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Chapter

## *Phytophthora* spp.: Economic Plant Threats in Egypt

Waleed Mohamed Hussain Abdulkhair

#### Abstract

The potato crop is exposed to infection with many fungal diseases including late blight, caused by *Phytophthora infestans*. The control of late blight disease requires an integrated management approach represented in cultivation control, plant resistance, and fungicide control. The citrus plants are infected by *Phytophthora nicotianae* that is causing root rot disease in Egypt. Three species of *Phytophthora* responsible for infection of citrus plants; *P. nicotianae*, *P. citrophthora*, and *P. palmivora*. Other pathogens associate *P. nicotianae* and form complexes or coinfection that release different diseases for citrus plants such as gummosis, *Phytophthora–Diaprepes* complex (PDC), and Huanglongbing syndrome (HLBS).

Keywords: oomycetes, pathogenicity, coinfection, citrus plants, potatoes

#### 1. Introduction

Plants are exposed to various biotic and abiotic stresses that affect their growth and yield. Biotic stress is represented by different microbial diseases that infect the plant. Therefore, the control of biotic stress is very urgent to maintain healthy crops Egypt suffers from accelerated population growth, which hinders economic development and constitutes an explicit threat to food security if it is not decisively controlled. FAO reported that "In the next decades, Africa will suffer from severe decrease of crops due to water deficiency, adverse weather events, pests and other factors, which lead to famines and drought".

Crops are exposed to microbial infections particularly fungal infections including *Phytophthora* infection as one of the main destructive phytopathogens. Among these crops, citrus is exposed to damage and subsequently high economic loss due to *Phytophthora* infection. – spp. infect different parts of citrus plants causing various diseases such as damping-off of seedlings, fibrous root rot, crown rot, and gummosis [1]. Ten species of genus *Phytophthora* are known to infect citrus plants around the world causing serious diseases such as gummosis, and root and fruit rots [2]. Chemical fungicides are one of the control measures, which provide an effective result, but their effects are not eco-friendly due to their harmful effects. Different fungicides, including Fosetyl-Al and Metalaxyl-M, may be used separately or by alternation to reduce the development of fungicide resistance [3]. Metalaxyl-M is usually used in East African countries to control the *Phytophthora* infection prevalence in potatoes [4, 5]. Climatic conditions play an important role in the prevalence of *Phytophthora* infection in citrus plants; i.e., the root infections caused by *P. citrophthora* are severe during spring and very mild during winter, on the other hand, the root infections caused by *P. nicotianae* are severe during summer and early autumn and very mild during winter [6].

Potato is one of the economic crops which are important for Egyptian exportation. This strategic and economic crop is exposed to complete damage by late blight disease caused by *P. infestans*, which is considered the most common disease for both potatoes and tomatoes worldwide [7–10]. Control of *Phytophthora* infection depends mainly on the contentious elimination of spoiled piles of potatoes and using a convenient means for both harvesting and storage [11]. Although different fungicides are used in the control of late blight, the phenyl amide fungicides such as Ridomil still the most effective ones [12, 13]. Nevertheless, the use of Ridomil leads to resistance development with frequent usage, so the strategy of Ridomil practice should be modified; i.e., the potatoes should be treated with Ridomil  $(0.75 \text{ kg ha}^{-1})$ followed by another dose (1.5 kg  $ha^{-1}$ ) to prevent both formations of late blight disease and resistance development [14–16]. In such a way, using fungicides as an effective treatment for late blight disease increases the productivity of potatoes by as much as 60% and therefore decreases the economic loss [17]. In this chapter, we will elucidate the harmful effects of *Phytophthora* spp. on crops in Egypt, and the followed recent methods to control its diseases to maintain sustainable agriculture and a strong economy, therefore.

