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EFFECT OF GUMMOSIS CAUSED BY *PHYTOPHTHORA* SPP. ON LEAF AREA AND TRUNK SIZE OF CITRUS IN DISTRICT SARGODHA, PAKISTAN

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ABSTRACT

Gummosis disease of citrus, caused by *Phytophthora* spp. is one of the most devastating diseases of citrus in Pakistan. Considering the menace of this disease, the present study was conducted to determine the disease incidence and its effect on the trunk size and leaf area of affected plants. A comprehensive survey was conducted in 75 citrus orchards in 3 tehsils (Sargodha, Bhalwal, and Kot Momin) of the district Sargodha for the prevalence of the disease. The diseased plants were marked from each orchard, trunk size was measured and leaf area was recorded using leaf area meter. The leaves from diseased plants of tehsil Sargodha showed the highest leaf area with an average of about 2685 mm^2 followed by Bhalwal 2548 mm^2. There was a huge difference in the trunk size of healthy and diseased trees. The highest average healthy trunk size (102 cm) was recorded in Kot Momin while the diseased trunk in Bhalwal was found 9 cm in size. Healthy leaf area and trunk size were not significantly different from tehsil to tehsil, but diseased trees were significantly different from each other at $P<0.05$. Gummosis is a widespread disease in citrus growing areas and continues to gain importance as a constraint in citrus production. Therefore, concerted efforts should be implemented towards educating farmers and extension personnel about the disease's spread and management.

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INTRODUCTION

Citrus belongs to the *Rutaceae* family which has 140 genera and 1300 species worldwide (Savita and Nagpal, 2012). Citrus genera (*Eremocitrus*, *Fortunella*, *Microcitrus* and *Poncirus*) are widely distributed across the monsoon region from west Pakistan to northcentral China and south through the East Indian Archipelago, to New Guinea and the Bismarck Archipelago, northeastern Australia, New Caledonia, Melanesia and the western, Polynesian islands (Wu et al., 2018). Citrus is the world's most valuable fruit crop, with an estimated yield of 128 million tons. Pakistan has favorable environmental conditions for fruit production (Iftikhar et al., 2020). The Punjab province accounts for a significant share (more than 98%) of Pakistan's total citrus production and acreage (FAO, 2018). Although Pakistan is among the top 13th leading citrus producing countries, the production of citrus in Pakistan is lesser than rest of the world (Ahmad et al., 2004; Iqbal et al., 2004b; Iqbal et al., 2004a; Iqbal et al., 2005a). Citrus production in Pakistan has been affected by many biotic and abiotic factors (Fateh et al., 2017; Iqbal et al., 2007; Irshad et al., 2012; Mukhtar et al., 2007; Saeed et al., 2019). Among biotic factors, gummosis caused by *Phytophthora* spp. debilitates the citrus trees. The most prominent *Phytophthora* spp.

involved in gummosis disease are *Phytophthora parasitica, P. citrophthora, P. nicotianae* and *P. palmivora* (Gade and Koche, 2012).

The epidemiological studies of *Phytophthora* infections in citrus fields were helpful to understand the dispersion pattern of *Phytophthora* spp. from disease foci and the significance of inoculum sources. Hence, the development of forecasting models could be used to predict and control gummosis disease of citrus (Gade and Koche, 2012). High levels of humidity and temperatures encourage gummosis disease. During grafting, when scion comes in contact with soil or irrigation water, *Phytophthora* spp. attacks the citrus plant.

The symptoms start emerging after a few days, such as root discoloration, yellowing and wilting of young plants, reduced feeder roots, lesion formation and the emergence of gummy material from them (Rajput et al., 2015). Gummosis can be controlled by using a variety of disease management strategies, including those based on host-plant interventions such as rootstock resistance and grafting and cultural approaches like soil preparation, fertilization and irrigation and management of the soil as well as weed control. In addition, systemic fungicides have been used to control it; however, this management strategy has only been partially successful. Hence, gummosis is the major problem of the citrus industry in Pakistan. Limited surveys regarding this were carried out in Pakistan. Therefore, in the present study, three tehsils of Sargodha districts were surveyed to check the morphological characteristics of citrus plants infected with gummosis and to map out the disease incidence so that appropriate research may be executed to combat this menace.

