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**

stone fruits
Incubation
isolates
diverse pathogen

**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 Anantnagand 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 Hawer’s (AH) media and incubated at 24±1°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 30µl of spore suspension. Inoculum concentration was adjusted to 10⁴ 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 gloeosporioides* 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   | Kherburgh| 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±10°C

| Isolates | Texture       | Colony Characters                                      | 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   | Velvetty       | 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

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.

References

Adaskaveg, J.E., Ogawa, J.M. and Butler, E. E. 1990. Morphology and ontogeny of conidia in Wilsonomyces carpophilus, gen. nov., and comb. nov., causal pathogen of shot hole disease ofPrunus species. Mycologist37: 275–290.

Ahmad, S. 1994. 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.

Azza, Azmy, M.K. and Korra, A.K.M. 2010. Management of Shot-Hole Disease of Stone Fruit Trees Caused By Stigmina carpophila. Journal of Plant Protection and Pathology 12: 973-989.

Dar, G.N. and Teng, R.K. 1979. Proceedings of first symposium on possible improvement in temperate fruit culture J&K State Srinagar, 13th September, pp. 14-16.

Grove, G.G. 2002. Influence of temperature and wetness period on infection of cherry and peach foliage by Wilsonomyces carpophilus. Canadian Journal of Plant Pathology 24: 40-45.

Hightberg, L.M. and Ogawa, J.M. 1986. Survival of shot hole inoculum in association with dormant almond buds. Plant Disease 70: 828–831.

Nabi, A., Shah, M.D., Padder, B.A., Dar, M.S., Bharti, V., Ahmad, M. and Sofi, S. 2018. Morphological characterisation and media preferences in Wilsonomyces carpophilus, the causal agent of shot hole disease of stone fruits in Kashmir. Journal of Pharmacognocy and Phytochemistry 7: 1326-1331.

Ogawa, J.M., and English, H. 1991. Diseases of temperate zone fruit and nut crops. University of California, Division of Agriculture and Natural Resources. 123 pp.

Raabe, R.D. 1959. Clasterosporium shot hole of catalina cherry. Phytopathology 49: 116.

Shaw, D.A., Adaskaveg, J.E. and Ogaw, J.M. 1990. Influence of wetness duration and temperature on infection of almond and development of shot hole disease of almond caused by Wilsonomyces carpophilus. Phytopathology. 80: 749–756.

Smith, C.O. and Smith, D.J. 1942. Host range and growth temperature relations of Coryneum beijerinckii. Hytopathology. 32: 221–225.

Sukumar, J. and Ramalingam, A. 1981. Detached leaf technique to study leaf spot and other foliar diseases of mulberry. Indian Phytopathology 34: 110-111

Torres-Calzada, C., Tapia-Tussell, R., Higuera-Ciapara, I. and Perez-Brito, D. 2013. Morphological, pathological and genetic diversity of Colletotrichum
species responsible for anthracnose in papaya (Carica papaya L). European Journal of Plant Pathology 135:67-79.

Tuite, J. 1969. Plant Pathologist Methods, Fungi and Bacteria. Burgess Publishing, Minnea Polis, pp. 239.

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