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IMPACT OF *PHYTOPHTHORA* SPP. ON MORPHOLOGICAL, PHYSICAL, AND BIOCHEMICAL PARAMETERS OF *CITRUS RETICULATA*

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ABSTRACT

Citrus gummosis, caused by *Phytophthora spp.,* is one of the economically critical fungal diseases prevailing in the major citrus-growing areas of the country. The fungus induces alterations in morphological and physiological parameters (leaf area, fruit weight, fruit volume, fruit length, fruit diameter, peel thickness, peel weight, rag weight, and juice weight). Therefore, the study was conducted to analyze the physical and biochemical parameters of citrus plants of various *Citrus reticulata* infected with phytophthora gummosis. Citrus leaves and fruits were collected to determine different morphological, physical, and biochemical parameters to compare the diseased and healthy citrus samples. There was a significant difference ($P > 0.05$) in physical parameters between infected and healthy citrus samples. The fruit size was significantly lower within the range of 29.1-35.4 cm^2 in the diseased plants as compared to healthy ones (37.3-43.6 cm^2). The fruit volume of infected samples, with a maximum difference of 37.1 cm3, was also recorded in infected and healthy samples. The infected citrus samples had lower fruit weights of 111.3–145.2 g than the healthy samples (147.8–175.9 g). The leaf area was significantly less for diseased plants (11.8–20.0 cm2) compared to 17.1-29.5 cm^2 for healthy samples. Other physical parameters were also altered, but not significantly. Similarly, biochemical parameters such as TSS to Acid ratio 77.9–86.8, Total Soluble Solids 11.3–11.6%, Vitamin C 55.4–77.9 mg/100 mL, total phenolic contents 288.8–341.9, total flavonoids 210.1–240.2, antioxidant activity 655.7–749.5, and pH 3.13–3.32 were found to be significantly lowered at $P > 0.05$ in diseased citrus samples as compared to those in healthy citrus samples with the mean values of 101.3–109.5, 12.2–12.4%, 91.5–123.5 mg/100 mL, 3636.1-421.2, 249.7–285.6, 749.6–867.7, and 3.52–3.81, respectively. The findings of the research revealed the devastating impact of *Phytophthora* spp. on *C. reticulata*, resulting in low yield and fruit quality.

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INTRODUCTION

Citrus (*Citrus reticulata*) belongs to the family *Rutaceae* and is one of the most important fruit tree crops in tropical and subtropical areas of the world. The origin of citrus is believed to be in China and the eastern regions, viz., Thailand, Malaysia, and India (Iftikhar *et al.*, 2022). Currently, China is the top citrus-producing country,