MATERIALS AND METHODS

Survey and data collection

A survey of citrus orchards was conducted in different fields of 3 major tehsils (Sargodha, Bhalwal and Kot Momin) of district Sargodha. The survey was laid out in such a way that 5 villages were selected per tehsil, and 5 fields per village were surveyed to collect data regarding leaf area and trunk sizes of citrus trees affected with gummosis (Table 1, Figure 1).

The salient feature of citrus gummosis i.e. sap oozing from small cracks in the infected bark, giving the tree a bleeding appearance, affected root system and yellowing was used as morphological markers to identify the diseased tree.

Table 1. Surveyed areas of three tehsils of Sargodha.

Tehsil	Village
Sargodha	Chak 68 SB
	Chak 84 SB
	Chak 88 NB
	Chak 38 NB
	Jhal Chakkian
Bhalwal	Chak 4 SB
	Chak 9 SB
	Chak 7 NB
	Chak 21 NB
	Chak 15 NB
Kot Momin	Lilliani
	Chak 12 SB
	Chak 21 SB
	Sultan Pura
	Chak 19

Leaf samples of the diseased tree were collected in polythene bags and shifted to the lab, where leaf area was measured with Leaf Area Meter (CI-202 Portable Laser Area Meter). Data regarding disease incidence were recorded by following formula;

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

Statistical analysis

Collected data were then organized and reviewed to fulfil the research objectives. Statistical tools such as average, standard deviation and standard error were used to analyze the data. The data were analyzed with Satistix V. 10 and the threshold for significance was set at $P<0.05$. Means were separated using Fisher's protected least significant difference (LSD) procedure (Steel et al., 1997).

RESULTS

The morphological factors, such as trunk size and leaf area were found smaller in infected samples than healthy ones. The leaf size was found to be significantly different in healthy and diseased ones. The data recorded were significantly different from orchard to orchard in each tehsil but not significant among the tehsils because of the least data differences.

Leaf area affected by gummosis

In Tehsil Sargodha, the maximum leaf area of healthy leaves was recorded in Chak 88 NB which was 2931.4 mm^2 followed by Jhal Chakkian having leaf area of 2879.5 mm^2. The lowest leaf area of diseased leaves was

noted from Chak 84 SB (1377.28 mm²) followed by Chak 38 NB (1398.44 mm²). On the other hand, the highest leaf area of diseased leaves (1882.3 mm²) was recorded from Jhal Chakkian (Figure 2).

