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Pathological diversity in *Wilsonomyces carpophilus* isolates on five different *Prunus* hosts

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Abstract
Wilsonomyces carpophilus is a necrotrophic plant pathogenic fungus that infects all the stone fruits including almonds among nut crops. Necrotrophic pathogens are developed in such a way that they kill their host swiftly to feed themselves and complete their lifecycle. Therefore to understand pathogenicity mechanism and necrotrophic life style of the fungus, we studied the pathogen on morphological and pathological grounds. A total of fifty isolates were tested for morphological and pathological characteristics. The pathogen isolated from different hosts showed difference in their morphological characters, however analysis of variance in pathogenicity test showed non-significant results.

Keywords: Pathological diversity, *Wilsonomyces carpophilus*, *Prunus*, lifecycle

Introduction
The stone fruits that includes peach, plum, cherry, apricot, nectarine and almond are important crops grown throughout the world and foremost growing countries are America, Australia, Afghanistan, China, Iran, Italy, Greece, France, New Zealand, Portugal, India and Central Asian countries of earlier USSR (Nabi *et al*. 2018) [5]. A number of biotic factors affect stone fruits among which shot hole disease caused by *Wilsonomyces carpophilus* is of paramount importance (Bird *et al*. 1995) [2]. The fungus was initially identified as *Clasterosporium carpophilum* however, the further studies identified it as *Stigmina carpophila* (Ellis 1959) [3]. Number of other synonyms such as *C. carpophila*, *Stigmina carpophila*, *Thyrostroma carpophilum* and *Wilsonomyces carpophilus* also exists for the pathogen. The disease is reported from Africa, Asia, America (North, South, and Central), Australia and Oceania (Väcäroju *et al*. 2008). Being a necrotroph it causes shot hole disease in all stone fruits worldwide (Adaskaveg *et al*. 1990) [1]. The intermittent outbreak of the disease results in 30 to 90% yield loss in cherry and in apricot, about 60.3% crop loss is recorded in Malatya province of Turkey (Nabi *et al*. 2018) [5]. The disease appear as small circular purple lesion with pale centre that gradually enlarges and become necrotic at the centre that ultimately fall down leaving a shot hole appearance (Shukla *et al*. 1984) [6]. Regardless of the enormous damage caused by the pathogen hardly any study exists in the literature. Therefore we studied the pathogen on morphological and pathological basis. Our aim was to deduce the difference in pathogenicity and morphology of the pathogen.

Materials and methods
Assessment of Morphological characteristics
Fifty isolates from five host species (*Prunus persica, Prunus domestica, Prunus armeniaca, Prunus avium and Prunus dulcis*) were used for assessment of morphological differences between isolates of different host species. The growth pattern, colony colour and texture of different host isolates was recorded. The cultures incubated at 24±1ºC under the 12 hour photoperiod were evaluated every seven days after inoculation. Conidial characteristics such as shape, size, colour and septation of different isolates were assessed on Asthana and Hawker's medium.

Pathogenicity test
Pathogenic variability of different isolates was studied by inoculating each isolate on their respective hosts and on other host species following detached leaf technique (Sukumar and Ramalingam 1981) [7]. Healthy leaves collected were thoroughly washed with sterilized water.

Sterilized moist chamber was formed for pathogenicity test, blotting papers were placed in Petri plates of 200 x 200 mm size along with sterilized glass slides. The leaves to be inoculated were placed on these glass slides to prevent any direct contact with moist blotting paper. Moist cotton was placed on petioles of leaves to maintain turgidity. Inoculations were carried out with spore (conidia) suspensions containing 10^5 spores/ml by drop placement method (Kanchana-Udomkan *et al*. 2004) [4] with help of 20µl micropipette. In this method, uniform sized drops of spore suspension were placed with the help of micropipette on upper and lower surfaces of the leaves with- and without- injury and control (check) was maintained by placing sterilized water drops on leaves instead of spore suspension. The study was carried out under aseptic conditions. The moist chambers were then incubated at 24±1 ºC with 12 hour photoperiod. Observations in terms of symptom development (incubation period) and lesion size (after one week) by different isolates were recorded and compared. The one way ANOVA (Analysis of variance) was used to determine pathological variability between the isolates of five hosts.

Results and Discussion

The fifty isolates raised on PDA medium showed significant difference in their growth pattern, colony colour, colony texture. Most of the isolates raised on PDA medium showed brownish coloration, however some isolates from cherry peach plum also showed dull white, grayish borders with green centres and white coloration. There was a significant difference between the growth pattern of pathogen isolated from different hosts some showed fluffy colonies and most of the isolates showed velvety growth pattern, The results showed coordination with previously reported researches (Ye *et al*. 2020) [9]. The pathogen produces light brown conidia with three to five septa. The shape of conidia is oval in latter stage, however aseptate circular initially. The average conidial size ranges from 16.54-39.20 x 6.90-14.98 µm.

On the basis of pathogenicity test we assessed pathological variability between the isolates of five different hosts, Incubation time varied from four to seven days for the development of disease symptoms. The lesions developed were brown with circular to slightly irregular shape. The *Prunus persica* isolates showed incubation period of 4, 4, 5, 5 and 4 days on Peach, Plum, Apricot, Sweet cherry and Almond hosts respectively. The other isolates showed same incubation period with some slight changes like cherry isolates usually took more time for disease development followed by almond. The incubation period of peach, plum and apricot were almost same (Nabi *et al*. 2018) [5]. We found probability value of 0.23 (p> 0.23) that indicates no significant difference in pathogenicity of different isolates of *W. carpophilus*.

Conclusion

The pathogen isolated from five different hosts showed difference in their morphological characters. There growth pattern, colony colour, conidial size showed considerable variation. However, the incubation period of the isolates varied with the hosts, though the one way ANOVA suggests there is no pathological variability between the isolates.

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