#### 2. Phytophthora

The phytopathogenic fungus *Phytophthora* spp. is unrelated to true fungi and belongs to oomycetes. This oomycete has mycelia which released branched sporangiophores, which produce lemon-shaped sporangia at their tips. This oomycete is characteristic of swellings produced by sporangiophores at the places of sporangia formation [18, 19]. Different species are belonging to the genus *Phytophthora* including *Phytophthora infestans* which have two mating types (A1 and A2) which are required to produce sexual spores known as oospores. P. infestans is the causative pathogen of potato late blight disease [18–20]. Oospores are the main reason for new strains' release of *P. infestans* worldwide, which are more deleterious than the old strains. Sexual reproduction begins with the growth of the two mating types adjacently, where the globose oogonium is developed above the antheridium due to the growth of female hypha through the young antheridium, which in turn fertilizes the oogonium to develop a thick-walled and hardy oospore. The germination of oospores is carried out by a germ tube that produces sporangia, which germinate entirely by releasing three to eight zoospores at temperatures up to 12 or 15°C, whereas above 15°C sporangia may germinate directly by producing a germ tube [19].

#### 3. Phytophthora diseases in Egypt

Egypt is an agronomic country that depends on the exportation of crops to develop its economy. Therefore, all agronomic pests including *Phytophthora* spp. which destroy the crops and other plants are threats and lead to decline of the economy. Potatoes and citrus plants are occupied the first ranks of Egyptian agronomic crops which export to different countries over the world, especially the European Union and Arabic Gulf countries. In Egypt, potatoes and citrus plants are usually attacked by *P. infestans* and *P. nicotianae*, respectively. These phytopathogenic fungi are very deleterious due to their severe effects and resistance ability for many fungicides. So, the control of these pathogens is a very urgent approach to maintain

healthy crops, food security, and a strong economy. In the last 5 years, the Egyptian government has succeeded to render the orange crop is the first exported citrus plant worldwide due to new strategies and sustainable agriculture programmes which are applied intensively.

#### 4. P. infestans

#### 4.1 Life cycle

Temperature plays an important role in its life cycle, where sporangia of P. infestans can germinate by a single germ tube at 13–21°C, and the emergence of late blight is accompanied by a moderate temperature (10–16°C) and high relative humidity [21, 22]. The fungal growth is inhibited at 30°C or more, and it can further sporulate when the temperature be relatively moderate with high relative humidity near 100% [19]. The emergence of lesions is the main symptom of late blight disease. These lesions have appeared as small circular or irregular with light to dark green color, and they are also water-soaked [22]. These lesions are appeared on both lower and upper leaves, where unfavorable and favorable climate conditions are present, respectively [22]. At high humidity, the lesions are fast enlarged with the formation of brown areas with the absence of clear borders. At these borders, a white zone (3–5 mm) of downy mildew growth has appeared on the lower sides of the leaves, and then all leaves are infected and die [19]. Therefore, there is a relationship between late blight infection and humidity, where the infection and symptoms appearance are accompanied by high relative humidity for a minimum of 7–10 hours. The late blight infection is distributed via spores scattering by wind and rain until reach healthy plants where the disease cycle begins again. The life cycle of P. infestans depends on two main environmental factors; humidity and temperature. The moderate temperature and high relative humidity intensively allow to formation and distribution of late blight infection. Many life cycles maybe happen in one season, and subsequently, the oomycetal infection is more prevalent even in the soil as a carrier for spores. The late blight disease can completely damage the potatoes within a few days or a few weeks when favorable environmental conditions are provided. On the other hand, late blight disease is attenuated and disappeared with dry weather, where the oomycetal activity is stopped and the existing lesions do not enlarge and turn black, curl, and wither [19].

The late blight infection begins with close contact with sporangia and tubers. Usually, late blight infection happens when sporangia are penetrating the tubers of potatoes. Developing and mature tubers are a target for sporangia; however, mature ones are a more favorable targets because they can cause cracks in the soil and give sporangia ready access. The moist soil accelerates the infection of tubers, which are usually appeared as irregular dark purple or brownish blotches. When the infected tuber is torn off, water-soaked, dark, reddish-brown infected tissues have appeared, and the oomycetal infection may be deeply extended into 5–15 mm in the tuber flesh. The coinfection may happen for the infected tubers, where other fungal and bacterial infections are present. The coinfection causes soft rots which characterize by a putrid taste and offensive odor [19, 22].