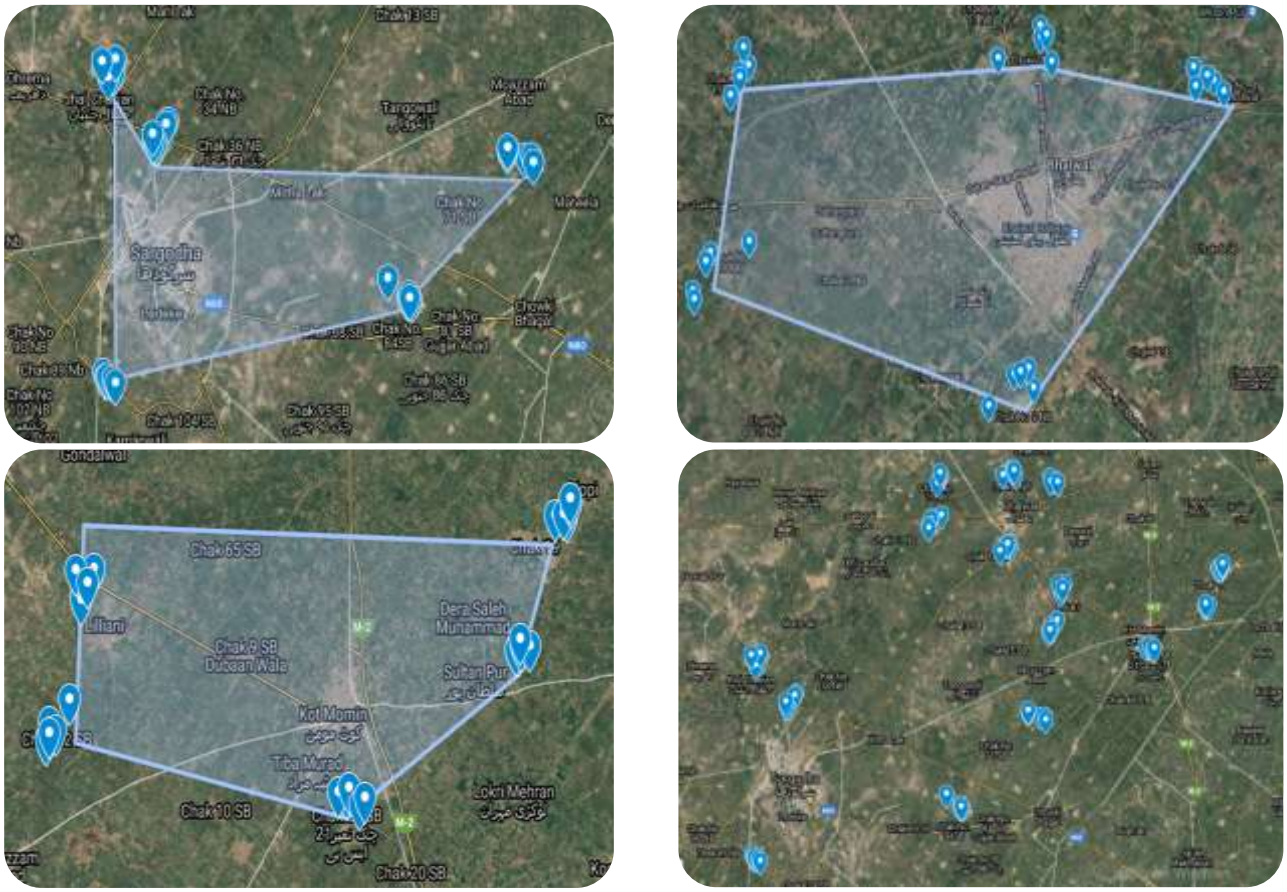

Figure 1. Polygonal view of surveyed citrus orchards of tehsil Sargodha, Bhalwal, Kot Momin and district Sargodha.

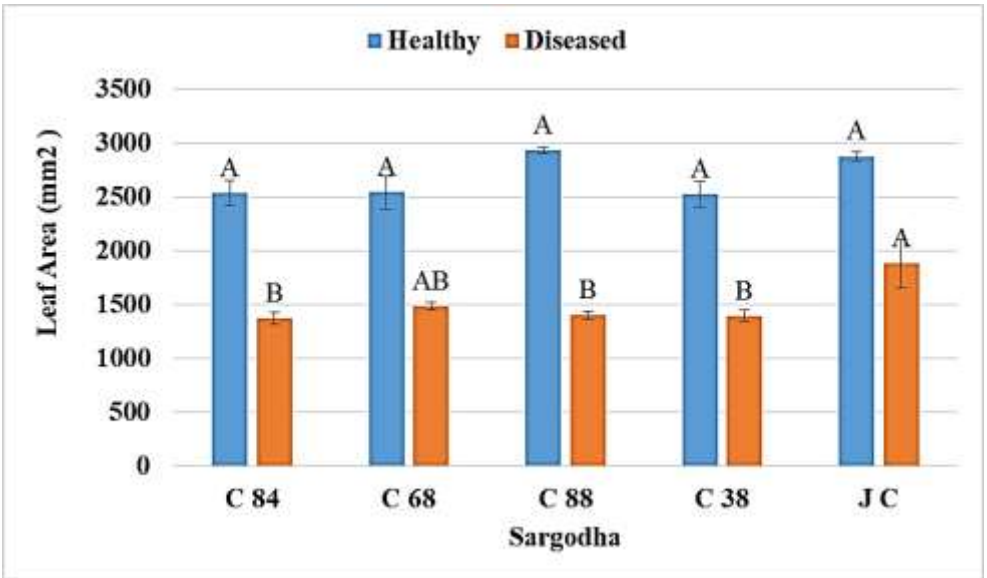

Figure 2. Leaf area of healthy and diseased citrus trees of tehsil Sargodha affected with *P. citrophthora.*

