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In-vitro evaluation of aggressiveness and chlorothalonil sensitivity in *Taphrina deformans* isolates from peach leaf curl disease in Swat

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Abstract

Peach (*Prunus persica* L.), a significant fruit crop in Swat, is frequently afflicted by peach leaf curl, a disease caused by the fungus *Taphrina deformans*. This study evaluates the in-vitro responses of six isolates of *T. deformans* to normal and chemical stress conditions. Isolates were cultured on Yeast Extract Peptone Dextrose Agar (YEPDA), both unamended and amended with 500 mg/L Chlorothalonil, with three replicates per condition. The colony diameter of each isolate was measured bi-weekly for two weeks. Disease incidence in peach orchards varied significantly across districts, with the highest recorded in Asharay (91.53%) and the lowest in Matta (21.34%). In unamended medium, Isolate Td5 (Matta) exhibited the highest aggressiveness, followed by Td3 (Null), while Td2 (Baidara) was the least aggressive. In the amended medium, Isolate Td6 (Bodigram) was the most aggressive, with Td5 (Matta) also showing high aggressiveness, and Td3 (Null) was the least aggressive. Sensitivity to Chlorothalonil varied among isolates; Td4 (Sambat) and Td3 (Null) were more sensitive, whereas Td6 (Bodigram) was the least sensitive. These findings highlight the variability in aggressiveness and fungicide sensitivity among *T. deformans* isolates, underscoring the need for tailored management strategies, including the strategic application of Chlorothalonil, to effectively control peach leaf curl in affected regions.

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Introduction

Peach (*Prunus persica* L.), a member of the Rosaceae family, originated in Persia (Mustapha *et al.,* 2024; Celiński *et al.,* 2024). Cultivated by the Romans during the time of Christ, peaches were disseminated throughout Europe as the Roman Empire expanded (Yancovic-Pakarati *et al.,* 2024). Today, peaches are widely grown in temperate regions globally. Since the 1980s, peach and nectarine production has surged, increasing from an average of 9,323 thousand tons (1989-1991) to 11,065 thousand tons in 1998 (Karim *et al.,* 2024). The Mediterranean region, particularly Italy, Greece, Spain, France, and Turkey, is a major production center, with Europe contributing nearly half of the global output (4,500 thousand tons). Spain and Italy are prominent producers in the European Union, while North America produces about 1,600 thousand tons. South American countries like Argentina, Brazil, and Chile show notable production growth, though they still lag behind Mediterranean nations (Anonymous, 1998).

In Pakistan, during 2010-2011, peach production reached 52,600 tons from 15,200 hectares. Cultivation areas include 100 hectares in Punjab, 5,600 hectares in Khyber Pakhtunkhwa, and 9,500 hectares in Baluchistan, yielding 500, 57,800, and 25,400 tons respectively. Nutritionally, peaches offer 39 kcal energy, 9.45 g carbohydrates, 0.91 g protein, 0.25 g fats, 1.5 g dietary fiber, and a range of vitamins and minerals including 326 IU Vitamin A, 6.6 mg Vitamin C, and 190 mg potassium (USDA National Nutrient Database). These nutrients help counteract oxidative stress and contribute to overall health. Peach productivity is challenged by various biotic and abiotic stresses, including pests and diseases. Major pests include the San Jose scale, peach tree borers, plum curculio, and peach twig borer. Diseases such as peach leaf curl, scab, brown rot, and bacterial spot also impact yields (Jim *et al.,* 2001). Peach leaf curl, caused by the fungus *Taphrina deformans*, is prevalent in Khyber Pakhtunkhwa, leading to reduced tree vigor, fruit quality, and yield. Recent increases in disease reports may be due to mild winters, which favor the fungus's survival (Ingham *et al.,* 2010). Symptoms include distorted, puckered, and reddish to purple leaves, stunted shoots, and reduced fruit set (Ogawa *et al.,* 1995).

The fungus overwinters in bark and bud scales, with infection occurring in spring under mild temperatures (50-70°F) and wet conditions. The disease disrupts normal plant cell development, causing secondary infections if weather conditions remain favorable (McManus and Hudelson, 2004). While peach leaf curl alone rarely kills trees, significant leaf drop can lead to drought stress and weather damage. To maintain tree health, proper irrigation, timely nitrogen application, and pruning are essential. Late summer fertilization should be avoided to ensure proper winter hardening (Moller *et al.,* 1979). As no peach varieties are immune to peach leaf curl, resistant varieties derived from Red Haven are preferred over those from Redskin. Effective management includes a single fungicide application in fall or early spring, with active ingredients such as chlorothalonil, copper (e.g., Bordeaux mixture), and ferbam (McCain *et al.,* 1979). Given the severity of peach leaf curl, this study aims to assess the incidence of the disease and conduct an in-vitro comparison of different Taphrina deformans isolates under normal and chemical stress conditions.

