Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

QUANTIFYING THE SPATIAL DYNAMICS AND SEVERITY OF CITRUS GUMMOSIS DISEASE IN SARGODHA

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A B S T R A C T

Citrus is the most valuable fruit worldwide because of its flavor, dietary and therapeutic features. Every citrus growing area face a menace of citrus gummosis disease caused by a fugal like organism i.e. *Phytophthora citrophthora*. Gummosis ranks among the main diseases that are responsible for the destruction of citrus orchards. The diseased plants exhibit decline symptoms along with chlorosis, twig die-back, discolored fruits and wilted leaf tips. The experiment was aimed to map out the spatial distribution of citrus gummosis disease in Sargodha region (Pakistan). The survey was carried out in 3 Tehsils of District Sargodha where 3 orchards were marked. The symptomatic samples were collected from the selected trees and pathogenicity tests was performed *in-vitro* for the confirmation of pathogen. In all orchards, 15 trees were chosen for the assessment of citrus gummosis disease severity by using a disease rating scale. In Sargodha, the mean disease severity was 44.71%, followed by Bhalwal (36.29%) and Kotmomin Tehsil (31.55%). This study is supposed to facilitate growers for the identification and prediction of gummosis outbreak so that appropriate management options may be opted.

Keywords: Screening, Citrus decline, Gum formation, Stramenopiles, Germplasm, Water molds.

INTRODUCTION

Pakistan is producing more than 30 types of fruits among which citrus is the leading one that constitutes about 30% of total fruit production in the country (Moriya *et al.,* 2021). The citrus industry is regarded as the 2nd largest global market of fruits. It is cultivated on a commercial scale in over 135 countries round the globe (Cheema and Jamali, 2020). Pakistan is ranked 12th in the world among all citrus producing countries and 1st in the production of kinnow. Citrus fruit production was estimated 101.5 million tons in the year 2018-2019 (FAO, 2021). Citrus fruit is considered as the best source of nutrients such as vitamins, amino acids, carbohydrates and phytochemicals. Citrus is a valuable fruit in treating skin, liver and heart diseases (Dananjayan *et al.,* 2022). Citrus industry is confronting danger because of many living and non-living factors. Among all the stresses, citrus gummosis ranks at prominent position in the destruction of citrus plantations. Gummosis is caused by an Oomycete *Phytophthora citrophthora* that belongs to kingdom Stramenopiles (Graham and Feichtenberger, 2015).

The salient features of gummosis disease are ooze of gum from the bark that later on dries, cracks and sloughs off. The canker like lesions spread and girdle the stem of the infected plant (Badnakhe *et al.,* 2018). Decline may occur rapidly within a year, especially under conditions favorable for disease development, or may take several years (Urashima *et al.,* 2018). The gum oozes out from the cracks and each crack contains a gum pocket from where it secretes out on the plant surface. The wood

Submitted: February 26, 2022
Revised: May 17, 2022
Accepted for Publication: June 15, 2022
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underneath the blisters shows a pinkish orange tone (Benfradj *et al.,* 2017). Annual yield losses due to citrus gummosis were recorded up to 25% which resulted in considerable monetary losses (Mounde *et al.,* 2009). Gummosis is amazingly disastrous fungal disorder that causes complete destruction of citrus orchards. In various experiments, it was noted that gummosis disease prevails in more than 90% orchards with almost 50% incidence.

Choudhari *et al.,* (2018) studied the epidemiology of citrus gummosis caused by Phytophthora spp. and indicated that all soil factors, humidity and rainfall have positive relationship with disease development. Rajput *et al.* (2020) studied the epidemiology, disease cycle, biology, spatio-temporal dynamics and management of gummosis diseases in various citrus cultivars. Thakre *et al.* (2017) evaluated different fungicides against *Phytophthora* sp. which were applied on the trunk of citrus tree up to 4 feet to control Phytophthora gummosis. Best result was obtained by using bordeaux mixture during August and September. Savita and Nagpal (2012) studied different diseases of citrus caused by Phytophthora sp. in India. Survey of different citrus nurseries and orchard were conducted and history, epidemiology, distribution, disease cycle, mode of action and spreading of Phytophthora spp. were discussed.