There are two types of sporangia germination of *P. infestans*; direct germination by a germ tube or indirect germination by zoospores formation. Secondary sporangia may be produced by germ tubes, and germinate at 7–13°C with the presence of free water on leaves, and form 8–12 motile zoospores per sporangium, which swim in the water and attach to the leaf surface and infect the plant. Zoospores liberate the germ tubes and by which infect the leaves either through stomata or by direct penetration, where the mycelia grow and release long-curled haustoria between the cells. The second phase of the life cycle starts in the moist soil bearing sporangia. Zoospores profusely germinate and penetrate the tubers through the wounds [21, 22].

#### 4.2 Control

The control of late blight infection depends on different management approaches including cultivation control, plant resistance and fungicides control.

#### 4.2.1 Cultivation control

Control of late blight disease can be achieved by using good agricultural practices as the most common control technique including using healthy seeds, elimination of all infected potatoes, avoidance of frequent or night-time overhead irrigation, using more conventional methods for both harvesting and storage, and using more effective fungicides and herbicides to destroy the dropped infected parts of potato plant [23]. As mentioned above, the prevalence of late blight disease depends on providing adequate weather conditions (moderate temperature and high relative humidity) despite these conditions being beyond the control. Nevertheless, some measures should be applied such as the selection of proper fields and taking into account the appropriate method, time and amount of irrigation. The proper field should have good water seepage, high drainage, free from inoculum sources (infected potatoes), and also free from the weeds which restrict the air movement within the canopy and block the access of fungicides to the leaves and tubers of potatoes, and alternative late blight hosts like hairy nightshade that supports the disease prevalence [21, 22]. Sprinkler irrigation is a more adequate method for potato cultivation because it does not provide sufficient humidity that allows fungal growth and late blight disease incidence. It is preferable to grow potatoes in rows parallel with the winds to improve the air circulation and therefore foliage dryness. Conscious cultivation of potatoes requires rooting of the vines before 2 weeks of harvest, and then spraying the foliage with fungicides to kill living late blight spores. The healthy tubers should be dried during and after storage, while the infected tubers should be separated from the healthy ones [24].

#### 4.2.2 Plant resistance

The plant resistance to late blight disease is another means for control that saves us from using fungicides and subsequently prevents or diminishes fungicide resistance. The plant resistance for late blight disease is an eco-friendly means, so the modern approach of potato cultivation depends on using resistant cultivars, which vary among each other in the resistance rate to late blight disease. Therefore, the pursuit of late blight resistance is a very urgent matter, so a lot of potato cultivars are genetically engineered to be resistant. Nevertheless, these genetically engineered cultivars can be destroyed by other new strains of *P. infestans* because the resistance is encoded by a single gene. So, using of polygenic (durable) resistant cultivars is very helpful to allow the production of healthy potatoes free from any deposits of fungicides. Although plant resistance technique is very effective for control of late blight disease, avoidance of cultivation inadequate weather for late blight emergence is required; as well as, frequent spray with an effective fungicide to completely prevent the growth of *P. infestans* [25].

#### 4.2.3 Fungicides control

Despite the two previous effective methods in controlling the spread of late blight that infects potato plants, using fungicides are very essential to completely prevent or eradicate the late blight disease. The fungicides are also used as prophylactic agents, where they are sprayed on healthy potatoes to prevent any growth chance of *P. infestans*. The use of fungicides must be frequent and periodically because they may be broken down by weather factors. There are two types of fungicides according to their mobility named protectant and penetrant. The protectant fungicides are usually used before the incidence of late blight infection because the already infected potatoes never get rid of the symptoms of late blight and the damaged tissues are never repaired. The penetrant fungicides are effectively used after the incidence of late blight infection. Both protectant and penetrant fungicides have broad-spectrum and systemic action, so they are powerful chemical control agents [26].

