

Original Research Article

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Study on Cross Infectivity of Different Isolates of *Thyrostroma carpophilum* on Stone Fruits in Kashmir Valley

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ABSTRACT

Thyrostroma carpophilum, a causal agent of shot hole of stone fruits was isolated from the leaves of different stone fruit trees (Peach, Plum, Apricot, Cherry and Almond) collected from various locations of district Srinagar and Anantnag. Twenty isolates were collected and their pathogenicity was proved. Significant variation in cultural characteristics such as colony texture, colour and shape was found among the isolates. Two isolates showed different growth pattern. Isolate TC-H6 showed fluffy with olivaceous green and TC-R16 showed velvety growth pattern. Incubation period was found maximum in case of peach (7 days) and minimum in case of apricot (2 days) when the isolates were inoculated on their respective hosts. Cross infectivity tests of different isolates was carried out by cross inoculation of isolates. The isolates obtained from the peach host (TC-S1, TC-H1, TC-K1 and TC-R1) were able to infect plum and apricot while as the isolates from plum (TC-S3, TC-H3, TC-K3 and TC-R3) were able to infect cherry. The isolates derived from cherry were able to infect apricot. However, isolates from apricot and almond did not show any cross infectivity when tested on other stone fruit hosts. The above results revealed that *Thyrostroma carpophilum* is highly diverse pathogen with wide host range and thus has ability to infect other trees of same family. Thus, *Thyrostroma carpophilum* has potential to overcome management strategies very rapidly.

Keywords

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Introduction

Stone fruits are prone to various diseases of which diseases of fungal origin are more destructive. Shot hole caused by *Thyrostroma carpophilum* is one of the important diseases of stone fruits and is a great threat to stone fruit industry in Kashmir valley. The disease

is reported to cause considerable yield losses of about 30-90 per cent under favourable climatic conditions (Dar and Teng, 1979). The disease is called shot hole because of the symptoms caused by fungus on the leaves of host trees. Ogawa and English, (1991) reported that the fungus survives as mycelium in twig cankers and blighted buds and under

favourable climatic conditions fungus is reportedly dispersed from twig cankers to infection courts by splashing of water droplets. Numerous studies have been made on the biology and epidemiology (Shaw *et al.*, 1990; Adaskaveg *et al.*, 1990; Grove, 2002), survival (Highberg and Ogawa 1986), host range (Smith and Smith, 1942) and disease control (Azza *et al.*, 2010) aspects of the disease. However, little work has been done on the cross infectivity of shot hole disease pathogen within the stone fruits. Therefore, an attempt was made to study the cultural characters, pathogenicity and host specificity of *T. carpophilum* isolates.

Materials and Methods

Collection, isolation, identification, purification and maintenance of pathogen

The infected leaves showing shot hole symptoms (Plate 1) were collected from different stone fruit trees such as cherry, plum, peach, apricot and almond from various locations of district Srinagar and Anantnag and brought to the laboratory for isolation of causal pathogen. The diseased samples were washed in running tap water and blotter dried.

Diseased leaf tissue along with some healthy portion was cut and surface sterilized in 0.1 per cent mercuric chloride for 20-30 seconds and then rinsed thrice with sterilized water to remove traces of mercuric chloride. The sterilized bits were blotter dried, transferred to petri plates containing Asthana Haver's (AH) media and incubated at $24 \pm 1^\circ\text{C}$.

In all twenty isolates (Table 1) were purified by single spore technique (Tuite, 1969) and maintained for further studies. The identification of pathogen was done on the basis of morpho-cultural characters. The Pathogenicity test of all the isolates was carried out to prove Koch's postulates by

using detached leaf technique (Sukumar and Ramalingum, 1981). Healthy leaves were collected, surface sterilized and then inoculated with 30 μl of spore suspension. Inoculum concentration was adjusted to 10^4 spores/ml by using hemocytometer. The test isolates were inoculated on their respective hosts. The inoculated leaves were placed in the moisture chamber and incubated at desired temperature till the symptom appearance.

Cultural Characters

Colony characters such as texture, colour, colony shape and type of margins of the isolates were ascertained by visual examination on AH media after 7 days of inoculation.

Cross infectivity of isolates on different host

The cross infectivity test of the collected isolates was carried out on different stone fruits. The healthy leaves from different hosts were brought to the laboratory and surface sterilized. The isolate collected from the particular host was cross inoculated on the leaves of other stone fruit trees to test its cross infectivity. The inoculated leaves were placed in moisture chamber and incubated at desired temperature.

Results and Discussion

Isolation and identification of causal pathogen

Isolation of the pathogen was made from the infected leaves of peach, plum, apricot, cherry and almond. The pathogen was purified and identified as *Thyrostroma carpophilum* (Lev.).

Pathogenicity Test

Pathogenicity test of all the isolates was

carried out on their respective hosts. All the isolates were pathogenic (Plate 2) with different incubation period. The maximum incubation period was found in case of peach (7 days) and minimum in case of apricot (3 days).