The average yield/acre of citrus in Pakistan is less as compared to other countries like China, America etc. Consequently, there is a need to conduct a multi-staged examination to find out potential reasons for citrus decay and track down a feasible, eco-friendly and handily marketed innovation that fits for our subsistence of agricultural system. The present experiment was performed to assess the distribution and severity of gummosis disease in 3 main citrus producing regions of Sargodha. The study would be helpful to develop forecasting models for prediction and management of Phytophthora diseases.

MATERIALS AND METHODS

Experimental site: The study was carried out during November to December 2020 for the determination of spatial distribution and gummosis severity in main citrus producing regions of Sargodha (Pakistan). The inspection was performed in 3 citrus orchards in Sargodha, Bhalwal, and Kotmomin. Sites were located at 32°7'51''N, 72°41'13'' E; 32°15'26'' N, 72°53'17'' E and 32°08' 15''N, 72°53'74'' E, Sargodha, Bhalwal and Kotmomin, respectively. Plants were selected for citrus gummosis disease severity by observing gum oozing from minute bark splits that resemble with plant bleeding, lesion on bark, leaves show nutritional deficiency symptoms. The samples of infected leaves and fruits were collected for *in-vitro* experiments from each surveyed field.

Isolation of Pathogen: Culture medium for the pathogen: PARP (Pimaricin, ampicillin, rifamicin and pentachloronitrobenzene) media was used for isolation of pathogen from collected samples

Table. 1. Ingredients for PARP medium

Sr. No.	Ingredients	Quantity
1	Pimaricin	0.4ml/L
2	Ampicillin	0.25g/L
3	Rifamicin	0.01g/L
4	PCNB	5ml/L
5	DMSO	1ml/L
6	Corn meal agar	17g/L

PDA medium for multiplication of the pathogen

Potato dextrose agar (PDA) was used for multiplication of *Phytophthora* spp. and for evaluation of different fungicides

Table 2. Ingredients for PDA

Sr. No.	Ingredients	Quantity
1	Potato starch	4g/ml
2	Agar	17g/ml
3	Dextrose	17g/L

Phytophthora spp. were isolated by using soil dilution plate isolation techniques.

Soil dilution plate: Soil dilution was prepared by taking 1g of soil and added to 9ml of distilled water. From 1st dilution, 1ml were added to another tube containing 9ml of distilled water, in this way 4 dilutions were prepared. From 4th dilution few drops of water were poured into petri plates having PARP media and plates were

incubated at 25ºC (Arshi and Nasreen, 2016).

Identification of the pathogen: Fungus identification was accomplished based upon morphological characteristics of *Phytophthora* spp. like colony color, shape, size, hyphal morphology, morphology of sexual structure such as oospores, antheridia, oogonia and morphology of sporangium. These characters were compared with reported literature (Drenth and Sendall, 2001).

Preparation of soil extract: The soil extract was prepaared by mixing 40 g of soil in 1L of water and continuously shaked at 200 rpm for 36 hours on electronic shaker. Soil solution obtained was filtered through whatman filter paper no. 1 for 3 times and preserved at room temperature for future use.

Production of sporangia: Twenty ml of soil extract was taken in petri plates and 4-5 bits from 7 days old culture was placed in it. These Perti plates were incubated in dark at room temperature for 2 days. Slides were made from this liquid and observed under microscope at 10x and 40x magnification.

Disease assessment: In each orchard of Sargodha, Bhalwal and Kotmomin, 15 trees were selected and gummosis severity was assessed. The occurrence of gummosis is determined by the portion of plants exhibiting symptoms of gummosis divided by total number of plants evaluated. Tree decline was scored on a 0-4 scale (Table 1) and disease severity index (DSI) was described according to following formula

$$\text{DSI} = \frac{\text{Sum of all ratings}}{\text{Total number of assessed plants x Maximum rating value}} \text{X } 100$$

Table 3. Disease rating scale for Gummosis

Scale	Percentage	Description
0	0	No symptoms
1	1-25%	Decline symptoms associated
2	26-50%	Widespread decline
3	51-75%	Decline of branches
4	76-100%	Dead tree

(Mekonen*et al.,* 2015).