with an annual production of 44.06 million tons (FAO, 2020). Major contributions to citrus production are from Brazil, Mexico, India, and Spain (Misachi, 2017). Citrus products and by-products have a great role in foreign revenue and increase income for the local citrus industry. There is a great role for citrus products and by-products in employment generation and an increase in income for the local citrus industry. Citrus exports also earn a lot of foreign exchange (Iftikhar *et al.*, 2009). Citrus fruits are highly nutritious, and aromatics have therapeutic values. It consists of vitamins, minerals, dietary fibers, phyto-phenolics like flavonoids, and phenolic acids, which provide various health benefits to humans when used as an anti-microbial, anti-tumor, and anti-inflammatory agent (Mahmoud *et al.*, 2019). Citrus is the best source of vitamin C, sugars, amino acids, and other nutrients. Anti-oxidants in citrus are vital in catalyzing our immune system to protect against life-threatening diseases (Rafiq *et al.*, 2018). Pakistan is blessed with soil and environmental conditions favorable for citrus growth. There was a decline in citrus production, and Pakistan was at the 16th rank by the end of 2020 with an annual production of 2.89 MMT (FAO, 2020). Different commercially grown Citrus cultivars in Pakistan are kinnow mandarin (*Citrus reticulata* Blanco.), Mosambi (*C. sinensis* (L) Osb.), grapefruit (*C. paradise* Macf.), lemon (*C. limon* (L) Burm), lime (*C. aurantifolia* (Christm) swingle.), and sweet lime (*C. limettioides* Tanaka). The Mandarin group (Kinnow and Feutrell's early) has a share of 80% of Pakistan's commercially available citrus cultivars (Iftikhar *et al.*, 2009). Citrus cultivation has been spread throughout the country, but over 95% of total citrus production is from Punjab province (Mubeen *et al.*, 2015b; Rasool *et al.*, 2020; Mubeen *et al.*, 2015a). In contrast to our climate and potential, the citrus production in Pakistan is significantly lower than in the major citrus-producing countries, including our neighbors China and India. The citrus production in Pakistan is estimated at 10–12 tons per hectare compared to other top citrus-producing countries, up to 26 tons per hectare (Iqbal and Kamal, 2014). This decline in citrus production is attributed to many biotic and abiotic factors. Wrong agronomic practices, faulty nursery management, harsh environmental conditions, and major insect pests and diseases are the major constraints in citrus production. Among the biotic factors, Citrus tristeza virus, Citrus greening disease, Citrus canker, and gummosis have been reported as major contributors to citrus decline (Iftikhar *et al.*, 2021; Sajid *et al.*, 2021; Mubeen *et al.*, 2024). Among the numerous fungal infections, soil-borne plant pathogens are threatening in a given location once they enter. Diseases in citrus like root rot, foot rot, crown rot, and gummosis are caused by *Phytophthora* spp. (Wagh *et al.*, 2018). Citrus gummosis due to *Phytophthora spp*. is one of the major constraints in citrus production. It is a soil-borne disease. Different species of *Phytophthora* have been reported in citrus-growing areas of the world and Pakistan as well (Asim *et al.*, 2018; Lad *et al.*, 2020). The commonly prevailing species in the citrus orchards are *P. citrophthora* and *P. nicotianae*. Gummosis by *Phytophthora* species has been identified as a significant blockade for citrus production, causing 10 to 30% of global citrus crop losses (Rajput *et al.*, 2020). *Phytophthora citrophthora* and *Phytophthora nicotianae,* var. *parasitica,* are widely distributed. Xylem vessels aid in the transportation of *P. citrophthora* to other plant parts (Das *et al.*, 2016). The fungus attacks the plant when soil is in contact with the scion (Timmer *et al.*, 2000). *Phytophthora* gummosis adversely impacts the physiological and biochemical processes in infected plants. Different types of symptoms include root discoloration, leaf yellowing, and the wilting of plants (Rehman *et al.*, 2022). Different biomolecules have vital roles in plant defense mechanisms. The biochemical pathway is disturbed by pathogen invasion. Phenolic compounds are vital in plant defense against pathogens (Roginsky and Lissi, 2005). Similarly, total soluble sugars are a structural component of plant growth (Loreti *et al.*, 2005). Mineral nutrients also play a role in plant defense. Chlorophyll and photosynthesis are affected by the attack of pathogens (Kobayashi and Masuda, 2019). *P. citrophthora* harms citrus plants' biochemical and mineral profiling under gummosis (Iftikhar *et al.*, 2022). Therefore, the study has been conducted to investigate the physiological and biochemical alterations in healthy and diseased citrus plants infected with *Phytophthora* gummosis.

MATERIALS AND METHODS

The following morphological, physical and biochemical parameters were determined;

Morphological and Physical Parameters

The following were the morphological and physical parameters for comparing gummosis-infected and healthy plants. Leaf area, fruit weight (g), fruit volume

(cm^3), fruit length (cm), fruit diameter (cm), peel thickness (cm), peel weight (g), rag weight (g), and juice weight (g) were taken as morphological parameters (Ahmed *et al.*, 2012).

Leaf area

A portable leaf area meter (Model LI 3100, LICOR, USA) was used to determine the leaf area meter. The leaf was placed inside the leaf area meter, and the leaf area was detected by the sensors.

Physical analysis of fruits

Fruits from gummosis-infected and healthy citrus plants were selected. The samples were washed thoroughly with distilled water to remove the dirt. Twenty fruits in one replicate from each plant sample were collected. The samples were replicated three times. Fruits were analyzed at the laboratory, Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan. A digital scale was used to measure the fruit weight (g). The fruit diameter, length, and peel thickness (cm) were measured through Vernier calipers. The displacement method was used to determine the fruit volume (cm3) (Ahmed *et al.*, 2012). Fruit peel weight and rag weight (gm) were measured on a digital scale.

