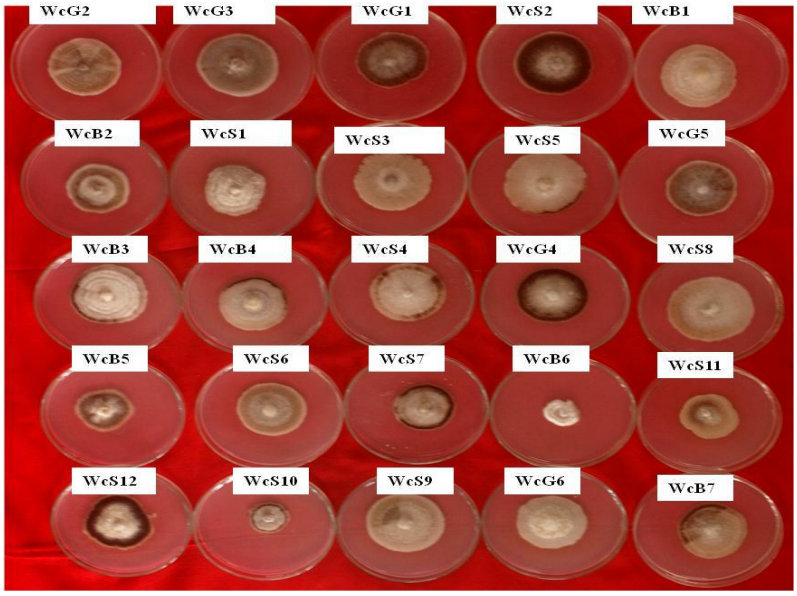

Plate 2: Colonies of *Wilsonomyces carpophilus* isolates on Potato dextrose agar medium

Table 2: Growth characteristics of different isolates of *Wilsonomyces carpophilus* on potato dextrose agar medium at 24±1^{0}C

Isolate$	Colony characters	
	Texture	**Colour**
WcS1	Flat cottony, irregular margins	Greyish white
WcS2	Flat cottony, uniform margins	Greyish centre surrounded by blackish outer region
WcS3	Vevetty, uniform margins, bearing spore mass	Dull white
WcS4	Fluffy, uniform margins, bearing spore mass	Whitish centre surrounded by olivaceous green outer region
WcS5	Fluffy, uniform margins, bearing olivaceous green fructifications	Dull white
WcS6	Fluffy, uniform margins	Whitish centre with olivaceous green outer region
WcS7	Fluffy, uniform margins	Light greyish centre surrounded by black region having spore mass
WcS8	Fluffy, uniform margins	Whitish
WcS9	Cottony, uniform margins	Dull white centre surrounded by olivaceous green region
WcS10	Fluffy, raised, irregular margins	Greyish
WcS11	Fluffy, irregular margins	Whitish
WcS12	Flat cottony, irregular margins	Dull white surrounded by blackish spore mass
WcG1	Fluffy, uniform margins	Greyish centre surrounded by greyish green outer region
WcG2	Flat cottony, uniform margins	Light green with whitish centre
WcG3	Velvetty, uniform margins	Greyish green
WcG4	Velvetty, uniform margins	Whitish centre surrounded by olivaceous green region containing spore mass
WcG5	Fluffy, uniform margins	Light greyish to olivaceous green
WcG6	Cottony, uniform margins	Dull white centre surrounded by light brown region
WcB1	Cottony with prominent zonations, uniform margins	Whitish
WcB2	Cottony, uniform margins	Whitish centre surrounded by greyish green region
WcB3	Fluffy with prominent zonations, uniform margins	Whitish
WcB4	Velvetty, uniform margins, bearing spore mass	Dull white
WcB5	Fluffy, uniform margins	Greyish white surrounded by olivaceous green region containing spore mass
WcB6	Cottony, irregular margins	Whitish
WcB7	Cottony, uniform margins	Whitish centre surrounded by olivaceous green region containing spore mass

Colony diameter

Isolates exhibited significant variations in terms of colony diameter (Table 3). The highest average colony diameter (43.4 mm) was recorded in WcS5 followed by WcS3. The least colony diameter (29.1mm) was recorded in WcB6 followed by WcS1 (31.5 mm) and WcS10 (31.9 mm). Isolates WcS4, WcS8, WcG1, WcG4, WcB1 and WcB3 were statistically at par in terms of colony diameter. Similarly isolates WcS2, WcG2, WcG3 and WcG5 were also statistically at par with each other.

