ECRONICON OPEN ACCESS

EC AGRICULTURE
Review Article

Study of *Phytophthora* Associated with Citrus Decline

Muhammad Asim, Zafar Iqbal, Waqas Raza*, Salman Ahmad, Muhammad Usman Ghazanfar and Mohsin Raza

Department of Plant Pathology, College of Agriculture, University of Sargodha, Punjab, Pakistan

***Corresponding Author**: Waqas Raza, Department of Plant Pathology, College of Agriculture, University of Sargodha, Punjab, Pakistan.

Received: May 28, 2019; **Published:** July 01, 2019

Abstract

Phytophthora is among the most influential disease causing organisms responsible for number of diseases in economically important plants all over the world. Tree and crop production losses occur from nursery to fruit production. It is need of time to manage these destructive plant pathogens to increase citrus production. Hence, present review focused on different aspects of pathogen observed by several scientists worked on it. Management can be performed better after exact knowledge of pathogen and its behavior.

Keywords: *Disease; Organism; Important; Review; Management*

Introduction

Phytophthora belongs to kingdom Chromista, class Oomycetes and order Peronosporales. There are four orders in class Oomycetes, among them order Peronosporales and Saprolegniales consist of important plant pathogens. Other two orders contain fungal like aquatic organisms [1]. Genus *Phytophthora* consist of 60 species and all are important plant pathogens. *Phytophthora* spp. are responsible for billions of dollar losses every year [2].

There are several species of *Phytophthora* that are responsible for citrus diseases but most important are *Phytophthora citrophthora* and *Phytophthora nicotianae* that are responsible for damping of young seedling as well as yield loss and slow decline of adult trees [3]. Gummosis is present in all citrus producing regions of the world and producing 10 - 30% losses every year [4]. Combination of high temperature and humidity is suitable for disease development in citrus orchard. This disease mostly attacks the plant when scion comes in contact with soil or when irrigated water touches the scion. Common symptoms of root rot in seedling include reduction in feeder rot, discoloration of roots as infected, yellowing and wilting of young plant as it appear water stressed. Collar rot disease infect the plant just below ground near soil level and causes bark infection, discoloration and cracking of bark which makes it easy to removes as well as infection move upward. Foliar symptoms are wilting, dieback of twigs and reduction of foliage. In case of brown rot of fruit after 3 to 5 days of infection, brown centered water soaked lesions appear on fruit, which spread and cover whole fruit. White mycelium produced behind the lesion if high humidity is available. *Phytophthora* spp. causes most serious and economically important diseases of citrus. Tree and crop production losses occur from nursery to fruit production. Disease caused by *Phytophthora* spp. are root rot, gummosis and brown rot of fruit. Most important species involve in citrus decline are *P. citrophthora* and *P. nicotianae.* Keeping in view pathogen devastating nature, therefore present review focused to study different aspects of pathogen for alternative management strategies.

Pathogen

Graham and Timmer [5] discussed citrus diseases cause by *Phytophthora* spp. in Florida. He discussed damping off, gummosis, foot rot, fibrous root rot and brown rot of fruits. Damping off affect new germinated seedling of all cultivar of citrus. If high moisture is avai-

lable infected seedling died rapidly. In gummosis and foot rot scion becomes infected close to ground level. Below ground, roots become rotten and above ground bark shredded out around infected stem. In case of fibrous root rot, cortex removed and whitish thin roots left behind, their ability for uptake of nutrient decreased which become the reason of reduction in fruit size and quantity. *Phytophthora* sp. also infects fruit by causing brown rot. Infected area on fruit becomes lathery and light brown as compared to healthy rind. Under high humidity whitish mycelium appeared on fruit. All these diseases mainly caused by *P. nicotianae* and *P. citrophthora*. *Phytophthora* spp. is good parasite but poor saprophyte. They form chlamydospores and oospores under unfavorable condition. Soil temperature, moisture and aeration affect *Phytophthora* spp. infection.