In Tehsil Bhalwal, the maximum leaf area of healthy leaves was recorded in Chak 12 NB which was 2712.22 mm^2 followed by Chak 7 NB, Chak 4 SB, Chak 9 SB and Chak 21 NB having leaf areas of 2673.24, 2628.18, 2409 and 2319.5 mm^2 respectively. The lowest leaf area of diseased leaves (1509.16 mm^2) was recorded from Chak 15 NB followed by Chak 21 NB (1569.16 mm^2). The highest leaf area of diseased leaves was recorded from Chak 4 SB (1882.3 mm^2) (Figure 3).

In Tehsil Kot Momin, the maximum leaf area of healthy leaves was recorded in Chak 19 which was 2684.84 mm^2 followed by Chak 21 SB, Laliani, Chak 12 SB and Sultan Pura having leaf areas of 2616.64, 2536.88, 2416.82 and 2086.86 mm^2 respectively. The lowest leaf area of diseased trees was recorded from Chak 21 SB (1152.6 mm^2) followed by Sultan Pura (1206.288 mm^2). The highest leaf area of diseased leaves was recorded from Chak 12 SB (1393.104 mm^2) (Figure 4).

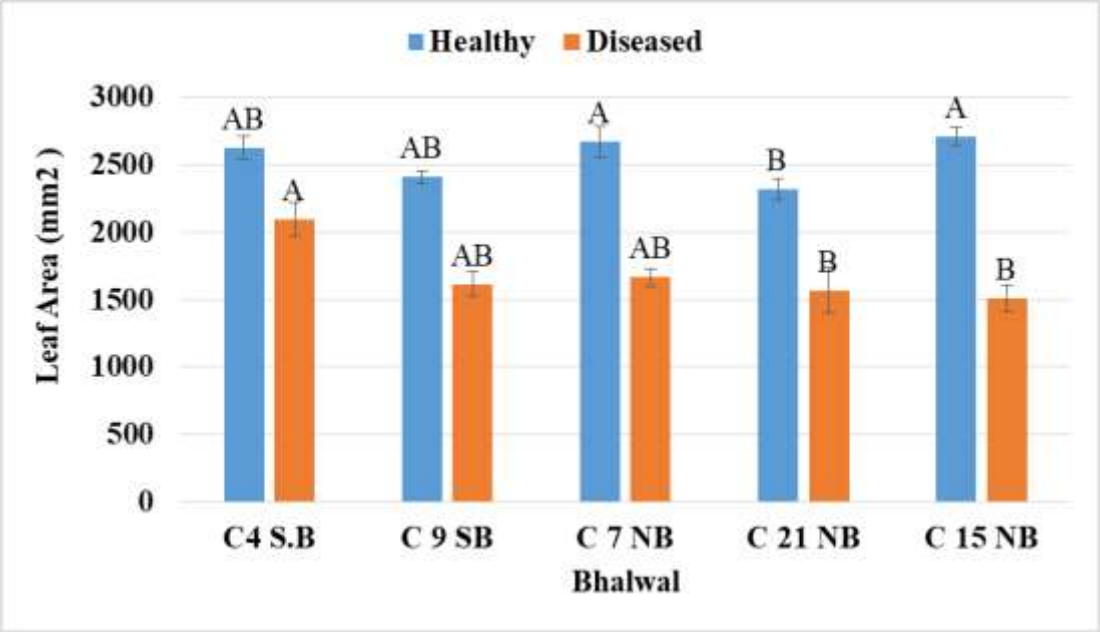

Figure 3. Leaf area of healthy and diseased citrus trees of tehsil Bhalwal affected with *P. citrophthora.*