Cultural Characteristics

The isolates from different locations cultured on Asthana Hawker's media showed significant variation in their texture, colony colour and shape. Most of the isolates showed fluffy type of growth followed by isolates that showed flat cottony growth (Table 2). Two isolates viz., TC-H6 and TC-K16 out of the entire twenty showed different growth pattern. Isolate TC-H6 showed fluffy with

olivaceous green and TC-R16 showed velvety growth pattern (Plate 3). Most of the isolates were with uniform margins except few (TC-S1, TC-S2, TC-S5, TC-H9, TC-K11, TC-R16, TC-R17 and TC-R20) with irregular margins. The colony colour of most of the isolates varied from whitish to dull white, however, it varied in some isolates from olivaceous green to greyish (Table 2). The colour of margins varied from light green to blackish due to spore mass. Nabi *et al.*, (2019) also reported the variation in colony characteristic of *Wilsonomyces carpophilus*. Torres-Calzada *et al.*, (2013) grouped *Colletotrichum gleosporoides* and *Colletotrichum capsici* into nine groups on the basis of colony characteristics.

Table.1 List of *Thyrostroma carpophilum* isolates collected from different locations of Srinagar and anantnag districts of Kashmir

District	Location	Isolate Number	Host
Srinagar	Shalimar	TC-S1	Peach
		TC-S2	Almond
		TC-S3	Plum
		TC-S4	Apricot
		TC-S5	Cherry
	Harwan	TC-H1	Peach
		TC-H2	Almond
		TC-H3	Plum
		TC-H4	Apricot
		TC-H5	Cherry
Anantnag	Kherbugh	TC-K1	Peach
		TC-K2	Almond
		TC-K3	Plum
		TC-K4	Apricot
		TC-K5	Cherry
	Ranbirpora	TC-R1	Peach
		TC-R2	Almond
		TC-R3	Plum
		TC-R4	Apricot
		TC-R5	Cherry

Table.2 Colony characteristics of different isolates of *Thyrostroma carpophilum* on Asthana Hawker's media at 24±10C

Isolates	Colony Characters		
	Texture	Colour	Shape
TC-S1	Smooth, Cottony	Dull white centre surrounded by greyish region having spore mass	Irregular margins
TC-S2	Fluffy	Greyish margin with off white centre	Irregular margins
TC-S3	Cottony	Greyish centre surrounded by black region with spore mass	Uniform margins
TC-S4	Flat, Cottony	Light green with dull centre	Uniform margins
TC-S5	Fluffy	Greyish centre with light green margin	Irregular margins
TC-H6	Fluffy, olivaceous green fructifications	White	Uniform margins
TC-H7	Flat with prominent zonations	White centre with green margin	Uniform margins
TC-H8	Fluffy	Whitish	Uniform margins
TC-H9	Fluffy	Greyish	Irregular margins
TC-H10	Cottony	Whitish centre surrounded light green region with spore mass	Uniform margins
TC-K11	Fluffy	Greyish to olivaceous green	Irregular margins
TC-K12	Cottony	Whitish centre surrounded with greyish region	Uniform margins
TC-K13	Cottony	Dull white centre surrounded by green region	Uniform margins
TC-K14	Flat cottony	Light green with dull white centre	Uniform margins
TC-K15	Cottony	Dull white centre surrounded by olivaceous green region	Uniform margins
TC-R16	Velvety	Dull white	Irregular margins
TC-R17	Flat Cottony	Dull white surrounded by greyish region	Irregular margins
TC-R18	Fluffy	Whitish surrounded by green region	Uniform margins
TC-R19	Cottony	Dull white	Uniform margins
TC-R20	Cottony, raised	Dull white surrounded by Light brown	Irregular margins

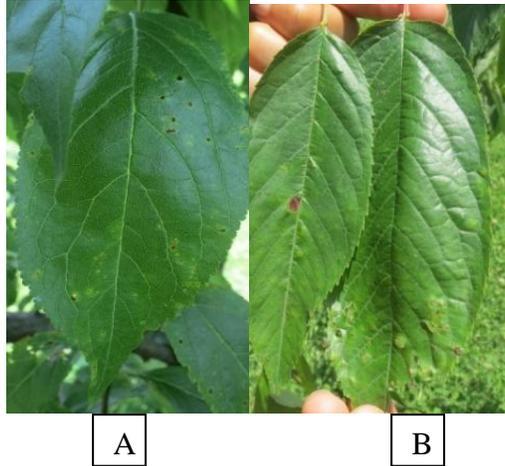


Plate.1 (A) Shot hole symptoms on Plum
(B) Shot hole symptoms on Cherry

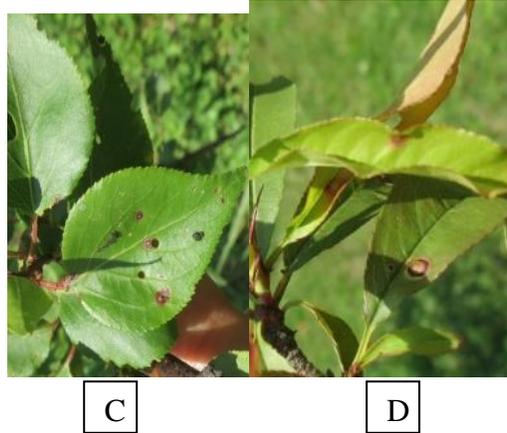


Plate.1 (C) Shot hole symptoms on Apricot
(D) Shot hole symptoms on Peach



E

Plate.1(E) Mycelium and spores of *Thyrostroma carpophilum* (Causal organism of Shot hole)