PDA (self-prepared) was the best medium followed by Asthana and Hawker's medium and PDA (Hi-Media). On PDA (self-prepared), the maximum and minimum colony diameter was recorded in isolates WcS8 and WcB6, respectively. On Asthana and Hawker's medium WcS5 showed maximum colony diameter and WcS1 showed minimum colony diameter. On PDA (Hi-Media), the maximum colony diameter was recorded in WcS5 and minimum in WcB2. Similar results were obtained by different workers in terms of media preferences by *W. carpophilus* (Shukla and Bhat, 1984 and Ahmad, 1994) [13, 1] but in our case, the number of isolated used in the study was more so as to authenticate the results.

Table 3: Colony diameter of different *Wilsonomyces carpophilus* isolates on different culture media

Isolate No.[$]	Colony diameter (mm) on different media after 10 days of incubation[*]			Mean
	Asthana and Hawker's	PDA (Hi-Media)	PDA (Prepared in Lab)	
WcS1	27.9	27.6	39.1	31.5[f]
WcS2	35.2	30.5	48.6	38.1[c]
WcS3	43.0	36.5	49.4	43.0[a]
WcS4	41.6	30.0	47.0	39.5[b]
WcS5	40.8	39.0	50.4	43.4[a]
WcS6	37.0	28.2	44.4	36.5[d]
WcS7	36.4	27.5	38.4	34.1[e]
WcS8	42.3	25.4	52.6	40.1[b]
WcS9	40.6	28.5	49.0	39.4[b]
WcS10	34.8	27.6	33.4	31.9[f]
WcS11	41.6	28.0	36.4	35.3[d]
WcS12	37.6	23.2	45.6	35.5[d]
WcG1	42.9	27.3	50.7	40.3[b]
WcG2	42.0	27.2	44.8	38.0[c]
WcG3	36.0	30.6	45.4	37.3[c]
WcG4	38.2	34.8	47.0	40.0[b]
WcG5	43.5	25.2	44.2	37.6[c]
WcG6	39.9	25.2	45.4	36.8[d]
WcB1	39.7	37.2	46.6	41.2[b]
WcB2	43.0	22.8	37.2	34.3[e]
WcB3	40.4	26.0	51.4	39.3[b]
WcB4	37.2	24.2	45.8	35.7[d]
WcB5	38.4	31.7	30.2	33.4[e]
WcB6	39.9	25.4	22.0	29.1[g]
WcB7	42.2	23.4	42.8	36.1[d]
Mean	39.3	28.5	43.5	
CD (0.05)	Isolate: 1.9 Media: 0.7 Isolate x Media: 3.3			

[*]Mean of 5 replications

Growth rate

The maximum growth rate (1.72-4.96 mm/day) was recorded on PDA (self-prepared) (Table 4) followed by Asthana and Hawker's medium (2.3-3.8 mm/day) (Table 5) and PDA (Hi-media) (1.6-3.5 mm/day) (6).

Table 4: Growth rates of different *Wilsonomyces carpophilus* isolates on Potato dextrose agar medium (Self prepared) at 24±1ºC