Juice extraction

The juice was extracted manually and filtered through a muslin cloth. Juice weight was used for further analysis. The juice percentage was calculated using a formula (Kubar *et al.*, 2018).

$$Juice\ \% = \frac{Juice\ weight\ (g)}{Fruit\ weight\ (g)} \times 100$$

Biochemical Parameters

The biochemical parameters, such as total soluble solids, vitamin C, total phenolic compounds, total acidity, total flavonoids, antioxidant activity, and pH, were used to analyze the juice analysis from infected and healthy plant samples. The already established and recommended methods were being followed to determine the biochemical parameters.

Determination of pH

The pH of each juice sample was determined by a digital pH meter (HANA 8520, Japan). Citrus fruit juice (40 mL) was taken in a 100 mL beaker, and a pH meter was used to find the pH according to the method given in the official analysis methods (AOAC, 2006). All determinations were performed in triplicate on diseased and healthy plants.

Total titratable acidity content of juice

The total titratable acidity was expressed as anhydrous citric acid on a weight basis. The acidity was determined following the method described by the Hortwitz (1960) method. The titration method was used to determine the titratable acidity. The indicator (phenolphthalein) was used to check the pink color. The results were expressed as grams of citric acid per 100 mL of juice (AOAC, 2003).

$$Acidity\ \%\ (citric\ acid) = \frac{Titer\ value\ (mL)\ \times 0.068}{10\ \times 100}$$

Determination of total soluble solids (TSS = Brix)

The juice's total soluble solids (TSS) were determined through a refractometer in an equivalent solution. The determination of TSS/Brix was carried out using the standardized method as described in "Official Methods of Analysis, 13th Edition" (AOAC, 2003).

Determination of the TSS-acid ratio

The TSS-acid ratio is an arithmetical proportion of soluble solids to citric acid. It indicates the balance between TSS and acidity. The ratio was determined by dividing the TSS value by the total acidity percentage using the methods described by Lacey *et al.* (2009).

$$TSS{:}\ Acid\ Ratio = \frac{TSS\ (Brix)}{Acidity}$$

Determination of vitamin C

Ascorbic acid (mg/100 mL) was determined following the Ruck method as described by Nweze *et al.* (2015). An indicator (1%) of starch was prepared, mixed well, and allowed to cool before use. 5 g of potassium iodide and 0.268 g of potassium iodate were dissolved in 200 mL of distilled water. 3M sulfuric acid (30 mL) was added to a beaker, increasing the volume to 500 mL. The standard vitamin C solution was prepared in 100 mL of distilled water by adding 0.250 g of ascorbic acid and increasing the volume to 500 mL. Pipetting methods were used to standardize the iodine solution with vitamin C. A blue-black color was observed during the titration of the 1% starch solution against the iodine solution.

Measurement of vitamin C

The amount of vitamin C was determined in mg/100 mL following the method as described by Kaleem *et al.* (2015). Each sample of juice in a 25-mL aliquot was added into a 250-mL conical flask using a pipette. Distilled water (150 mL) and ten drops of a 1% starch indicator solution were added to the flask. The standardized iodine solution (0.00125 mol/L) was put

into a burette. Each sample was titrated with the iodine solution and identified as having a dark blue-black color. The titration was repeated with further aliquots of the sample solution.

$$Ascorbic\ acid\ (\frac{mg}{100}mL) = \frac{Conc.mg/mL\ standard}{Sample\ weight} \times 1000$$

Determination of total phenolic content

The total soluble phenolic compounds were estimated using the Folin-Ciocalteu reagent method, as described by Singleton *et al.* (1999). The standard of gallic acid was 0.1 g/100 mL of ethanol, and the results were expressed as mg GAE (gallic acid equivalents)/100 g FW. Where FW is the fresh weight of the sample. One mL of the sample was taken in the test tube, and the same volume of 10% Folin solution was added and left for 6 minutes. Similarly, 20% Na_2CO_3 (4 mL) was added and left for 60 minutes at 30 °C. Absorbance was taken at 760 nm.

Determination of total flavonoid content

Flavonoid contents were determined by the method described by Kim *et al.* (2002). Deionized water (12.5 mL) was added to a 2.5 mL sample and mixed with 750 µL of 5% sodium nitrite. 10% aluminum chloride (1500 µL) was added after 6 minutes. 1.0 mL of 1 M sodium hydroxide (1 M) of volume after 5 minutes. 2.5 mL of distilled water was added to make the final sample volume, and absorbance was measured at 415 nm. Catechin (in ethanol) was used as a standard, and the results were expressed as µg of (+)-catechin equivalent (CE) per g of sample.