Dandurand and Menge [6] studied influence of *Fusarium solani* on citrus root growth and population dynamic of *Phytophthora parasitica* and *Phytophthora citrophthora.* Feeder roots were collected from California and few other areas for the isolation of *F. solani.* Soil was collected from same field for isolation of *P. parasitica* and *P. citrophthora* through soil dilution plate method. Fungi were identified by colony morphology. Citrus plants were grown in sandy loam soil which was autoclaved two times to minimize *F. solani* contamination. Chamber in which plant were grown was fumigated with methyl bromide and plants were watered with distilled water. Soil was amended with *F. solani* spores at the concentration of 4 × 104 and plants were also sprayed with water having spore of *F. solani.* For control treatment soil and plants were sprayed with equal amount of sterile water. After 30 days, plants were watered with water having spore of *P. parasitica* and *P. citrophthora*. Ten plants from every treatment were harvested after every four days up to 28 days. Shoot portion of the plant was separated from roots and feeder root and different parameter were measured like shoot weight, root weight and length, number of new feeder roots and new tips of feeder roots. Result showed that in *F. solani* infested soil, *P. parasitica* density was 43% less as compare to control treatment but there was no negative effect on *P. citrophthora* population. There was 42% reduction in new tip emergence but no effect on root weight on plant grown in soil infested with *F. solani* spores.

Smiely., *et al. [*7] reported collar rot and root rot diseases of ornamental plants caused by *Phytophthora* spp. were discussed. *Phytophthora* is water mold so in poorly drained soil its disease causing ability is high. *Phytophthora cactorum* causes collar rot while *P. cinnamomi* is causal organism of root rot. Symptoms include small leaves, stunting growth, chlorosis, reduced shoot growth and dieback. Infected root are dark brown in colour. For diagnosis, pathogen can be cultured on growth media and by using ELISA test. Management includes good cultural practices and use of effective fungicides.

Masek and Coutinho (2001) assessed pathogenic association of *Phytophthora* and *Pythium* spp. with citrus was assessed. For this purpose, disease sample were collected from infected orchards and nurseries. The number of samples collected from each site varied depending upon the number of infected trees. Bark from infected collar region and rhizosphere soil from these trees was collected, samples were placed in plastic bag and isolation was carried within 24h. Isolation from infected tissues was carried out by direct planting method on PARP and PARPH media. For isolation 425g soil was saturated in 350 ml water and was baited with citrus leaf pieces. Container was incubated in darkness for 72h. Leaf pieces were taken and placed on selective media and incubated for 72h in dark. Small pieces of agar were taken from growing colonies and transferred to CAM and PDA for growth and identification. For two and four days plates were incubated at 20°C for *Pythium* and *Phytophthora spp*., respectively.

Andres., *et al*. [8] checked the pathogenicity of *Phytophthora nicotianae* against pepper in Spain. Samples were collected from different location. Nineteen isolates were isolated on V8 media and sporangium was induced on 1% potassium nitrate under UV light, length and width of sporangia of each isolate were measured. Pathogenicity test were perform on host plant at 6 leaf stage. For this purpose, host plant was grown under controlled condition in growth chamber at 22ºC. Inoculums were prepared on solution of 1% potassium nitrate in petri dishes and when abundant sporangia were formed transferred to distilled water. There were 20×10^3/ml zoospores in water which were used for inoculation. Pathogenicity tests confirmed the pathogenicity of *Phytophthora nicotianae* under high temperature. Koch postulates confirm that *P. capsici* and *P. nicotianae* were responsible for root and collar rot in Spain.

Uddin., et al. [9] assessed Phytophthora citrophthora phylogenetic relationship on the basis of internal transcribed region. For this purpose sample from citrus and other host plants from different areas in Japan were collected and pathogen was isolated on V8 media. DNA was extracted by using commercially available extraction solution. From 1 week old culture mycelium were scraped and preserved in

1.5 ml micro tube in 100ul solution and incubated for 10 minutes at 100ºC. After centrifugation for 3 minutes at 15000 rpm, supernatant was stored at -20ºC. Extracted DNA was amplified by using forward and reverse primer through PCR. Product obtained through PCR was purified through PCR cleaning kit. For the assessment of phylogenetic relationship between the isolate from Japan and isolates from other localities was done by constricting phylogenetic tree through maximum parsimony method.