Isolate No.[$]	Growth rate (mm/day) at different incubation periods (day)*									Mean
	2	3	4	5	6	7	8	9	10	
WcS1	4.17	4.33	3.83	4.00	3.00	3.00	3.00	2.83	3.00	3.46[j]
WcS2	4.17	4.83	4.33	5.00	5.00	5.00	4.33	4.00	4.00	4.52[d]
WcS3	3.50	4.00	5.00	5.00	5.00	5.00	4.17	5.00	5.00	4.63[c]
WcS4	4.00	4.00	4.00	4.00	5.00	5.00	5.00	4.00	4.00	4.33[e]
WcS5	4.00	5.00	4.50	5.00	5.17	5.00	5.17	4.00	4.00	4.65[c]
WcS6	3.17	4.00	4.00	4.00	4.00	5.67	4.00	4.00	4.00	4.09[h]
WcS7	2.83	3.00	3.00	3.00	4.00	4.00	4.33	3.67	3.00	3.43[j]
WcS8	4.33	4.00	5.33	5.00	5.50	5.00	5.50	5.00	5.00	4.96[a]
WcS9	4.50	3.83	4.00	5.00	5.00	5.00	5.00	5.00	4.00	4.59[d]
WcS10	2.67	3.17	3.00	3.00	3.00	3.00	3.00	3.00	2.00	2.87[m]
WcS11	3.00	3.17	3.00	3.00	4.17	4.00	3.00	3.00	3.00	3.26[l]
WcS12	3.33	4.00	4.00	4.00	4.33	5.00	5.00	4.00	4.00	4.19[g]
WcG1	4.17	4.17	5.50	5.00	5.00	5.00	5.00	5.00	4.00	4.76[b]
WcG2	3.00	4.00	4.00	4.00	4.00	5.00	5.00	4.00	4.00	4.11[g]
WcG3	4.50	4.00	6.00	5.00	4.00	4.00	3.50	3.17	3.17	4.15[g]
WcG4	4.00	5.00	5.00	5.00	5.00	4.00	4.00	4.00	3.00	4.33[e]
WcG5	4.67	5.00	6.00	5.00	4.33	3.00	2.67	3.00	2.33	4.00[i]
WcG6	4.00	4.00	4.00	4.00	5.00	5.00	3.67	4.00	4.00	4.19[g]
WcB1	3.00	4.67	5.00	5.00	5.00	4.00	4.00	4.00	4.00	4.30[e]
WcB2	3.00	3.00	3.00	4.00	4.00	4.00	3.00	3.00	3.00	3.33[k]
WcB3	4.17	5.00	5.00	5.17	5.00	5.00	5.00	5.00	4.00	4.81[b]
WcB4	3.00	3.00	4.17	4.17	4.00	5.00	5.00	5.00	5.00	4.26[f]
WcB5	2.33	3.17	3.00	3.17	3.00	2.00	2.00	2.00	2.00	2.52[n]
WcB6	1.50	2.00	2.00	5.00	2.00	2.00	2.00	2.00	1.00	1.72[o]
WcB7	2.67	1.00	3.00	4.17	4.17	6.00	4.00	4.00	4.00	3.93[i]
Mean	3.5	3.9	4.2	4.3	4.3	4.27	4.01	3.75	3.50	
CD (0.05)	Isolate: 0.09 Days: 0.06 Isolate x days: 0.3									

[*]Mean of 5 replications

Table 5: Growth rate of different *Wilsonomyces carpophilus* isolates on Asthana and Hawker's medium at 24±1°C