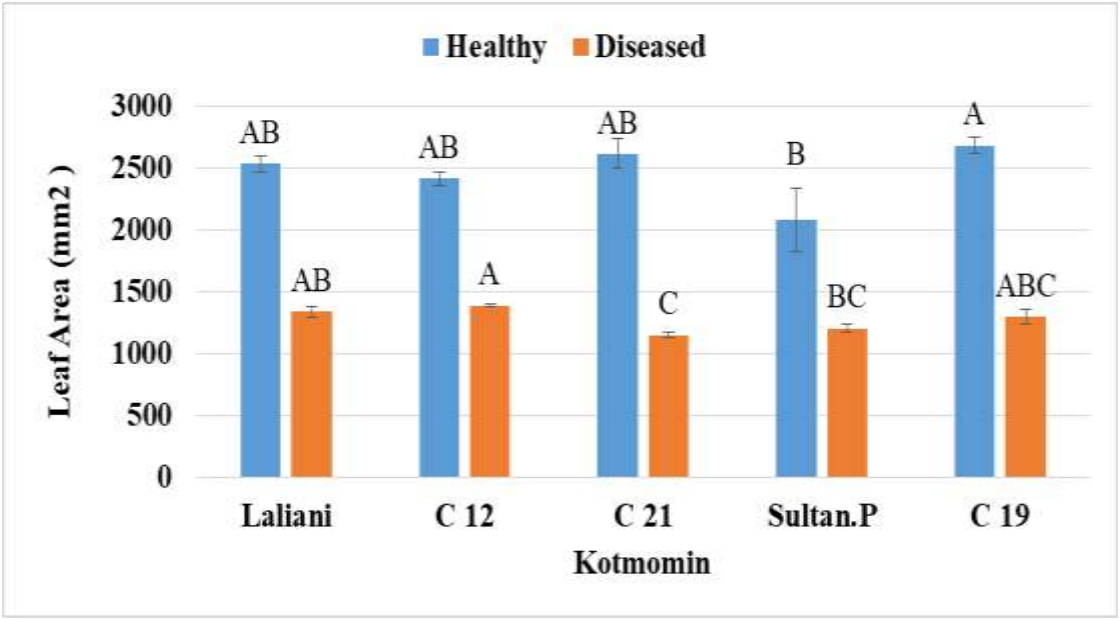

Figure 4. Leaf area of healthy and diseased citrus trees of tehsil Kot Momin affected with *P. citrophthora.*

After recording the whole readings of different orchards of villages, Tehsil Sargodha gave the highest leaf area in average of about 2685.368 mm² followed by Bhalwal 2548.428 mm². Kot Momin had least leaf area of diseased trees, having 1277.7836 mm² than Bhalwal and Sargodha. Healthy leaf area was not significantly different from tehsil to tehsil, but diseased trees are significantly different, having $P<0.05$ (Figure 5).

Trunk size affected by gummosis

The highest trunk size of healthy trees was recorded in Chak 88 NB of Tehsil Sargodha whose diameter was 106.6 cm and the least was recorded in Chak 68 SB and Jhal Chakkian, both having trunk size of 96.4 cm. Contrarily, in case of diseased trees, Chak 68 SB had the least trunk size of 83.8 cm while Chak 88 NB had the highest one of 90.8 cm (Figure 6).