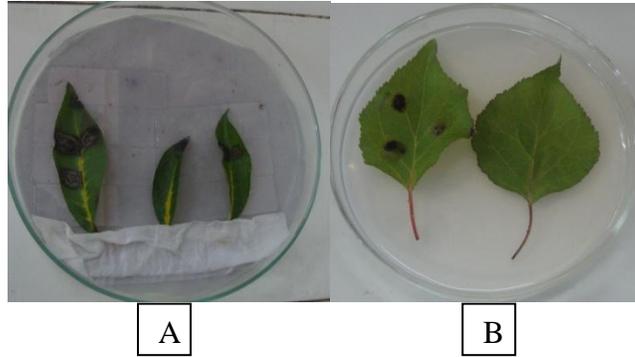


Plate.2 Pathogenicity test of .A. Peach isolate (TC-S1). B. Apricot isolate (TC-S4)

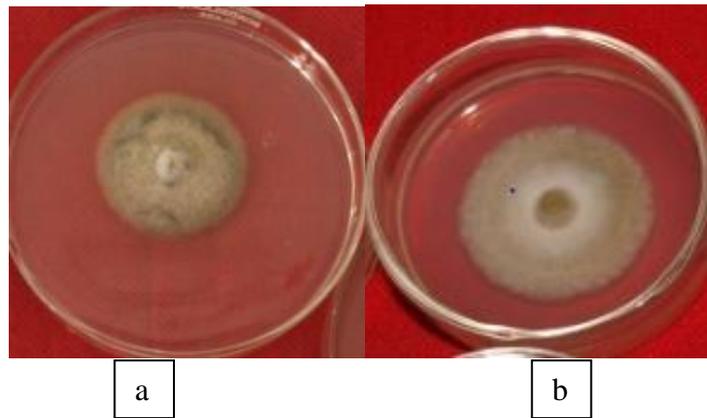


Plate.3 Growth pattern and texture of (a.) Isolate TC-H6 (b.) Isolate TC-R16

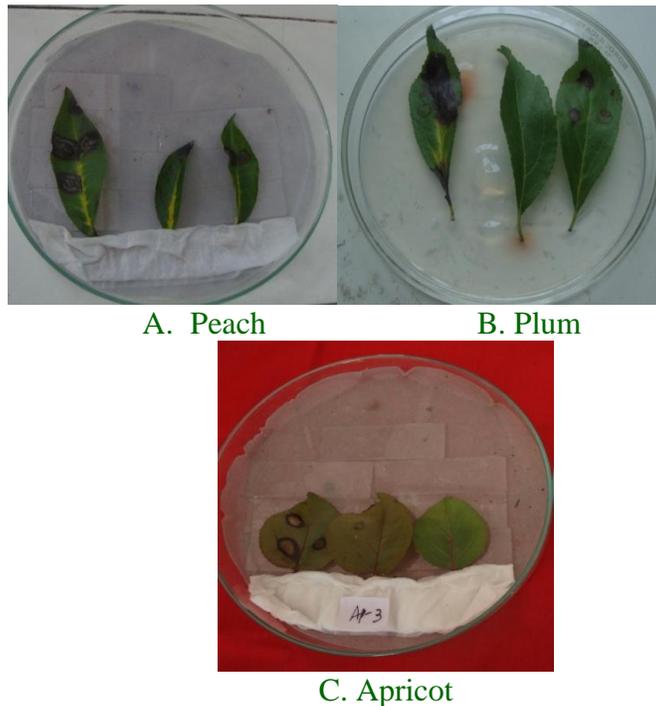


Plate.4 Cross infectivity of isolate TC-S1 on (a.) Peach (Host) (b.) Plum (c.) Apricot

Cross-infectivity

Cross infectivity of the isolates was carried out by cross inoculation of isolates on other stone fruit trees. The isolates obtained from peach host were able to infect plum and apricot (Plate 4). The isolate obtained from plum (TC-S3, TC-H3, TC-K3 and TC-R3) were able to infect cherry while as the isolates derived from cherry plant were able to infect apricot leaves. However, isolates obtained from apricot and almond was unable to infect other hosts. Raabe (1959) successfully proved pathogenicity of *Wilsonomyces carpophilus* isolates from peach, almond and catalina cherry by cross inoculations on each host. Similarly, Ahmad (1994) reported cross infectivity of four isolates of *W. Carpophilus* on different stone fruits by using detached leaf technique.

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