Isolate No.[$]	Growth rate (mm/day) at different incubation periods (day)*									Mean
	2	3	4	5	6	7	8	9	10	
WcS1	2.0	4.3	1.9	1.9	1.2	1.0	2.7	2.9	2.5	2.3[d]
WcS2	3.9	2.5	4.2	3.6	4.2	1.6	3.0	2.2	2.8	3.1[c]
WcS3	4.2	2.7	4.2	5.1	4.0	3.2	4.0	3.8	4.2	3.9[a]
WcS4	4.3	3.2	4.1	2.7	4.1	3.7	4.3	4.0	3.2	3.7[a]
WcS5	3.8	3.4	4.3	4.7	2.8	3.9	3.3	3.5	4.0	3.7[a]
WcS6	4.1	3.5	2.5	4.0	2.6	2.9	2.5	4.0	3.0	3.2b[c]
WcS7	4.3	2.5	3.2	4.9	2.1	3.4	2.5	3.2	3.3	3.3b[c]
WcS8	5.1	3.0	3.5	4.8	2.7	3.7	4.6	4.2	2.9	3.8[a]
WcS9	3.9	3.2	4.5	4.0	3.7	3.5	2.9	3.7	3.2	3.6[b]
WcS10	2.7	4.0	3.0	3.6	4.6	3.3	2.1	1.8	2.8	3.1[c]
WcS11	4.2	3.1	4.1	3.8	3.3	4.2	4.0	4.0	3.0	3.7[a]
WcS12	2.3	4.7	3.8	2.2	3.2	2.6	4.8	3.0	3.0	3.3b[c]
WcG1	4.1	3.3	3.7	4.1	3.4	3.4	3.6	4.2	4.1	3.8[a]
WcG2	4.2	2.9	3.5	4.7	3.2	3.8	3.5	4.0	3.8	3.7[a]
WcG3	4.0	2.6	3.8	3.9	2.4	3.4	2.7	3.2	2.7	3.2b[c]
WcG4	3.8	2.8	4.7	3.2	3.2	3.2	3.8	3.7	2.0	3.4[c]
WcG5	5.1	4.2	2.8	5.3	3.3	2.9	2.9	4.5	4.0	3.9[a]
WcG6	4.1	3.2	4.0	3.6	4.2	3.1	3.7	2.7	3.6	3.6[b]
WcB1	5.4	3.5	3.6	4.7	3.5	3.0	3.5	2.7	2.6	3.6[b]
WcB2	3.8	3.0	4.8	4.0	2.9	3.5	2.8	5.5	4.1	3.8[a]
WcB3	4.0	3.9	3.8	4.2	3.7	3.5	3.4	2.4	3.7	3.6[b]
WcB4	3.2	2.6	3.2	4.1	1.5	2.9	2.8	4.1	3.6	3.1[c]
WcB5	3.9	2.3	5.6	2.9	3.6	3.9	2.5	3.5	2.5	3.4[b]
WcB6	3.6	3.5	3.5	3.3	3.9	3.3	3.7	3.0	3.3	3.5[b]
WcB7	3.7	3.0	4.3	3.8	3.9	3.8	2.9	4.2	3.6	3.7[a]
Mean	3.9	3.2	3.8	3.9	3.2	3.2	3.3	3.5	3.3	
CD (0.05)	Isolate: 0.3 Days: 0.2 Isolate x days: 0.8									

*Mean of 5 replications

On PDA (self-prepared), the maximum average growth rate (4.96 mm/day) was observed in WcS8 followed by WcB3 (4.80 mm/day) and WcG1 (4.76 mm/day) where as WcB3 and WcG1 were at par with each other (Table 4). The lowest average growth rate (1.72 mm/day) was observed in WcB6 followed by WcB5 (2.52 mm/day). The overall growth rate increased upto sixth day of incubation and then started decreasing afterwards. Moreover, growth rates of different isolates peaked at different intervals.

On Asthana and Hawker's medium the maximum average growth rate (3.9 mm/day) was observed in WcS3 and WcG5 followed by WcB2 (3.8 mm/day), WcG1 (3.8 mm/day), WcS8 (3.8 mm/day), WcS4 (3.7 mm/day), WcS5 (3.7 mm/day), WcS11 (3.7 mm/day), WcG2 (3.7 mm/day) and WcB7 (3.7 mm/day) and were at par with each other (Table 5). The minimum average growth rate (2.3 mm /day) was observed in WcS1 followed by WcS2 (3.1 mm /day), WcS10 (3.1 mm /day) and WcB4 (3.1 mm/day) and these were at par with each other. No increasing or decreasing trend was observed in overall growth rate over a period of 10 days but fluctuations were observed.

On PDA (Hi- media) the maximum average growth rate (3.5 mm/day) was observed in WcS5 followed by WcS3 (3.2 mm/day) and WcG4 (3.1 mm/day) whereas WcS3 and WcG4 were at par with each other (Table 6). The minimum average growth rate (1.6 mm/day) was observed in WcB7 followed by WcB2 (1.7 mm/day), WcB4 (1.7 mm/day), WcB6 (1.8 mm/day), WcS8 (1.9 mm/day), WcG5 (1.9 mm/day) and WcG6 (1.9 mm/day) and were at par with each other. The overall growth rate was maximum on 2nd day of incubation, decreased on 3rd day and again increased on 4th day of incubation. The growth rate started decreasing from 4th day onwards and tending to stop after 9th day of incubation.