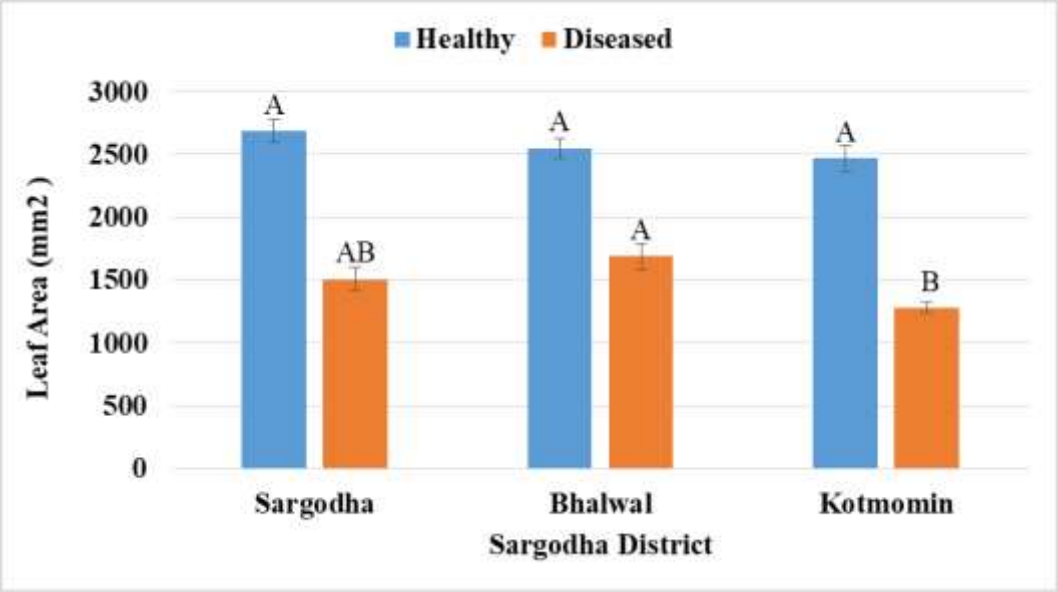

Figure 5. Leaf area of healthy and diseased citrus trees of district Sargodha affected with *P. citrophthora.*

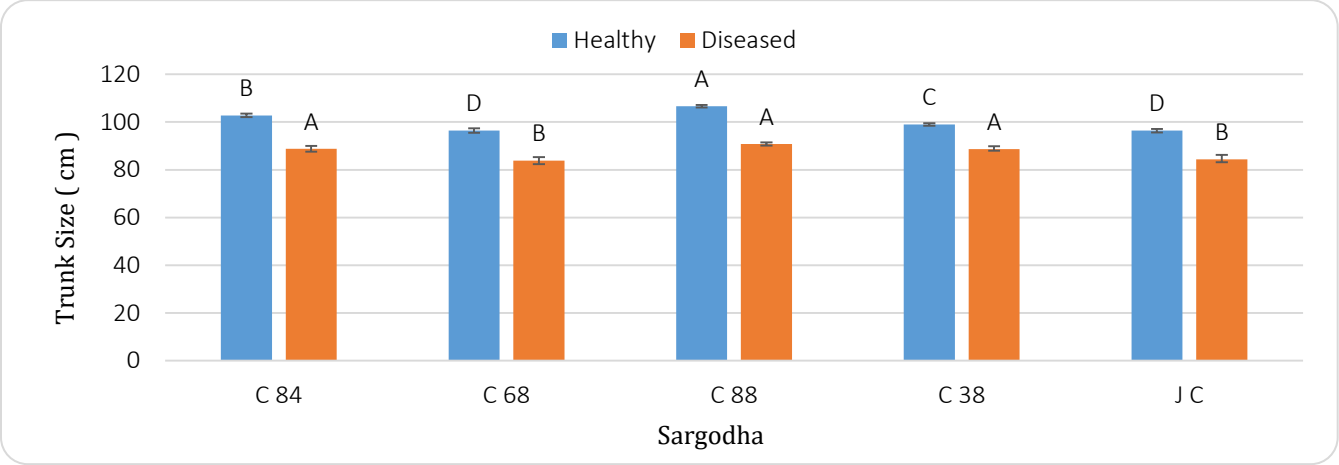

Figure 6. Trunk size of healthy and diseased citrus trees of tehsil Sargodha affected with *P. citrophthora.*

In Tehsil Bhalwal, the highest trunk size of healthy trees was recorded in Chak 4 SB with a diameter of 105.4 cm and least was recorded in Chak 15 NB having 96.4 cm. On the other hand, in case of diseased trees, Chak 9 SB had the least trunk size of 84.8 cm while Chak 4 SB and Chak 7 NB had the highest ones of 90.4 cm (Figure 7).

The highest trunk size of healthy trees in Tehsil Kot Momin was recorded in Laliani whose diameter was 104.2 cm and the least being recorded in Chak 19 had 99 cm. However, in case of diseased trees, Chak 12 SB

showed the least trunk size of 82.2 cm whereas Laliani had the highest one of 89.7 cm (Figure 8). The overall trunk size in district Sargodha between healthy and diseased trees is given in figure 9.