#### 5. P. nicotianae

#### 5.1 Description

The main pathogen of citrus plants in Egypt is *P. nicotianae* or also called *P. parasitica*. This phytopathogenic oomycete was firstly isolated from tobacco in Indonesia in 1896s. The oospores are produced from both antheridia and septate oogonia. The spherical, ovoidal, or ellipsoidal sporangia are present with one to three sharp papillae. The intercalary or terminal spherical chlamydospores and arachnoid mycelia are also present. Although *P. nicotianae* can infect around 90 different plant families, the citrus plants are still the main target of it. So, this pathogen causes a high loss of citrus plants to reach to 15% in Egypt and subsequently negatively affects the Egyptian exports from citrus plants and in turn leads to significant economic loss. The isolation of *P. nicotianae* from the soil can be carried out by different methods, but the baiting method is the most common and effective one. *P. nicotianae* can be identified either by classical methods depending on morphology determination or by genetical methods depending on SSCP fingerprinting and ITS sequencing. The eradication of *P. nicotianae* depends on the same methods mentioned above especially using effective fungicides such as Metalaxyl-M and phosphonate.

#### 5.2 Pathogenicity spectrum

The genus *Phytophthora* has a wide array of species that reaches 120 due to the improvement of identification tools and methods, a wide survey of natural habitats, and reports of new diseases [27, 28]. This blossoming may increase in the next years where it has been reached 600 species [29]. Therefore, the host plants of *Phytophthora* genus are also extending to be about 4400 hosts [30]. The pathogenicity spectrum is differed from one species to another and subsequently influence on agronomic productivity. *P. nicotianae* is characterized by its wide pathogenicity spectrum against 90 families of plants particularly citrus ones and causes a high loss of productivity. *P. nicotianae* is widely distributed worldwide especially in temperate countries like Egypt and infects a considerable number of plants causing huge economic loss [31, 32]. This pathogen causes many plant diseases including brown rot, foot rot, root rot, gummosis, and black shank of tobacco [33].

#### 5.3 Pathogenicity behavior

The phytopathogenic P. nicotianae usually infects the roots of different plants and other parts such as leaves, stems, and fruits and causes crown rot disease. The pathogenicity behavior of *P. nicotianae* is hemibiotrophic; i.e., the pathogenicity is accomplished through two steps; the first one is called biotrophy that implies the pathogen intimately contacts with the healthy tissues at the early stages of infection, and the second step is called necrotrophy in which the pathogen penetrates the tissues and profusely grows and causing tissue wilting and death. P. nicotianae has sporangiophores that bear multinucleate sporangia, which directly germinate in the proper weather and produce wall-less zoospores which are uninucleate and possess two flagella to can migrate until contact with the host tissues. Once contact is done, zoospores form a cell wall and cysts, which in turn germinate to form germ tubes by which they can penetrate the plant tissues [34, 35]. Moreover, P. nicotianae can reproduce sexually by formation thick-walled oospores as a net result of male and female gametangia fusion. Sexual reproduction leads to high genetic variation which in turn leads to releasing of novel pathogenicity and virulence factors. The main habitat of oospores is the soil where they can persist for several years until germination and formation of germ tube that penetrates the host tissues and causes the diseases.

Under hard extreme environmental conditions, *P. nicotianae* produces thickwalled asexual structures called chlamydospores, which persist in the soil very long time reach to several years. Chlamydospores can actively germinate at moderate and high temperatures, while they are dormant in low temperatures as the same with oospores. Therefore, *P. nicotianae* can tolerate the hard weather of the winter by both oospores and chlamydospores which be dormant in rhizospheres of host plants. Some animals like termites and snails are good vectors for both oospores and chlamydospores, which survive in their gastrointestinal tracts and feces [36]. It is argued that, *P. nicotianae* can reproduce sexually and asexually according to weather conditions; i.e., under proper conditions, they usually reproduce by sexual propagules, but under unfavorable conditions, they tend to asexual reproduction to tolerate the hard conditions. So, the life cycle of *P. nicotianae* includes both sexual and asexual propagules [37].

#### 5.4 Coinfection

The coinfection implies the association of *P. nicotianae* as a soil-borne pathogen with other pathogens which together infect the host-plant tissues and cause unprecedented diseases such as the new citrus diseases, which are somewhat incurable diseases.