RESULTS

The results of the current experiment have shown that gummosis disease of varied severity prevailed in all the orchards where survey was performed. The gum formation and cracks were noticed on rootstock, stems, and branches of the affected plants in all orchards.

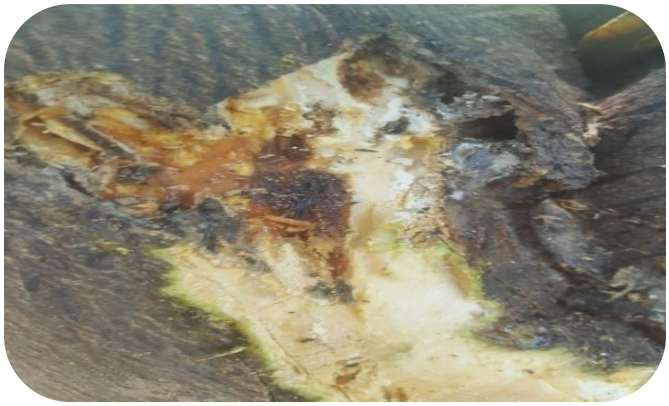

Figure 1. Gum exuding from trunk of citrus tree

In the present study, the gummosis disease was prevalent at all the sites with maximum disease severity (44.71%) was recorded in Sargodha followed by Bhalwal (36.29%) and Kotmomin (31.55%) (Figure 2). As far as the orchard wise disease severity was concerned in all 3 Tehsils of District Sargodha; the maximum disease severity was recorded in orchard 1 of Sargodha followed by orchard 3 and orchard 2. The highest disease severity was 53.72% and the lowest was 36.15% in orchard 1 and 2, respectively (Figure 3). Bhalwal was the 2nd most gummosis disease affected Tehsil; the maximum severity was recorded in orchard 2 followed by orchard 3 and orchard 1. The highest gummosis severity was 41.63% in orchard 2 and the lowest was 28.57% in orchard 1 (Figure 4). Tehsil Kotmomin, was least affected area by citrus gummosis disease; where maximum disease severity was recorded in orchard 1 followed by orchard 3 and orchard 2 respectively. The highest gummosis severity 35.83% and the lowest was22.54 % (Figure 5). The overall data of gummosis disease severity was

recorded on fortnight intervals which showed an increasing trend with the passage of time. The collective disease severity from all Tehsils and orchards showed that minimum disease severity (18.57%) was recorded after 1st date of recording while maximum (46.87%) after the last one (Figure 6). The gummosis percentage on the marked plants was positively increased after each observation.