Materials and methods

Survey and sample collection

In 2016, a survey was conducted across six different localities in the district of Swat (Table 1) to assess the incidence of peach leaf curl disease and to collect leaf samples for isolating *Taphrina deformans* (Fig. 1). Disease incidence (DI) was determined by calculating the percentage of infected plants out of the total number observed in each peach orchard. Leaf samples exhibiting symptoms of peach leaf curl were collected in plastic bags and transported to the Plant Pathology Laboratory at The University of Agriculture, Peshawar, Pakistan. Upon arrival, the samples were stored at 4°C until further processing.

Table 1. *Taphrina deformans* isolated from different localities of Tehsil Matta District Swat

Isolate number	Location
1	Asharay
2	Baidara
3	Null
4	Sambat
5	Matta
6	Bodigram

Fig. 1. Leaf curl disease in peach plant

Growth media for Taphrina deformans and method of inoculation

Three types of media were utilized for culturing *Taphrina deformans*: Yeast Extract Peptone Dextrose (YEPD), Yeast Extract Peptone Dextrose Agar (YEPDA), and Yeast Dextrose Agar (YDA). These media were prepared according to standard procedures with the following compositions expressed in grams per liter: 1% yeast, 2% peptone, 2% dextrose, and 2% agar. Peach leaf samples were cut into approximately 1 cm² pieces and surface sterilized by immersing them in a 0.1% mercuric chloride solution for 15-30 seconds. After sterilization, the samples were rinsed twice in sterilized distilled water for one minute each. The leaf pieces were then blotted dry using sterile tissue paper and aseptically transferred to Petri dishes containing the prepared media, with three pieces per dish. The Petri dishes were sealed, labeled, and incubated at 25°C for two weeks. To ensure the removal of residual chemicals, temporary slides of the leaf tissue were also prepared for examination (Fig. 2).

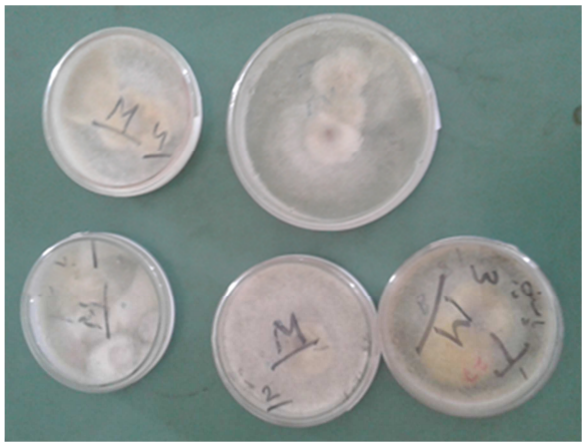

Fig. 2. Growth media for *Taphrina deformans* and method of inoculation

Comparison of *Taphrina deformans isolates*

Six isolates of *Taphrina deformans* were cultured on fresh Yeast Extract Peptone Dextrose Agar (YEPDA) medium, both unamended and amended with 500 mg/L Chlorothalonil, just before pouring into Petri dishes. Each isolate was tested on both media types in triplicate using a Completely Randomized Design (CRD). The Petri dishes were sealed with parafilm, labeled, and incubated at 25°C for two weeks. Colony diameters of *T. deformans* were measured weekly along two perpendicular lines, and the average diameter was calculated. The growth of the fungus was monitored regularly. The best growth was observed on YEPDA, while the YDA and YEPD media were discarded. Pure cultures from each locality were prepared on six separate plates. Temporary slides were also prepared under aseptic conditions to confirm the pathogen.

Disease incidence (%) of peach leaf curl

Disease incidence was calculated using the following formula:

DI=Total number of plants/Number of infected plant s ×100

Data analysis

The data were analyzed using the Analysis of Variance (ANOVA) technique appropriate for a CRD two-factor factorial experiment. Statistical analysis was performed using Statistix 8.1, with the least significant difference (LSD) calculated at a 5% probability level (Jan *et al.,* 2009).