Determination of total antioxidant activity

The antioxidant capacity was determined using the method described by Prieto *et al.* (1999). DPPH free radical scavenging activity was measured through the method reported by Yi *et al.* (2009). 4 mL of reagent solution was mixed with 0.4 mL of sample. After incubating for 95 min at 90 °C, the absorbance was measured at 695 nm. A blank solution with reagent and methanol was run, and absorbance was measured at the same wavelength. Ascorbic acid and Trolox were used to make a standard calibration curve, and the results were expressed as µg ascorbic acid equivalent (AAE)/g sample and µmol TE/g sample. All determinations were carried out in triplicate. 0.1 g of ascorbic acid was dissolved in 100 mL of methanol, and a standard solution was prepared from the stock solution.

RESULTS

Morphological and physical parameters

A significant difference was observed in fruit diameter (cm) collected from all tehsils except Bhalwal and Silanwali ($P > 0.05$). The mean fruit diameter was lower in the diseased plants than in the healthy plants in all locations, but the differences are not statistically significant in the case of Bhalwal and Silanwali ($P > 0.05$). The fruit size was significantly ($P< 0.05$) lower in the diseased plants (29.1-35.4 cm^2) than in the healthy plants (37.3-43.6 cm^2) in all locations. This indicates that the disease significantly negatively impacts fruit size. The fruit volume was also significantly lower in the diseased plants than in healthy plants in all locations except Bhalwal. The maximum difference (37.1 cm^3) in fruit volume collected from diseased and healthy plants was recorded from Bhera tehsil (Table 1). A significant difference was recorded in fruit weight collected from diseased and healthy plants in the case of all tehsils. The diseased plants had a lower weight of fruits than the healthy plants in all tehsils, with a range of 111.3–145.2 g for diseased plants compared to 147.8–175.9 g for healthy plants. The average values of juice percentage of diseased plants were generally lower than those of healthy plants in the case of Bhalwal and Kotmomin, while no significant difference in fruit juice percentage was recorded in Bhera, Sargodha, and Silanwali tehsils. The range of juice percentage for diseased plants was 50.0-53.1%, compared to 56.4-58.8% for healthy plants. The leaf area of diseased plants was significantly ($P< 0.001$) lower than that of healthy plants in all tehsils. The range of leaf area for diseased plants was 11.8–20.0 cm2, compared to 17.1–29.5 cm^2 for healthy plants. The greatest difference in leaf area was observed in Kotmomin, where the mean value of diseased plants was only about a quarter of that of healthy plants (Table 1). No significant difference ($P > 0.05$) in peel thickness was found in all tehsils except Kotmomin, where peel thickness was significantly lower (0.26 cm) in diseased fruits in comparison to healthy fruits (0.30 cm). The peel weight was slightly lower for diseased fruits than for healthy fruits collected from Bhalwal, Kotmomin, and Silanwali. There was no significant difference ($P< 0.05$) in the fruit rag weight of diseased and healthy fruits in the case of all tehsils (Table 1).

Table 1. Morphological and physical parameters (means±SE) of kinnow collected from diseased (*Phytophthora citrophthora*) and healthy plants from five tehsils of Sargodha district.