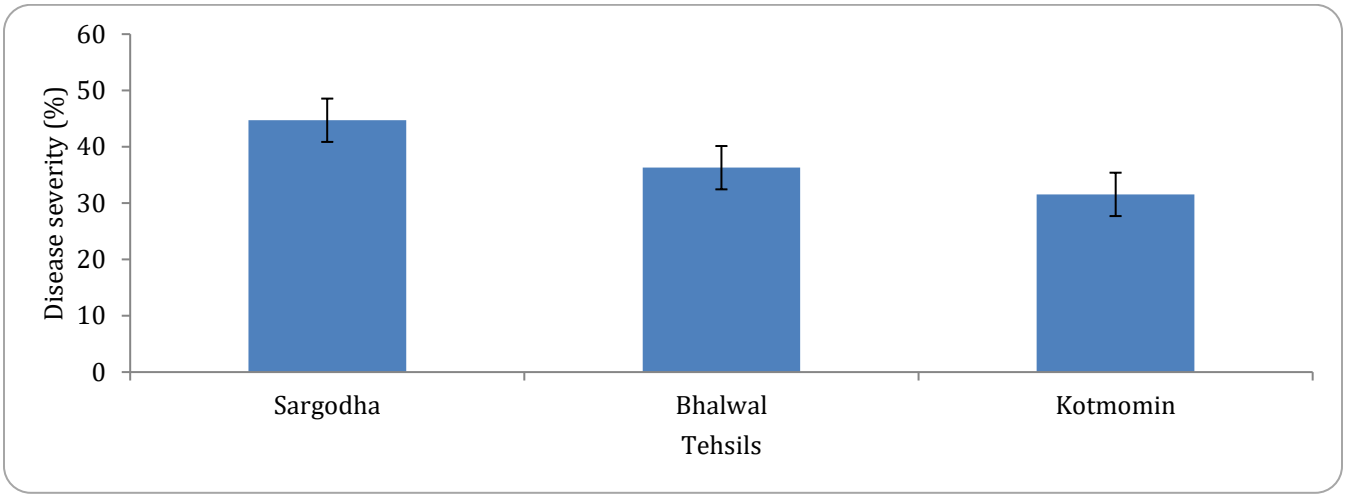

Figure 2. Overall comparison of citrus gummosis disease (mean±SE) severity in Sargodha, Bhalwal, and Kotmomin

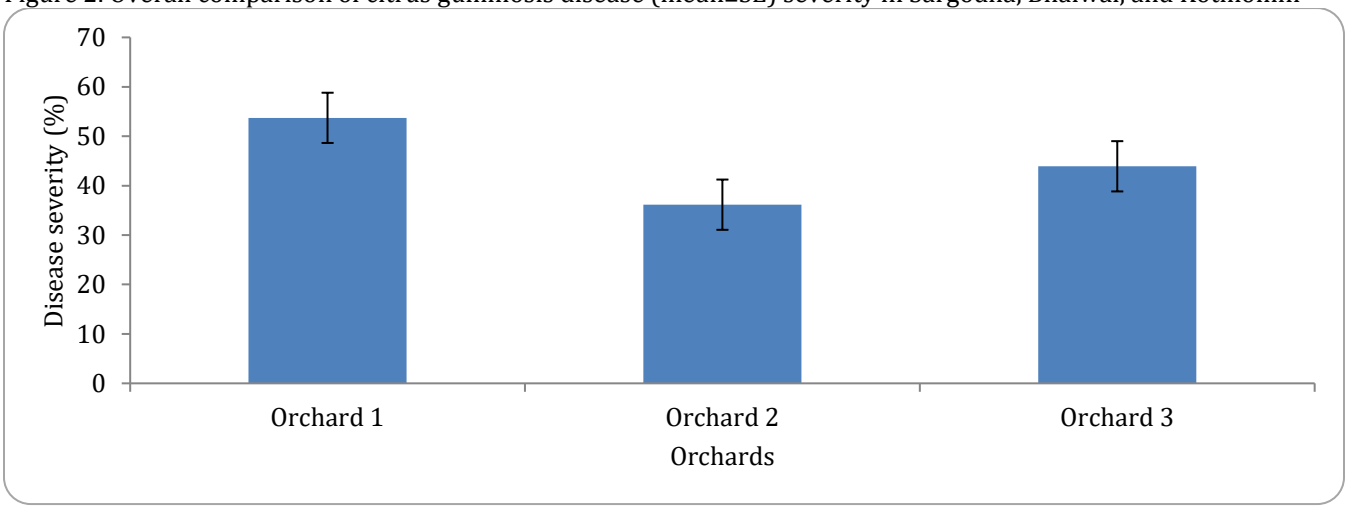

Figure 3. Citrus gummosis distribution in Sargodha (mean±SE) severity in different orchards