Table 6: Growth rate of different *Wilsonomyces carpophilus* isolates on Potato dextrose agar medium (Hi-Media BioSciences) at 24±1^{0}C

Isolate No.[$]	Growth rate (mm/day) at different incubation periods (day)*									Mean
	2	3	4	5	6	7	8	9	10	
WcS1	3.6	2.8	2.5	1.6	1.6	2.2	1.7	1.8	2.3	2.2[d]
WcS2	5.6	4.7	6.2	4.2	2.4	0.3	0.0	0.0	0	2.6[c]
WcS3	6.1	4.4	6.2	3.0	3.9	2.2	1.8	1.2	0.1	3.2[b]
WcS4	4.0	2.7	4.7	2.2	2.1	2.6	1.5	1.6	1.2	2.5[c]
WcS5	6.1	4.5	6.0	5.0	4.2	2.8	1.3	1.8	0.2	3.5[a]
WcS6	5.6	4.1	4.0	2.2	1.9	0.9	0.9	0.6	0.6	2.3[d]
WcS7	4.9	4.2	4.7	2.8	1.3	0.6	0.2	0.7	0.1	2.2[d]
WcS8	5.1	4.5	3.1	1.5	1.2	0.8	0.8	0.0	0.0	1.9[e]
WcS9	5.7	4.1	4.6	2.9	1.5	1.1	0.2	0.5	0.1	2.3[d]
WcS10	2.4	1.6	2.8	2.4	2.5	2.7	2.2	2.0	2.0	2.3[d]
WcS11	3.8	3.9	2.5	2.4	2.6	2.0	1.6	0.8	0.8	2.3[d]
WcS12	3.6	3.0	2.8	1.6	1.2	2.0	0.8	0.8	0.2	1.8[e]
WcG1	5.9	4.3	4.1	2.6	0.6	0.4	0.1	0.0	0.0	2.0[d]
WcG2	5.5	3.6	3.3	2.2	1.6	0.3	1.1	0.7	0.0	2.0[d]

WcG3	5.8	3.7	5.2	3.3	2.1	1.7	1.0	0.5	0.2	2.6[c]
WcG4	7.3	4.0	6.5	3.4	2.9	0.8	2.2	0.4	0.0	3.1[b]
WcG5	6.0	3.6	4.3	1.7	0.5	0.4	0.3	0.0	0.0	1.9[e]
WcG6	5.3	4.0	1.8	2.0	1.2	0.2	0.6	1.0	0.6	1.9[e]
WcB1	5.5	4.4	6.1	5.0	2.9	1.1	2.7	1.4	0.2	3.3[a]
WcB2	3.9	2.6	2.8	3.2	2.1	0.4	0.1	0.0	0.0	1.7[e]
WcB3	6.1	4.0	4.7	1.8	1.9	0.1	0.1	0.0	0.0	2.1[d]
WcB4	5.0	3.6	3.0	2.2	0.4	0.6	0.4	0.0	0.0	1.7[e]
WcB5	6.3	4.2	6.0	3.3	1.7	1.9	0.6	0.0	0.2	2.7[c]
WcB6	4.9	3.6	3.7	1.9	1.0	0.8	0.2	0.0	0.0	1.8[e]
WcB7	5.1	3.6	2.9	1.0	1.1	0.1	0.6	0.0	0.0	1.6[e]
Mean	5.2	3.7	4.2	2.6	1.9	1.2	0.9	0.6	0.4	
CD (0.05)	Isolate: 0.4 Days: 0.2 Isolate x days: 1.1									

*Mean of 5 replications

Significant variations were observed in growth rates of different isolates of *W. carpophilus* on different culture media. Razdan and Puttoo (1987) [9] reported non-significant variations among seventeen isolates of *Wilsonomyces carpophilus* with respect to location or host type involved but Ahmadpour *et al*. (2009) [2] confirmed our results by reporting significant differences in sporulation and vegetative growth rate of *W. carpophilus* isolates. Growth rates of different isolates varied from one medium to another medium, therefore, it becomes difficult to standardise phenotypic characterization in pathogens.

Thus, *W. carpophilus* isolates show remarkable morphological diversity. This variation may be attributed to adaptation of *W. carpophilus* to different hosts and this variation implicates the presence of different pathotypes in the population. Further research is needed to study the diversity of this fungus using molecular markers.