Parameters	Tehsil	Bhalwal	Bhera	Kotmomin	Sargodha	Silanwali
Fruit diameter (cm)	Diseased	6.62±0.17	6.26±0.06	5.78±0.17	6.48±0.09	6.16±0.24
	Healthy	7.38±0.30	6.68±0.15	6.80±0.09	7.34±0.22	6.77±0.14
	t-test	-2.24^{ns}	-2.66**	-5.31***	-3.60**	-2.20^{ns}
Fruit size (cm^2)	Diseased	34.8±1.50	32.0±0.52	29.1±0.96	35.4±0.62	32.3±1.50
	Healthy	43.6±2.00	37.3±1.30	37.9±1.00	41.7±1.20	38.6±1.10
	t-test	-3.54**	-3.86**	-6.27***	-4.63**	-3.42**
Fruit volume (cm^3)	Diseased	152.3±9.80	119.7±4.10	130.9±7.10	152.4±4.60	140.4±4.10
	Healthy	180.7±9.60	156.8±11.0	156.8±2.50	171.1±6.30	157.5±5.90
	t-test	-2.07^{ns}	-3.19**	-3.45**	-2.40**	-2.38**
Fruit weight (g)	Diseased	140.7±8.30	111.3±4.20	118.2±2.10	145.2±4.40	129.0±2.80
	Healthy	175.9±11.0	147.8±9.50	145.8±4.40	166.8±5.30	151.7±5.80
	t-test	-2.49**	-3.51**	-5.68***	-3.13**	-3.51**
Juice percentage (%)	Diseased	50.3±1.10	50.0±2.0	52.6±1.10	53.1±1.50	53.1±1.40
	Healthy	56.8±2.20	56.9±4.30	56.4±1.20	58.8±3.2	56.9±1.20
	t-test	-2.57**	-1.48ns	-2.39**	-1.60ns	-2.03ns
Leaf area (cm^2)	Diseased	20.0±0.32	11.8±0.38	16.8±0.17	19.9±0.97	17.8±0.46
	Healthy	27.83±0.47	17.1±0.48	25.2±0.29	29.5±0.26	25.3±0.44
	t-test	-13.73***	-8.50***	-24.51***	-9.58***	-11.81***
Peel Thickness (cm)	Diseased	0.27±0.014	0.27±0.007	0.26±0.011	0.29±0.019	0.22±0.006
	Healthy	0.31±0.013	0.30±0.016	0.30±0.015	0.37±0.031	0.26±0.014
	t-test	-1.82ns	-1.73ns	-2.55**	-2.11ns	-2.13ns
Peel Weight (g)	Diseased	32.7±0.32	29.8±1.90	27.4±0.69	35.4±2.10	29.2±1.20
	Healthy	36.5±1.00	35.2±3.30	36.7±2.70	38.8±2.40	34.5±1.30
	t-test	-3.62**	-1.41ns	-3.34**	-1.04ns	-2.98**
Fruit Rag Weight (g)	Diseased	35.4±2.30	25.8±1.40	25.1±1.30	30.8±1.40	27.2±0.66
	Healthy	38.5±1.90	27.5±2.50	30.9±2.40	34.4±2.30	33.2±3.40
	t-test	-1.06ns	-0.57ns	-2.11ns	-1.35ns	-1.73ns

ns = non-significant, ** = P< 0.05, *** = P< 0.01

Biochemical Parameters

Looking at the TSS/Acid ratio, it can be observed that the mean values for healthy fruits were significantly (P<0.001) higher than those for diseased fruits in all tehsils. This suggests healthy fruits have a higher TSS/Acid ratio (101.3–109.5) than diseased fruits (77.9–86.8). Similar results were recorded for juice vitamin C and juice pH. The vitamin C content in the juice of healthy fruits was higher (91.5–123.5 mg/100 mL) than that of diseased fruits (55.4–77.9 mg/100 mL) in all tissues. In terms of juice pH, the mean values for healthy fruits were significantly (P< 0.01) higher than those for diseased fruits in all tehsils except Bhera (Table 2). Our findings showed significant differences between the diseased and healthy fruits regarding their titratable acidity, except in the case of Sargodha tehsil. However, a significant (P<0.05) difference in total antioxidant activity and total flavonoid content in the diseased and healthy fruits was observed in all tehsils. The diseased fruits had significantly lower antioxidant acidity (655.7–749.6) than healthy fruits (749.6–867.7) in all locations. Similarly, diseased fruits had significantly lower total flavonoid content (210.1–240.2) compared to healthy fruits (249.7–285.6) (Table 2). The mean total phenolic content was higher in healthy fruit samples (363.1–421.2) compared to diseased fruit samples (288.8–341.9) in all five tehsils, with a significant difference (p<0.01). This suggests that healthy fruit may contain higher levels of phenolic compounds, associated with various health benefits such as antioxidant and anti-inflammatory effects (Table 2). On the other hand, the mean total soluble solids (TSS) values were higher in healthy fruit

samples (12.2-12.4%) compared to diseased fruit samples (11.3-11.6%) in all five tehsils, with a significant difference ($p<0.01$). This suggests that healthy fruit may have a higher sugar content, as TSS measures the sugar concentration in fruit juices (Table 2).