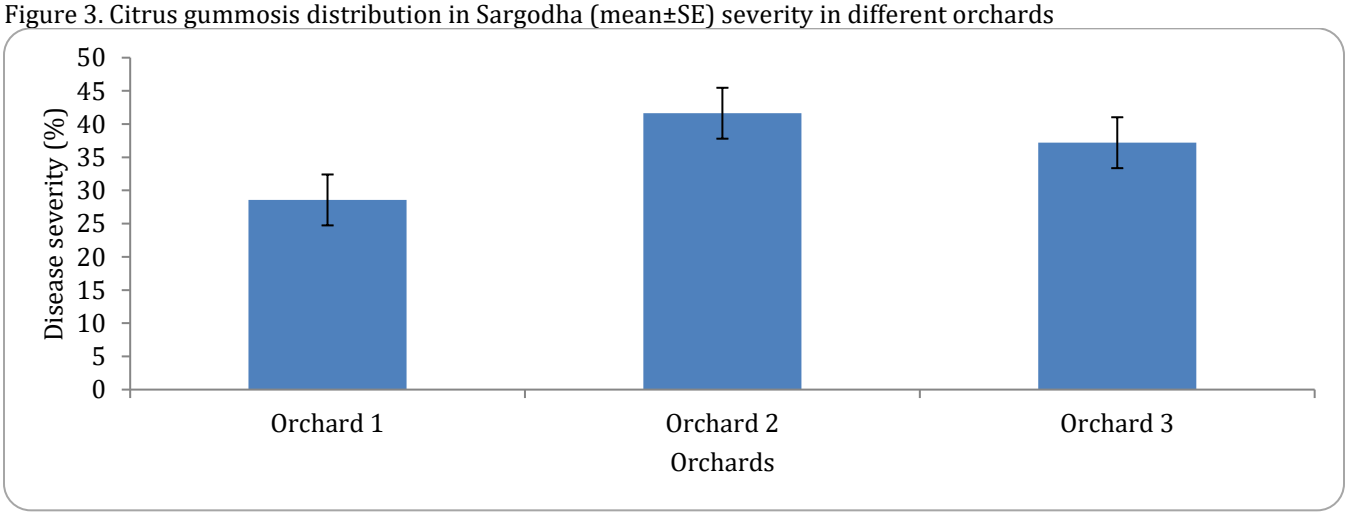

Figure 4.Citrus gummosis distribution in Bhalwal (mean±SE) severity in different orchards

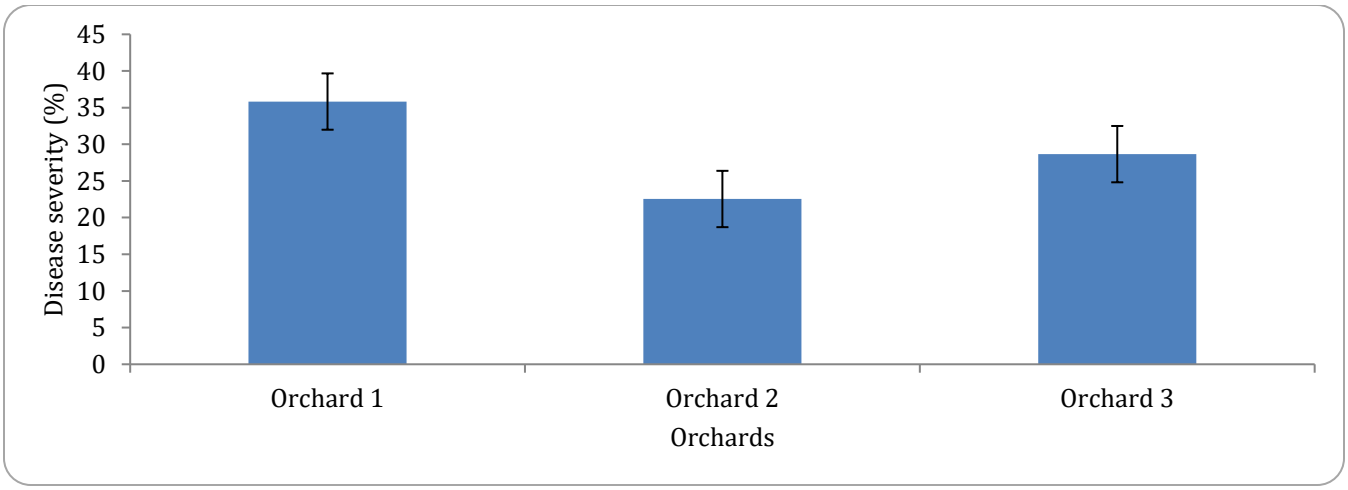

Figure 5. Citrus gummosis distribution in Kotmomin (mean±SE) severity in different orchards