#### 5.4.1 Phytophthora gummosis

Citrus plants represent a high economic value in Egypt, so they must be protected from all destructive pathogens especially *P. nicotianae*. Citrus plants may usually be infected by 10 species of *Phytophthora*, the severe diseases are incident by only three species namely *P. nicotianae*, *P. citrophthora* and *Promecotheca palmivora*, but *P. nicotianae* is the most destructive one not only in Egypt but all over the world where it causes root rot, foot rot, and gummosis [38]. The coinfection is usually accompanied by the life cycle of *P. nicotianae*, where it is associated with *P. palmivora* in warm countries like Florida or Southern Asia, and it is associated with *P. citrophthora* in the Mediterranean countries and causing branched cankers [39, 40]. *P. citrophthora* is active in spring, while *P. nicotianae* is active in the summer and early autumn causing root infection. Therefore, *P. nicotianae* requires a high temperature more than *P. citrophthora* for growth and activity. *P. nicotianae* is a dominant phytopathogenic fungus in Brazil, Egypt, South Africa, and Tunisia. Fungicides, resistant plants, and sanitary practices are good measures for the control of *P. nicotianae* [41].

#### 5.4.2 Phytophthora: Diaprepes complex (PDC)

*Diaprepes* is the polyphagous root weevil found in citrus areas due to the distribution of infected nursery stock. The roots of citrus plants are being infected by the larvae of the weevil which are feeding on root tissues until be dilapidated and then be died. On the other hand, the infection with *Diaprepes* is followed by the infection with *P. nicotianae* through the injured roots predisposed to *Phytophthora* infections, and PDC is formed, which is an incurable disease that is very difficult to control [42].

#### 5.4.3 Huanglongbing syndrome (HLBS)

There is another coinfection include *P. nicotianae* called HLBS that is more incurable than PDC. The name of this disease returns to the citrus greening or yellow dragon disease which is the oldest citrus disease worldwide. Although, this disease is widely distributed in most citrus areas over the world, the Mediterranean Basin region, Australia and Japan are less affected [43]. HLBS is usually transmitted by both psyllid vectors and grafting. Three varieties are belonging to unculturable Gram-negative bacterium; Candidatus liberibacter. The first variety was isolated from Africa; C. liberibacter africanus and designated as (CaLaf), the second variety was isolated from Asia; C. liberibacter asiaticus and designated as (CaLas), and the third and last variety was isolated from America; C. liberibacter americanus and designated as (CaLam) [44]. The effect of this disease is very severe because it causes vascular decline, reduces both fruit size and quality, and completely kills the trees [45]. The seriousness of this disease returns to no resistant plants are found and also no efficient management program is currently available, so the plant losses may reach 100% locally [46]. Moreover, the pathogenic bacteria attack all parts of the host plant and cause complete damage particularly if the coinfection included P. nicotianae is incident. Accordingly, the control of this disease mainly depends on the eradication of psyllid vectors and following the sanitary practices including removal of infected trees, which may be avoided by following a good nutrition program. Furthermore, effective mefenoxam-based fungicides can be used as helpful agents to get a ride of this dreaded plant disease. This control strategy has two disadvantages: high cost and inefficiency with mild or long persistence [3, 47].

#### 6. Methodology

#### 6.1 Isolation of pure cultures of *Phytophthora* spp.

Isolation of *Phytophthora* spp. is usually carried out by using Rye B Agar medium [48] (60 g rye grain, 20 g glucose/sucrose, 15 g agar, and 1.0 L distilled water, pH 6.8 ± 0.2). The rye grains were soaked in distilled water and left at room temperature for 36 hours. The supernatant was poured off and kept in a separate vessel, while the distilled water was added to the settled grains and heated in a water bath at 50°C for 3.0 hours. The mixture was filtered through a sieve and the grains were discarded. The filtrate was mixed with the kept supernatant, glucose/sucrose, and agar, and the

distilled water was added until the volume reached 1.0 L, and then agitated thoroughly. The mixture was autoclaved at 121°C for 20 minutes. The autoclaved medium was left to be cooled at 45°C, and 3 ml of a stock rifamycin (30  $\mu$ g/ml) and 1 ml of stock piramycin (10  $\mu$ g/ml) were added and mixed thoroughly.