Table 2. Biochemical parameters (means ± SE) of Kinnow collected from diseased *Phytophthora citrophthora* and healthy plants from five tehsils of Sargodha district.

Parameters	Tehsil	Bhalwal	Bhera	Kotmomin	Sargodha	Silanwali
TSS/Acid ratio	Diseased	86.7±1.30	86.8±2.0	77.9±0.64	86.2±2.60	78.6±1.50
	Healthy	109.5±0.70	104.1±1.90	105.2±2.30	101.3±4.00	104.9±3.40
	t-test	-15.4***	-6.22***	-11.3***	-3.19**	-7.04***
Juice Vitamin C (mg/100 mL)	Diseased	71.1±5.80	75.0±2.00	55.4±2.90	77.9±5.10	55.9±2.90
	Healthy	117.9±1.80	115.7±9.10	91.5±0.50	123.5±8.80	92.3±1.10
	t-test	-7.71***	-4.35**	-12.3***	-4.48**	-12.1***
Juice PH	Diseased	3.32±0.02	3.38±0.05	3.16±0.01	3.40±0.07	3.13±0.01
	Healthy	3.55±0.03	3.52±0.09	3.79±0.03	3.62±0.05	3.81±0.01
	t-test	-5.64***	-1.34ns	-16.7***	-2.49**	-32.1***
Titratable acidity (%)	Diseased	0.13±0.002	0.13±0.003	0.15±0.008	0.13±0.005	0.15±0.002
	Healthy	0.11±0.001	0.12±0.002	0.12±0.002	0.12±0.004	0.12±0.004
	t-test	6.63***	3.78**	9.8***	1.66ns	6.55***
Total antioxidant activity	Diseased	708.5±28.0	721.2±21.0	655.7±20.0	749.6±7.70	656.7±25.0
	Healthy	867.7±31.0	844.0±22.0	749.6±26.0	847.3±13.0	764.1±21.0
	t-test	-3.82**	-4.09**	-2.86**	-6.40***	-3.28**
Total flavonoid content	Diseased	214.7±6.40	210.1±11.0	211.5±3.30	240.2±8.00	217.4±6.90
	Healthy	264.3±5.10	249.7±5.70	256.4±11.0	285.6±11.0	260.8±7.600
	t-test	-6.02***	-3.23**	-3.80**	-3.35**	-4.23**
Total phenolic content	Diseased	326.7±5.90	288.8±16.0	330.7±7.50	341.9±6.80	329.3±7.70
	Healthy	421.2±2.10	388.7±7.80	374.0±5.60	415.0±14.0	363.1±4.80
	t-test	-14.5***	-5.62***	-4.64**	-4.75**	-3.70**
Total soluble solids (TSS=°Brix) (%)	Diseased	11.6±0.09	11.5±0.01	11.5±0.04	11.3±0.21	11.6±0.04
	Healthy	12.4±0.09	12.2±0.02	12.3±0.05	12.2±0.13	12.2±0.05
	t-test	-5.98***	-23.9***	-8.87***	-3.89**	-8.20***

ns = non-significant, ** = P< 0.05, *** = P< 0.01

DISCUSSION

The intensity of root rot in citrus was also recorded based on levels of visible symptoms that showed dulling, yellowing, and browning of leaves (Wagh *et al.*, 2018). Fruit morphological and physiological parameters, ultimately fruit production, are found to be significantly reduced on trees infected by this disease (Mekonen *et al.*, 2015). Due to the drastic effects of disease on plant vigor, less leaf area, low fruit weight, and low fruit volume were observed in diseased plants. Our study showed that the leaf area of diseased plants in all the tehsils was significantly less as compared to that of healthy plants. Similarly, fruit weight and volume were also decreased in the diseased plants of Bhera and Silanwali compared to healthy citrus samples. Fruit diameter was found to be minimal in diseased plants concerning their healthy plants`s fruits, so the same was found for fruit size. Lower fruit diameter, fruit weight, and peel thickness values in diseased plants are attributed to the fungal attack. Similarly, the proportion of fruit juice and juice percentage in diseased and healthy plants was also significantly different. Diseased plants had a lower proportion of fruit juice and juice percentage as compared to healthy ones at all the locations. All the morphological abnormalities are due to the blockage of the main vessels of the plant, which resulted in less sap transport, ultimately affecting plant growth, yield, and fruit quality. *Phytophthora* species have been reported to destroy the feeder roots and foliage of citrus plantations. Morphology and physiological processes have also been altered in