Results

Percent disease incidence of peach leaf curl

The maximum disease incidence of 91.53 % was recorded at Asharay followed by Baidara 87.50% while the minimum DI of 21.34 % was registered at Matta (Table 2).

Table 2. Percentage incidence of peach leaf curl disease at different locations of Tehsil Matta district Swat

Isolate number	Location	Disease incidence (%)
1	Asharay	91.53
2	Baidara	87.50
3	Null	74.87
4	Sambat	78.92
5	Matta	21.34
6	Bodigram	51.43

Comparison of Taphrina deformans isolates

Table 3 presents the mean colony diameter of *Taphrina deformans* after one week of incubation. Among the six isolates tested, isolate 5 exhibited the most vigorous growth, with a colony diameter of 5.82 cm, which was statistically comparable to isolate 6, which had a diameter of 5.42 cm. In contrast, isolate 2 showed the smallest colony diameter of 2.67 cm. regarding the growth media, the largest colony diameter (6.32 cm) was observed on the unamended medium, while the medium amended with 500 mg/L Chlorothalonil resulted in the smallest colony diameter of 2.21 cm. The interaction between isolates and media had a significant impact on colony diameter (Table 3). The largest diameter (8.50 cm) was recorded for isolate 5 on the unamended medium after one week. Conversely, the smallest diameter (0.83 cm) was observed for isolate 3 on the medium amended with Chlorothalonil, which was statistically similar to the 0.93 cm diameter measured for isolate 4 on the same amended medium (Fig. 3).

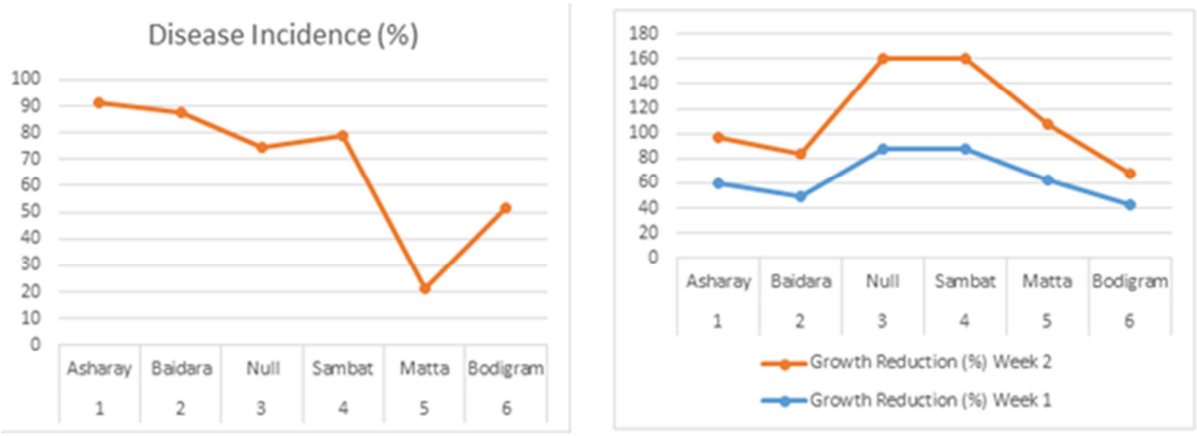

Fig. 3. The mean colony diameter of *Taphrina deformans* isolates after one week of incubation, showcasing the effect of different growth media and fungicide treatment

Table 3. Colony diameter of *Taphrina deformans* after one week of inoculation

Isolates	Media		Mean
	Amended media	Unamended media	
1	2.08 ef	5.13 cd	3.60 bc
2	1.80 ef	3.55 de	2.67 c
3	0.83 f	6.58 abc	3.70 bc
4	0.93 f	7.83 ab	4.38 ab
5	3.15 de	8.50 a	5.82 a
6	4.50 cd	6.33 bc	5.42 a
Mean	2.21 b	6.32 a	

CV= 2.064

LSD $_{(0.05)}$ value for isolates = 1.5098

LSD $_{(0.05)}$ value for Media = 0.8717

LSD $_{(0.05)}$ value for Isolates*Media = 2.1352

Means followed by different letters are significantly different using LSD test at 5% significance level.