Alvarez., *et al*. [10] isolated Phytophthora nicotianae from lavender and rose merry in Spain which is the cause of rot and collar rot of these plants. Samples were collected from infected plants washed and disinfected with 70% ethanol. After drying, sample, were cut into small pieces and inoculated in petri plates containing PARBPH media. Plates were incubated in dark at 24ºC. For pure culture, after growth, hypheal tip was transferred on PDA and V-8 media. Identification of isolates was performed on morphological basis as well as on the basis of mycelial character, morphology, dimension and production of antheridia, oogonia and sporangia. For production of conidia, soil extract was used. DNA sequencing of six isolates, three from rosemary and three from lavender, were also carried out. Pathogenicity test of 18 isolates were performed on their host plants. After comparison with Phytophthora spp. available in literature, it is revealed that, isolated specie was Phytophthora nicotianae.

Orlikowski., *et al.* [11] conducted different bait experiment to detect weather water is source for spreading of *Phytophthora* spp. to infect horticulture plants in Poland. Leaf of rhododendron was used as bait in different water bodies located at different location in the country. Three canals, four rivers and three reservoirs were inspected. Study showed that water sources have no influence on pathogen presence. Pathogen presence depends on the presence of host plant in the locality of water bodies.

Yaseen., *et al.* [12] investigated about dominant *Phytophthora* spp. in citrus grove on Syria. In July and August survey of different areas were conducted for the collection of diseased samples. From 55 orchard located in two different provinces more than two hundred samples of roots and soil were collected. From soil pathogen was isolate on selective media for *Phytophthora* spp. and inoculum density was measured. For isolation from roots, roots were separated from soil and washed thoroughly under tap water and after drying on blotting paper cut into small pieces. In five petri dishes 125 specimens were placed on specific media and plates were incubated at 20ºC for three to six days. From growing colonies *Phytophthora* spp. were identified on morphological bases. Some colonies were shifted on PDA for further identification. For comparison two strain of *Phytophthora citrophthora* and *P. nicotianae* CBS 274-33 and SCRP115 were used respectively. For confirmation of morphological identification molecular characterization was performed. Two *Phytophthora* sp. *P. nicotianae* and *P. citrophthora* were successfully isolated. Result of this study clearly indicated that *P. citrophthora* was predominant species in citrus orchard of Syria.

Molina., *et al.* (2010) isolated the causal agent of crown rot of red pepper that is *Phytophthora nicotianae* in Spain. Infected samples were collected from infected field that were consisted of soil, roots and aerial parts. Causal agent was isolated on PDA and V8 media and colony characteristics were observed. Morphological characteristics were observed by using microscope. Growth rate at different temperature was also assessed. Pathogenicity of fifteen isolates of *Phytophthora* was checked. Plants were grown and inoculated at 2, 3 leaf stage.

Savita and Nagpal [13] studied different diseases of citrus causes by *Phytophthora* sp. 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* sp. are damping off, gummosis, root rot and brown rot. There were three main species of *Phytophthora* that were responsible for citrus diseases these are *Phytophthora parasitica, P. citrophthora* and *P. palmivora*. In this study history, Epidemiology, distribution, disease cycle, mode of action and spreading of *Phytophthora* sp. were also discussed.

Mounde., *et al*. [14] morphologically characterised and identified the *Phytophthora* spp. associated with citrus gummosis in Kenya. Nine soil and fifteen bark sample were collected from 70 orchards. From soil, pathogens was isolated by following the method used by

Campbell and Hendrix and on PARBPH medium and for further identification pathogens were transferred to corn meal agar and V8 juice. From bark, pathogen was isolated of amended corn meal agar medium. After isolation pathogen was identified on the basis of morphological characteristics, colony morphology, dimension and morphology of sporangia oogonia and antheridia. One isolate of *P. syringae* 13 isolates of *P. nicotianae* and 45 *P. citrophthora* were tested for their virulence against lemon fruit, most virulent isolates of these species were used for pathogenicity test against rough lemon. When all isolates were identified to specie level, *P. syringae* was 2%, *P. nicotianae* was 22% and *P. citrophthora* was 76%. All the *Phytophthora* isolates were pathogenic to lemon.