citrus under Phytophthora spp. attack (Badnakhe *et al.*, 2018). Leaf area and trunk size of the Citrus plants infected with Citrus gummosis caused by *P. citrophthora* were significantly less as compared to healthy Citrus plants in Sargodha, Punjab, Pakistan (Rehman *et al.*, 2022). Similarly, Citrus's decreased plant growth and root morphology were noticed during the infection with *Phytophthora* root rot (Tian *et al.*, 2018). Our results are in close conformity with the previously cited literature. There is scanty literature on the detailed morphological and physical parameters of citrus plants and fruits infected with *P. citrophthora*. However, alterations in morphological and biochemical parameters in citrus infected with citrus greening disease and citrus bent leaf viroid have also been observed (Sajid *et al.*, 2022; Bakhtawar *et al.*, 2022). Our results are from previous literature, but we have a novelty regarding the detailed physical parameters of Citrus infected with *P. citrophthora.* Biochemical parameters like the juice pH of diseased plants were more acidic as compared to the healthy ones. Juice acidity contents, TSS values, TSS/acid ratios, juice vitamin C content, total Phenols, flavonoids, and antioxidants were significantly lower in diseased plant samples than in samples from healthy plants. Citrus has many phenols and antioxidants to manage the adverse effects of plant diseases. Antioxidants play an important role in defense against pathogen attacks. The antioxidant enzymes activate the plant defense system against pathogenic attacks. The antioxidants also mitigate the harmful effects of ROS produced by different *Phytophthora* species (Lin *et al.*, 2014). Total soluble sugars were vital to plant growth (Loreti *et al.*, 2005). The sugars were also known to have a role in stimulating host defense mechanisms against fungal pathogens. The host metabolism is interrupted by the uptake of sugars and other metabolites in response to pathogen attack (Chen *et al.*, 2010). Our results were from the studies of Iftikhar *et al.* (2022). They also reported low values of total soluble phenols, total sugars, and antioxidants in diseased plants as compared to healthy ones, as our study reported the same. Flavonoid levels were also changed after the infection by *P. citrophthora* in citrus, causing brown rot in citrus (Agrios, 2005). The TSS/acid ratio was also significantly lower in Citrus samples infected with Citrus greening disease (Sajid *et al.*, 2022). Although scanty literature is available concerning biochemical changes in citrus plants infected with citrus gummosis caused by *P. citrophthora* globally and in Pakistan, Therefore, we have an opinion and conclusion that this fungus also has the same impact as other pathogens of citrus, like citrus greening bacterium (Sajid *et al.*, 2022) and Citrus viroid (Bakhtawar *et al.*, 2022). Although studies have been carried out on the physiological and biochemical alterations in the Citrus plants infected by different pathogens, few studies are available concerning the impact of *Phytophthora* species on Citrus physiology and biochemical profiling. Therefore, our results have novelty and new future research areas.

CONCLUSIONS AND FUTURE PROSPECTS

Based on the study, it is concluded that *Phytophthora* spp., the cause of citrus gummosis, significantly induced the morpho-chemical changes in the infected citrus samples as compared to healthy ones. Alteration in the morphology and physiology of infected citrus samples with *Phytophthora gummosis* leads to the expression of characteristic symptoms. Moreover, in the future, these alterations will need to be understood for the molecular basis of pathogenesis, which will also aid in the development of specific and sustainable control measures for *Phytophthora* spp., a cause of citrus gummosis. More varieties are needed to be screened to measure the resistance against *Phytophthora* spp.

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AUTHOR'S CONTRIBUTION

Shazia Hanif and Muhammad Asim performed the experiments. Shazia Hanif and Mustansar Mubeen: writing the original draft; writing; reviewing and editing; and analyzing the data. Abdul Ghani and Yasir Iftikhar; supervision; project administration; and resource acquisition. Muhammad Nadeem validated and finalized the manuscript. Malik Abdul Rehman: corrections and editing of the of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

CONFLICT OF INTEREST

The authors declare that the review was written without any commercial or financial relationships that could be construed as a potential conflict of interest.

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