The infected part of plant with *Phytophthora* spp. was cut into small fragments, which were placed into the healthy part susceptible to infection. The two parts were fastened back together with a rubber band and incubated at 16°C for 4–9 days. The infected part was cut into small fragments by a sterile scalpel. These fragments were disinfected in 70% ethanol for 15–20 seconds and then de-aerate in 0.1% sublimate of HgCl<sub>2</sub> for 30–45 seconds, or in 1% sodium hypochlorite for 180 seconds. The disinfected fragments were rinsed three times with sterile distilled water, and placed onto the agar surface of Rye B medium containing antibiotics and maintained in dark at 16°C for 10 days. The colonies of *Phytophthora* were examined microscopically by a lens (400×). The sporangia of *Phytophthora* appeared in different shapes according to the species [49].

#### 6.2 Storage of *Phytophthora* spp.

#### 6.2.1 Using agar slopes under paraffin oil or water

The pure culture of *Phytophthora* spp. was grown on Rye B Agar medium for 2 weeks. The agar surface loaded by the growth of *Phytophthora* spp. was flooded with sterile paraffin oil or water and stored at 4–7°C. The sub-culture must carry out at least once every 3 years.

#### 6.2.2 Using liquid nitrogen

The pure culture of *Phytophthora* spp. was grown on Rye B Agar medium for 10–14 days at 16°C. The growth was cut into discs by a sterile cork-borer, and placed in 1.5 ml cryovials containing a sterile 15% dimethyl sulfoxide solution (DMSO), and mixed thoroughly until be immersed. The temperature of cryovials containing *Phytophthora* discs was decreased gradually ( $-1^{\circ}C/min$ ) for at least 4 hours at  $-70^{\circ}C$  using a specified device for this purpose. The frozen growth was transferred into liquid nitrogen ( $-196^{\circ}C$ ). Due to the toxic effect of DMSO on the growth of *Phytophthora* spp., the growth discs must be quickly thawed and rinsed carefully and thoroughly with sterile distilled water when out of the liquid nitrogen.

#### 6.3 Preparation of Phytophthora inoculum

The mycelial growth of *Phytophthora* spp. was placed on the agar surface of Rye B Agar medium between two slices (1 cm) of susceptible host plant for infection. The two slices never completely cut off from each other to be closely attached with the moist medium to enhance the pathogen proliferation. The agar plates were incubated at 16°C for a week at high relative air humidity (80–100%) until thick mycelia appeared on the upper surface of the top slice. The sporangia of *Phytophthora* were collected from the mycelia by a brush and washed with deionized water. The hemocytometer was used to prepare the required inoculum concentration (50 sporangia  $\mu$ l<sup>-1</sup>). The inoculum was left at 7°C for 2 hours, and at room temperature for half an hour to increase the liberation of zoospores from sporangia. During the test, the inoculum should be constantly and gently agitated to prevent sporangia sedimentation and therefore zoospores accumulation that provides an adverse event on the solution surface [50].

#### 7. Conclusions

The potato is one of the economic crops in Egypt that participates in the providing of local food security and valuable national income through the exportation of agronomic crops. The production of potatoes should be provided with complete care that includes the use of modern safe methods that prevent or treat diseases that affect it, including late blight. Using of effective fungicides is one of these methods which protect from and eradicate the infection of *P. infestans*. The integrated disease management strategy is the best method to control the prevalence of late blight due to the development of new fungicides-resistant strains. The citrus plants are also economic crops in Egypt and require complete care to prevent the growth of the main destructive pathogen called *P. nicotianae* that cause different diseases especially through its association with other pathogens (coinfection).

#### Acknowledgements

The author sincerely thanks everybody who introduced the required assistance and was helpful to complete this work.

#### **Conflict of interest**

The author declares no conflict of interest.

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