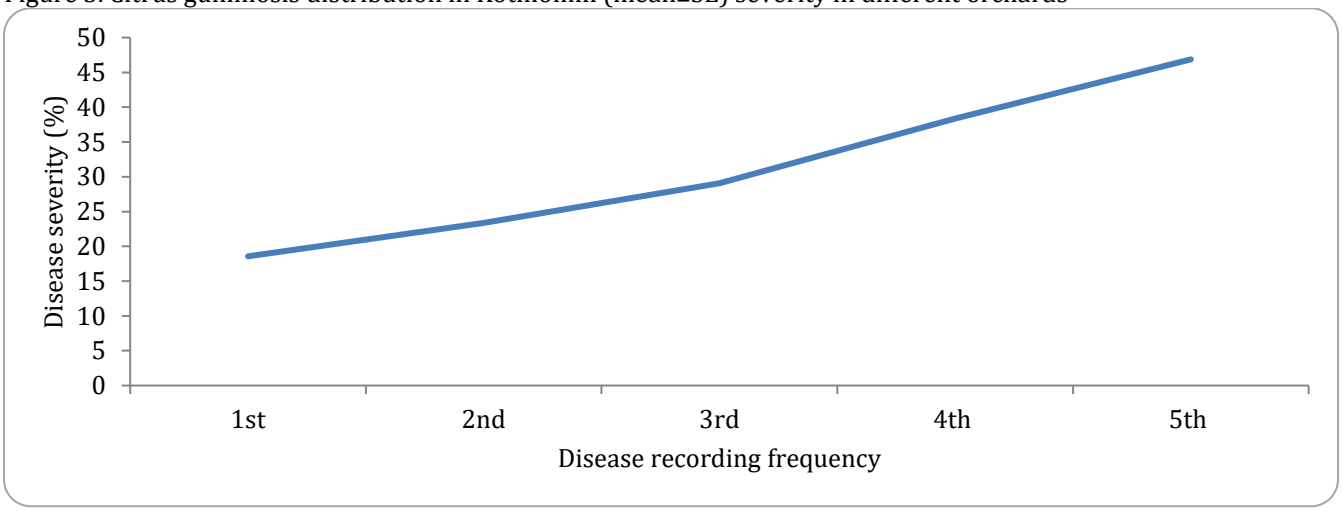

Figure 6. The overall disease severity trend on temporal basis

DISCUSSIONS

Gummosis caused by *Phytophthora citrophthora* is a vital risk in citrus production in Pakistan. The assessment of citrus gummosis disease in varied locations of District Sargodha would pave for its sustainable management. The maximum severity of gummosis was recorded from Tehsil Sargodha followed by Bhalwal and Kotmomin. The diseased samples were collected and pathogenicity tests were performed for the confirmation of causal organism i.e. *Phytophthora*. Maseko and Coutinho (2001) assessed pathogenic association of *Phytophthora* and *Pythium*spp. with citrus gummosis. Andres *et al.* (2003) checked the pathogenicity of a stramenopile *Phytophthora nicotianae* against pepper in Spain. Uddin *et al.* (2007) assessed *Phytophthora citropathora* phylogenetic relationship on the basis of internal transcribed region. Alvarez *et al.,* (2007) isolated *Phytophthora nicotianae* from lavender and rose merry in Spain which is the cause of rot and collar rot of these plants. Orlikowski *et al.* (2009) directed diverse analysis to distinguish climate water is hotspot for spreading of Phytophthora spp. to taint agriculture plants in Poland. Yaseen *et al.* (2010) investigated about dominant *Phytophthora*s pp. in citrus grove on Syria. The study clearly indicated that *P. citrophthora* was predominant species in citrus orchard of Syria. Savita and Nagpal (2012) studied different diseases of citrus caused by Phytophthora spp. in India. Survey of different citrus nurseries and orchard were conducted. In different areas there were 20 to 100% affected plants. Disease percentage was different in different citrus varieties. Different diseases caused by Phytophthora spp. are damping off, gummosis, root rot and brown rot. There were 3 main species of Phytophthora that were responsible for citrus diseases these are *Phytophthora parasitica*, *P. citropathora* and *P. palmivora*. In this study history, epidemiology, distribution, disease cycle, mode of action and spreading of Phytophthora spp. were also discussed.