Acknowledgement

The authors are highly thankful to University Grants 468 Commission, New Delhi and Department of Biotechnology, Govt. of India, New Delhi for their financial support.

References

1. Ahmad S. Studies on shot hole disease of almond and other stone fruits caused by *Stigmina carpophila* (Lev.) Ellis. M. Sc. thesis submitted to Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, 1994.
2. Ahmadpour A, Javan-Nikkhah M, Ghosta Y, Fatahi R. Study on some biological and morphological characteristics of *Wilsonomyces carpophilus* in West Azerbaijan. Rostaniha. 2009; 10:91-109.
3. Anderson HW. Diseases of fruit crops. McGraw-Hill Book Company, Inc. New York, 1956, p. 501.
4. Dar GN, Teng RK. Proceedings of first symposium on Possible improvement in temperate fruit culture J&K State Srinagar, 13th September, 1979, 14-16.
5. Ellis MB. *Clasterosporium* and some allied Dematiaceae-Phragmosporae II. *CMI* Mycological Paper, 1959; 72:1-75.
6. Hawksworth DL. The magnitude of fungal diversity, the 1.5 million species estimate revisited. Mycology Research, 2001; 105:1422-1432.
7. Malik VS. Biodiversity: A treasury of billion dollar molecules. In: Proceedings of International Conference. Integrated Plant Disease Management For Sustainable Agriculture, Indian Phytopathological Society, IARI, New Dehli, India, 2000; 1:109-117.
8. Meena BS. Morphological and molecular variability of rice blast pathogen Pyricularia grisea (Cooke) Sacc. MSc. thesis submitted to University of Agricultural Sciences, Dharwad, India, 2005.
9. Razdan VK, Puttoo BL. Cultural characteristics of some isolates of *Stigmina carpophila* causing shot hole of almonds and apricots in Kashmir. International Journal of Tropical Plant Diseases. 1987; 5:103-110.
10. Samuel G. On the shot hole disease caused by Clasterosporium carpophilum and on the shot hole effect. Annals of Botany, 1927; 41:375-404.
11. Sarma BK, Singh UP, Singh KP. Variability in Indian isolates of Sclerotium rolfsii. Mycologia 2002; 94:1051-1058.
12. Shah MD, Verma KS, Singh K, Kaur R. Morphological, pathological and molecular variability in *Botryodiplodia theobromae* (Botryosphaeriaceae) isolates associated with die-back and bark canker of pear trees in Punjab, India. Genetics and Molecular Research 2010; 9:1217-1228.
13. Shukla HP, Bhat GN. Morphology and physiology of *Stigmina carpophila* causing shot hole disease of almond. Indian Journal of Mycology and Plant Pathology. 1984; 14:178.
14. Sofi TA, Beig MA, Dar GH, Ahmad M, Hamid A, Ahangar FA, *et al*. Cultural, morphological, pathogenic and molecular characterization of *Alternaria mali* associated with *Alternaria* leaf blotch of apple. African Journal of Biotechnology. 2013; 12(4):370-381.
15. Thakur RP. Pathogen diversity and plant disease management. Indian Phytopathology, 1999; 52:1-9.
16. Torres-Calzada C, Tapia-Tussell R, Higuera-Ciapara I. and Perez-Brito D. Morphological, pathological and genetic diversity of *Colletotrichum* species responsible for anthracnose in papaya (*Carica papaya* L). European Journal of Plant Pathology. 2013; 135:67-79.
17. Tovar-Pedraza JM, Ayala-Escobar V, Segura-Leon OL. *Thyrostroma carpophilum* causing apricot shot hole in Mexico. Australasian Plant Disease Notes, 2013; DOI 10.1007/s13314-013-0089-7.
18. Tuite J. Plant Pathologist Methods, Fungi and Bacteria. Burgess Publishing, Miniea Polis, 1969, 239.
19. Walker SL, Leath S, Hagler JWM, Murphy JP. Variation among isolates of *Fusarium graminearum* associated with *Fusarium* head blight in North Carolina. Plant Diseases. 2001; 85:404-410.