Colony diameter (cm) of Taphrina deformans after two weeks of inoculation

The mean data pertaining colony diameter of *Taphrina deformans* after two weeks of inoculation is presented in Table 4. Among the six-isolate tested, isolate 6 was found to be the most vigorous with a

colony growth of 6.94 cm. The minimum colony diameter recorded in isolate 2 was 3.24 cm which is statistically similar to the colony diameter (3.69 cm) of isolate 4. In case of media used the maximum colony diameter (7.02 cm) after two weeks of inoculation was noted in the unamended media while the media amended with fungicide gives the minimum colony diameter of 3.24 cm. As regarding the interaction between isolates and media the maximum colony diameter (8.78 cm) was noted for isolate 5 in unamended media after two week of inoculation while the lowest (1.13cm) colony diameter at the second week of inoculation was measured for isolate 2 in media amended with fungicide chlorothalonil, which is statistically at par with isolate 4 in amended media (1.58 cm) (Table 4).

Table 4. Colony diameter of *Taphrina deformans* after two week of inoculation

Isolates	Media		Mean
	Amended media	Un-amended media	
1	3.70 def	5.92 abcd	4.81 bc
2	1.13 f	5.35 cd	3.24 c
3	2.21 ef	8.37 ab	5.29 abc
4	1.58 f	5.80 bcd	3.69 c
5	4.88 de	8.78 a	6.83 ab
6	5.95 abcd	7.93 abc	6.94 a
Mean	3.24 b	7.025 a	

CV= 2.064

$LSD_{(0.05)}$ value for isolates = 2.0602

$LSD_{(0.05)}$ value for Media = 1.1894

$LSD_{(0.05)}$ value for Isolates*Media = 2.9135

Means followed by different letters are significantly different using LSD test at 5% significance level

Discussion

Peach (*Prunus persica*), a key stone fruit, is primarily temperate in nature and is believed to have originated in China, with further development in Persia (Jahan and Khan, 2008). In Khyber Pakhtunkhwa, peaches and nectarines are among the top fruit crops produced. Peach leaf curl, a disease caused by the fungus *Taphrina deformans*, is prevalent on peach and nectarine trees throughout the region. Severe outbreaks of this disease can significantly diminish tree vigor, fruit quality, and overall yield. Recent reports indicate an increase in peach leaf curl cases, likely due to milder winters that have favored the survival and proliferation of the pathogen (Ingham *et al.,* 2010). In this study, six isolates of *Taphrina deformans* were purified and cultured on both amended and unamended media. On unamended media, the colony diameter of the pathogen ranged from 6.32 cm to 7.02 cm, indicating robust growth. In contrast, on amended media containing a fungicide, the colony diameter ranged from 2.21 cm to 3.24 cm, reflecting a significant reduction in growth. The mycelia of the fungus displayed a color gradient from white cottony to orange-yellow.

The observed reduction in fungal growth on amended media is attributed to the efficacy of the fungicide. Chlorothalonil, used at a concentration of 200 ppm, significantly inhibited fungal expansion. During the first week of incubation, chlorothalonil reduced fungal growth by up to 59.45%. However, in the second week, the growth reduction decreased to 37.29%. This decrease in effectiveness over time can be attributed to the fungal population's increase and potential development of resistance to the fungicide. As the pathogen population grows, its ability to withstand the fungicide improves, leading to diminished efficacy and increased fungal growth despite treatment. This underscores the need for integrated disease management strategies to effectively control peach leaf curl.

Conclusion

Peach leaf curl is widespread in the district of Swat, with the highest incidence observed at Asharay (91.53%), followed by Baidara (87.50%), and the lowest at Matta (21.34%). Among the isolates tested on unamended medium, Isolate Td5 (Matta) exhibited the highest aggressiveness, followed by Isolate Td3 (Null), while Isolate Td2 (Baidara) was the least aggressive. When grown on amended medium, Isolate Td6 (Bodigram) showed the greatest aggressiveness, with Isolate Td5 (Matta) also demonstrating significant aggression, whereas Isolate Td3 (Null) was the least aggressive. In terms of fungicide sensitivity, Isolate Td4 (Sambat) was the most sensitive to Chlorothalonil, followed by Isolate Td3 (Null), while Isolate Td6 (Bodigram) displayed the lowest sensitivity. These findings highlight the variability in aggressiveness and fungicide resistance among

different *Taphrina deformans* isolates. To effectively manage peach leaf curl disease, it is crucial to develop and implement targeted management strategies, including the use of Chlorothalonil fungicide.

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