Ahmed., *et al.* [15] studied the most prevalent *Phytophthora* species in citrus nursery of Egypt. Sample were collected from two nurseries, one was located in desert area and other in delta. Nursery in desert area was well managed and nursery in delta area was deficient in management. Volkamariana, sour orange and lemon root stock were collected from first nursery while in second nursery only volkamariana root stock was available. Chemical and physical analysis of the growing media showed that carbon to nitrogen ratio was higher in first nursery. Pathogen were not present in soil collected from sour orange but presence was detected in volkamirana and lemon soil sample from first nursery. Inoculum density in second nursery soil was higher. Pathogen could not isolated from roots collected from first nursery but pathogen was present in roots collected from second nursery.

Mahdavian and Javadi (2014) in Mazandran province of Iran studied crown and root rot of citrus. Survey of different orchard was conducted and disease samples were collected from infected orchard. Isolation was performed from disease specimen. Isolated pathogen were *Phytophthora nicotianae* and *P. citrophthora.*

Khan., *et al*. [16] conducted study for the identification and characterization of pathogen that involve in quality degradation of citrus fruit. For this purpose sample were collected from selected orchard of Sargodha region. For isolation of fungal pathogen collected sample were cut into small pieces of 2 - 3 mm and surface sterilized by 85% ethanol. Pieces were placed on PDA in petri plate incubated for 5 ¬- 7 days at 25 - 30°C and plates were examined daily to check growth of organism. The growth of the colonies were observed and recorded. Fungal culture was then purified by single spore isolation technique. Different fungi were identified up to genus level by their macroscopic and microscopic characteristics.

Hung., *et al.* [17] identified *phytophthora* spp. as causal organism of root rot of citrus in Thailand. 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. Pathogenicity test was performed on pomelo seedling. DNA sequence was also studied for identification of pathogen. Total 6 isolates were isolated, among them three were fast growing and remaining was slow growing.

Boudoudou., *et al*. (2016) studied the influence of *Phytophthora* spp. citrus root stock influence of *Phytophthora* spp. in Gharb region, Morocco. From 17 years old rootstocks of 16 different citrus verities soil sample were collected at depth of 5 - 20 cm and 1 meter away from the trunk. These samples were brought into lab and isolation of *Phytophthora* spp. were made on specific media BARPHY 72 and incubated at 28ºC in dark. After 48 hours it was transferred to test tubes having CMA. Total numbers of *Phytophthora* spp. propagules were calculated. Pathogen was identified by morphological character by dimension of sporangia, by presence of chlamydospores and on the basis of taxonomic criteria. Result indicated the presence of *P. citrophthora* and *P. parasitic,* presence of *P. citrophthora* was dominant over *P. parasitic,* no other strain was observed. These pathogens were tested against four root stocks and results indicated that, propagules presence were maximum in rhizosphere soil around *Citrus volkameriana* and minimum in soil around sour orange.

Thakre., *et al*. [18] evaluated different fungicides against *Phytophthora* sp. in Madhya Pradesh, India. They prepared different pastes by using different fungicides and applied on the trunk of citrus tree up to 4 feet to control *Phytophthora* gummosis and for control no treatment were applied. At 30 days interval four applications were used and size of the lesion recorded. Diseases development started in September and maximum lesion development was in September and November. Best result was obtained by using Bordeaux mixture during August and September.

Chaudhari., *et al.* [19] 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 stu-

died. 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.

Discussion

Phytophthora diseases are serious threat to citrus groves. *P. nicotianae* and *P. citrophthora* are two most important causal organisms of citrus diseases. Citrus gummosis, root rot and brown rot of fruit are diseases caused by these species. Gummosis is present in all citrus producing regions of the world and producing 10-30% losses every year. *Phytophthora* is causing more than 90% collar rot and 66% root rot in woody plants. These figures show that *Phytophthora* is one of the most damaging plant pathogen. There are several characteristics of *Phytophthora* which makes it worst plant enemy. These characters include its ability to spread through water and air, rapid production of inoculum, production of motile zoospores, production of chlamydospores and oospores for its survival outside plant tissues, wide host range, availability of host plant all around the year, ability of single species to cause diseases in multiple hosts and ability to cause more than one disease in single host.