Mounde *et al.* (2012) morphologically characterised and identified the Phytophthoras pp. associated with citrus gummosis in Kenya. Ahmed *et al.* (2012) studied the most prevalent Phytophthora specie in citrus nursery of Egypt. Khan *et al.,* (2015) conducted study for the identification and characterization of pathogen that involved in quality degradation of citrus fruit. Roberts *et al.* (2015) discussed the methods to avoid from *Phytophthora* are disease free transplanting material, treatment of seed with fungicide, well drained planting sites, sterilization of equipment's and tray, disinfestations of workers hand, fumigation of the soil, collection of infected fruit from the field and use of broad-spectrum fungicides.

Hung *et al.,* (2015) identified *Phytophthora*s pp. as causal organism of root rot of citrus in Thailand. The samples were collected from infected plants and pathogen was isolated on specific media. To study different morphological characters, colonies were grown on PDA, V8 and corn meal agar media. Benfradj *et al.* (2017) studied the influence of Phytophthora spp. on citrus root stock in Gharb region, Morocco. Result indicated the presence of *P. citropathora* was dominant over *P. parasitica*.

Choudhari *et al.* (2018) studied epidemiology of citrus gummosis caused by Phytophthora spp. in Nagpur, India. Six different locations were selected for the collection of data. Different environmental factor like, temperature, humidity, rainfall and soil moisture were studied. Rainfall was recorded without any interval with wireless and temperature was recorded after every thirty minutes. Along with these factor soil factor like soil pH, soil EC and moisture were also studied. These entire factors were correlated with disease incidence. Result indicated that all soil factors, humidity and rainfall have positive relationship with disease development and negative relationship with temperature. Disease incidence and severity were higher in December when humidity, soil moisture and rainfall was higher but temperature was low.

Mohite *et al.* (2016) described the fate of citrus orchards decline by integrating images data from satellite and artificial machines along with on field morphological traits. The most effective average 85.59% was received with artificial neural network (ANN) classification. Campanella *et al.*(2002) described that saline irrigation water results in salt accumulation that enhances fungal sporulation while soils with abundant calcium create unfavourable conditions for the water moulds. Graham and Timmer (2006) concluded from multiple studies that the injured roots excrete exudates that attract fungal spores and subsequent infection process is initiated. Savita *et al.* (2012) elucidated the disease cycle of gummosis fungus which starts with liberation of zoospores from sporangium and subsequent formation of mycelia whose growth is enhanced under nutrient deficiency. Timmer *et al,* (2006) stated that irrigation and infected propagative material is the primary cause for gummosis disease incidence. The irrigation water is vital driving force in the spread of fungal spores because it is a water loving pathogen. According to Brasier (2000) climate change also have a significant position in the emergence of pathogen and its altered activities. Wagh*et al.* (2018).inferred that *Phytophthora* population is directly proportional to rainfall and relative humidity while inversely proportional to temperature. The gummosis disease was more in August due to high rainfall and relative humidity while minimum incidence was recorded in May due to high temperature and low relative humidity. Choudhari *et al.* (2018) studied the spatial and temporal distribution of gummosis disease and recommended to avoid from low lying unlevelled fields and areas with high humidity and low temperature in order to avoid from the soil borne water loving pathogens.

CONCLUSIONS

Gummosis causes a remarkable quantitative and qualitative losses in citrus. The disease was significant at all locations of Sargodha with almost more than 45% severity. The spatial distribution of gummosis disease can be used for the development of predictive models and sustainable disease management. These results could also be utilized for disease mapping which would be helpful in preventive measures against the gummosis diseases in these sites.

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Moman Khan	:	Statistical analysis
Ashara Sajid	:	Proof reading
Saira Azmat	:	Helped in data collection
Salman Ghuffar	:	Helped in data recording and pathogenicity
MustansarMubeen	:	Wrote manuscript