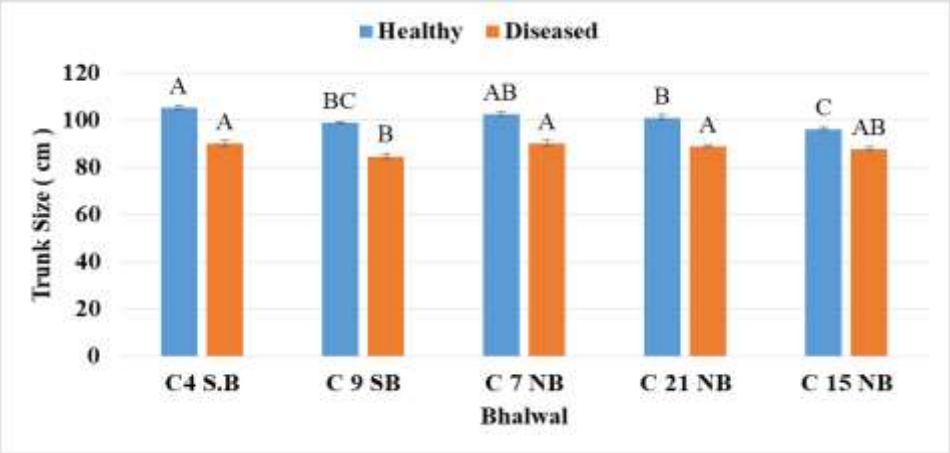

Figure 7. Trunk size of healthy and diseased citrus trees of tehsil Bhalwal affected with *P. citrophthora*.

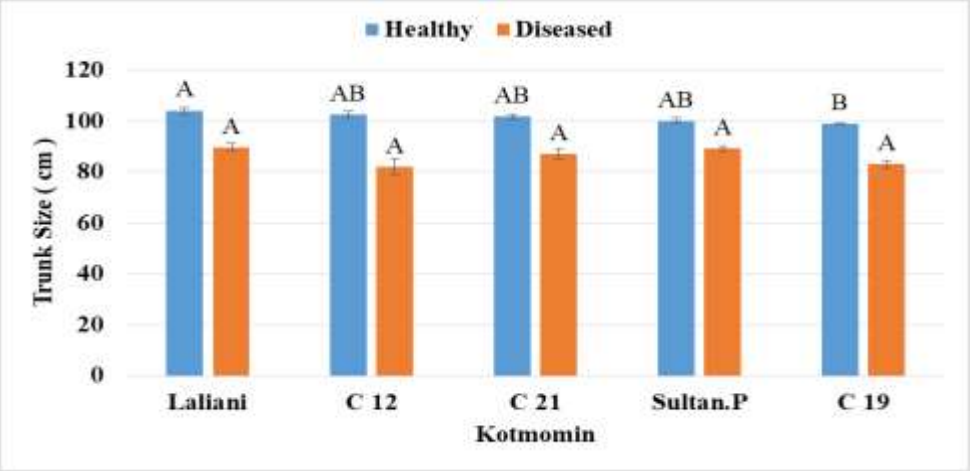

Figure 8. Trunk size of healthy and diseased citrus trees of tehsil Kot Momin affected with *P. citrophthora*.

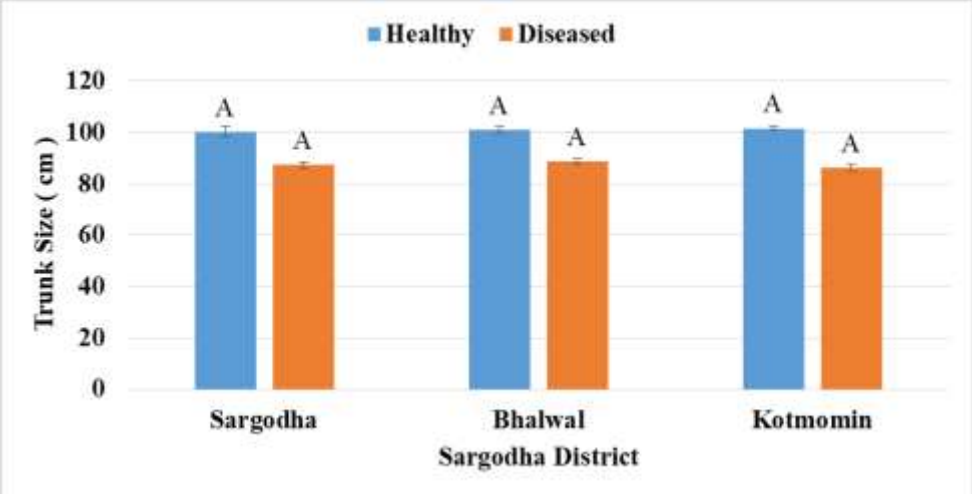

Figure 9. Trunk size of healthy and diseased citrus trees of district Sargodha affected with *P. citrophthora*.