Conclusion

Phytophthora spp. is growing concern to citrus production. Without managing this pathogen it is not possible to attain enough good quality citrus yield. So, it is need of time to overcome the effect of this pathogen by using integrated diseases management strategies.

Acknowledgement

The authors are thankful to the support provided by Higher Education Commission, Islamabad, Pakistan for funding the project (No. 20-3559/NRPU/R&D/HEC/14/1156) for Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha.

Conflict of Interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

Bibliography

1. Hawksworth DL., *et al*. "Dictionary of the fungi. No. C/589.203 H3" (1995).
2. Drenth A and Guest DI. "Diversity and management of Phytophthora in Southeast Asia". Australian Centre for International Agricultural Research (ACIAR) (2004).
3. Erwin DC and Ribeiro OK. "Phytophthora diseases worldwide". American Phytopathological Society (APS Press) (1996).
4. Mounde LG., *et al*. "Occurrence and distribution of citrus gummosis (Phytophthora spp.) in Kenya". *African Journal of Horticultural Science* 2 (2009).
5. Graham JH and Timmer LW. "Phytophthora diseases of citrus". *Plant Diseases of International Importance* 3 (1992): 250-269.
6. Dandurand LM and Menge JA. "Influence of Fusarium solani on Citrus Root Growth and Population Dynamics of Phythophthora parasitica and Phytophthora citrophthora". *Ecology and Epidemiology* 83.7 (1993): 767-771.
7. Smiley ET., *et al*. "Phytophthoru Root Rot and Collur Rot of Landscupe Plunts" (1999).
8. Andres JL., *et al*. "Phytophthora nicotianae pathogenic to pepper in northwest Spain". *Journal of Plant Pathology* 85.2 (2003): 91-98.
9. Uddin AF., *et al*. "Phylogenetic relationship of Phytophthora citrophthora isolates based on r DNA internal transcrined spacer sequence analysis". *International Journal of Integrative Biology* 1.3 (2007): 150-156.
10. Álvarez LA., *et al*. "Characterization of Phytophthora nicotianae isolates causing collar and root rot of lavender and rosemary in Spain". *Journal of Plant Pathology* 89.2 (2007): 261-264.

11. Orlikowski LB., *et al*. "Water as the source of Phytophthora spp. pathogens for horticultural plants". 28.3 (2009): 145-151.

12. Yaseen T., *et al*. "Phytophthora citrophthora is the predominant Phytophthora species in Syrian citrus groves". *Phytopathologia Mediterranea* 49.2 (2010): 205-211.

13. Savita VG and Nagpal A. "Citrus diseases caused by Phytophthora species". *GERF Bulletin of Biosciences* 3.1 (2012): 18-27.

14. Mounde LG., *et al*. "Morphological characterization and identification of Phytophthora species causing citrus gummosis in Kenya". *African Journal of Food, Agriculture, Nutrition and Development* 12.7 (2012).

15. Ahmed Y., *et al*. "Phytophthora nicotianae is the predominant Phytophthora species in citrus nurseries in Egypt". *Phytopathologia Mediterranea* 51.3 (2012): 519-527.

16. Khan A., *et al*. "Pathogens identification and charactrization that compromised citrus fruit quality in selected orchards of Sargodha". *International Journal of Environmental Science and Technology* 3.4 (2015): 54-59.

17. Hung PM., *et al*. "Identifications of Phytophthora spp. causing citrus root rots in Thailand". *Journal of Agricultural Technology* 11.8 (2015): 1897-1910.

18. Thakre B., *et al*. "Use of Suitable Fungicides for the Control of Gummosis Caused By (Phytophthora sp.) on Nagpur Mandarin in Satpura Plateau of Madhya Pradesh, India". *International Journal of Current Microbiology and Applied Sciences* 6.7 (2017): 2395-2400.

19. Choudhari RJ., *et al*. "Epidemiological Relations to Phytophthora Spp. Causing Citrus Root Rot in Nagpur Mandarin". *International Journal of Current Microbiology and Applied Sciences* 6 (2018): 406-417.

Volume 5 July 7 2019