DISCUSSION

Gummosis caused by *Phytophthopra* spp. is present in all citrus producing regions of the world and producing 10-30% losses every year (Mounde et al., 2008). *Phytophthopra* spp. are responsible for billions of dollar losses every year. This pathogen is among the most influential disease causing organisms responsible for number of diseases in economically important plants all over the world like citrus, rubber, cocoa, coconut, papaya, forest trees, tomato, potato and pepper (Drenth and Guest, 2004). In this study, a survey to determine the trunk size and leaf area of citrus gummosis was conducted in three different tehsils (Sargodha, Bhalwal, and Kot Momin) of Sargodha district. In general, the stem was painted with Bordeaux pastes and *T. harzianum* and *P. fluorescens*. The reduction in symptoms was mild to moderate (Gade and Koche, 2012). Phytophthora affects the root system and shows yellowing symptoms on leaves, which affect the plant's physiological processes (Badnakhe et al., 2015). The lesions caused to the trunk and roots by *Phytophthora* spp. lead losses in production, foot and root rot, brown fruit rot, canopy discoloration and leaf yellowing (Zhang et al., 2019). When compared to Kot Momin and Bhalwal tehsils, the results of this study showed that the affected leaf size in Sargodha tehsil is more affected than Kot Momin and Bhalwal and trunk size in Bhalwal is more affected than Sargodha and Kot Momin.

Citrus gummosis is the emerging and devastating citrus disease now a days associated with *Phytophthora* spp; a soil borne fungus (Assuah et al., 1999) and *Lasiodiplodia theobromae* in Chile (Guajardo et al., 2018). Gummosis has been reported to cause approximately 30 percent per year of yield losses (Mounde et al., 2008). Gummosis is a highly damaging disease that can lead to the total decline of orchards (Mekonen et al., 2015). In 2002, Naqvi and Singh (2002) stated that the spread of this pathogen is very rapid and that it is a soil-borne fungus that makes it difficult to monitor and handle. They said that once *Phytophthora* enters into a field, it establishes itself, becoming an endemic issue. In infested orchard soil, it is measured in the term of propagule count up to 250-350/cc. In an orchard, the propagule count can normally be around 1-20/cc, but in less time, it can reach 100-200/cc.

The feeder and fibrous root can have a major effect on the population exceeding 10 propagules/cc, causing their decay, resulting in sudden plant decline. A single sporangium releases 5-40 zoospores. The production and germination of sporangium depend on the soil, water and potential temperature. The zoospores that are produced from sporangia are motile and show flagella movement in the water at a short distance and are carried to longer distance through soil water. These zoospores are attracted by root exudates and they swim toward them and encyst upon contact.

The cyst upon germination penetrates into the cortex directly or through wounds, natural openings. Zoospores have the capability to infect any part of the plant if the surface remains wet for at least 18 hours. It has the ability to form a germ tube that can penetrate into the leaves, roots, twigs or fruits even in the absence of wounds (Graham and Timmer, 2006). This study suggests a comprehensive awareness-raising and training programme for farmers and frontline extension workers on how to recognize, manage, and control citrus gummosis. Farmers should be involved in the evaluation of improved rootstocks and clones, with farmers playing an active role in facilitating technological transfer of materials with desired qualities such as high yields and gummosis resistance.

CONCLUSION

The study has given new and quantitative data regarding progress of gummosis disease in citrus orchards of Sargodha. This study provides information regarding the methods and sampling designs required to quantify and evaluate the effects of climate, new management strategies, and integrated gummosis disease management programs in reducing gummosis threat.

AUTHORS' CONTRIBUTION

All the authors equally contributed in surveys, data collection, diagnosis, data analysis, and manuscript write up and formatting.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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