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REVIEW PAPER

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Downy mildew (*Pseudoperonospora Cubensis*); A devastating phytopathological issue of Muskmelon: Review

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Abstract

Downy Mildew of Muskmelon is one the most devastating fungal phytopathological issue. *Pseudopernospora Cubensis* is an obligate parasite and has worldwide distribution. It is reported in more than 70 different countries with diverse climatic conditions with a wide host range that can infect more than 20 different genera of cucurbits. The aim of this review is to summarize all recent advances in research about *P. cubensis*, Disease symptoms, mode of infection, mode of spread, Epidemiology, Disease cycle, and management (complete guide) for future strategies and development of new varieties having resistance against disease and best fungicides for management of pathogen population.

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Introduction

Muskmelon *(Cucumis melo* Linn*)* is the most important fruit crop of Pakistan and is locally known as Kharbooza. It belongs to the family *Cucurbitaceae* that consists of 118 genera and 825 different species (Milind and Kulwant, 2011). Muskmelon is a frost-tender annual crop with squashy hairy trailing stem and clasp tendril. It bears huge encircling to lobed leaf and yellow unisexual flowers with a size of 2.5 cm (1 inch) across. Botanically, fruits are berry which is commonly known as pepo and differ greatly in size, surface, texture, shape, flavor, and flesh color depending upon the variety. The weight of fruit usually ranges between 1-4 kg (2-9 pounds). It is grown as summer fruit and vegetable all over the world. It is mostly cultivated in a warm climate in tropical and subtropical regions of the world and serves as a major food source. Muskmelon ranked 4th among fruits (Parveen *et al.,* 2012). It is grown on an area of 37776 hectare with the production of 551886 tons in Pakistan (Agricultural Statistics of Pakistan, 2017-2018). Throughout the world, it attained significant importance due to its high nutritive value with extraordinary production potential. Different types of nutrients are present in its pulp and seeds. The fruit of muskmelon contains carbohydrates (5%), water (90%), vitamin and protein (1%), while seed contains (40-44%) oil (Parveen *et al.,* 2012). Muskmelon is an excellent source of amino acids and B-carotene. It is a very good source for anti-cancer, anti-diabetic and antimicrobial activity (Milind and Kulwant, 2011). In addition, fruits also contain potassium and folic acid, as well as many compounds beneficial for human health (Lester and Hodges, 2008). Muskmelon is attacked by various bacterial, fungal and viral pathogens which cause various diseases like Downy mildew (*Pseudoperonospora cubensis*), Bacterial wilt due to (*Erwinia tracheiphila*), Powdery mildew caused by (*Erysiphe cucurbitarum*), Alternaria leaf spot caused by (*Alternaria cucumerina*), Anthracnose caused by *(Colletotrichum lagenarium),* Fusarium wilt (Vine wilts) caused by *(Fusarium oxysporum*). Among all diseases, Downy mildew is the most destructive and damaging disease, which mainly affects foliar plant parts. Its causal agent is an Oomycete fungus *Pseudoperonospora cubensis* (Savory *et al.,* 2011). The disease was reported in 1868, first time in "Cuba" by Berkeley on a plant species name "cubensis". At the start, the pathogen was placed in the genus *Peronospora*. The pathogen is an obligate parasite, needs living host tissue for growth and reproduction. It can damage more than 50 plant species of 20 different genera (Lebeda and Urban, 2007). Disease plays a vital role in the quality and yield reduction by the destruction of plant leaves, reduction in the foliar canopy. The infected Plants remain to stunt with immature fruit. In a severe attack, plants and fruit may also die. The aim of this review is to summarize all the information from the last 20 years of research about *P. cubensis*, Disease symptoms, mode of infection, mode of spread, Epidemiology, Disease cycle, and management (complete guide) for future strategies to develop new hybrids and best fungicides in case if pathogen spread in the field to reduce pathogen population below economic losses. Information about downy mildew pathogen, *Ps. cubensis*, including taxonomy, disease development, and management strategies, are summarized, which will be beneficial for new researchers (Table 1).

Brief description about the biology of Pseudoperonospora cubensis

P. cubensis requires living host tissues for its survival and reproduction that's logic behind pathogen comes in the definition of obligate parasite or biotroph. Obligate parasitic characteristics of pathogen reveal that it must overwinter in the region free from hard frost (Southern Florida), including the area where wild cucurbits are being cultivated. The wind is a source for the dispersal of spores from neighboring plants and fields to long distances. Completion of the infection process results from the appearance of symptoms in a short period of 4 to 12 days. Moist and cool conditions are conducive for the reproduction of *P. cubensis*. The pathogen can thrive and do well under a wide range of environmental conditions. Temperature up to 15 °C with the presence of 6 to 12 hours of moisture is the optimum condition for sporulation of *P. cubensis*. High temperature (35 °C) at day time is not favorable for pathogen development while at night temperature become low, which is feasible for the pathogen. Thick-walled resting spores of fungus named oospores are rare with a poorly studied role in nature Nowicki, Marcin *et al.* (2013).

Taxonomic status of Pseudoperonospora cubensis

This pathogen *P. cubensis* belongs to Kingdom Stramenopila, which consists of five acknowledged species: *Ps. cubensis, Ps. humuli, Ps. cannabina, Ps. celtidis and Ps. Urticae* (Young-Joon *et al.*, 2005). Originally the name '*Peronospora cubensis*' was discovered firstly in Cuba by Berkeley and Curtis in (1868). In 1903 *Ps. cubensis* was reclassified after complete observations of sporangia germination as phylum Oomycota; subphylum; Peronospororomycotina Class; Peronosporomycetes (Oomycetes) Order: Peronosporals (downy mildews) Family: Peronosporaceae Specie: *Pseudoperonospora cubensis*. Savory *et al.*, (2010). Recent research on different isolates for *P. Cubensis,* which were inoculated on six different species of cucurbits, shows that the host cellular matrix plays a vital role to influence five different morphological criteria mainly: Sporangiophore length, ultimate branchlets length, sporangial length and width, the ratio between sporangial length and width (Runge and Thines, 2012). This variation is due to the phylogenetic relationship in species of Pseudoperonospora.

Mechanism of infection and perpetuation of Pseudoperonospora cubensis

Although the liberation and dispersion of sporangia take place at low moisture present on the leaf surface, leaf wetness is also an essential factor for spore germination of pathogens that can infect host plant tissues. The optimum temperature for pathogen infection is 15˚C, with a minimum of 2-hour leaf wetness is necessary for infection. Sporangia can also germinate and cause infection with a high level of inoculums at a temperature from 5 to 28 °C (Lebeda and Cohen, 2011). Longer periods of leaf wetness are crucial for disease development, even in the presence of less inoculum. The incubation period may depend upon photoperiod, temperature, duration of leaf wetness and inoculums concentration. The sporangia take 4 to 12 days to germinate and produce 2-15 motile spores through cleavage of the cytoplasm. These are zoospores, biflagellate and preferentially bathes for stomatal openings from the encyst (Lebeda and Cohen, 2011). These encysted zoospores germ tubes produce an apersoria. From that appressorium a penetrating hypha is developed and enters into the leaf tissue through stomatal openings. In the Hyaline coenocyte's hyphae grow intercellularly through palsied tissues and mesophyll cells consequently. Branched haustoria are formed through these mesophyll cells and then invaginate the plant cell membrane. Voglmayr *et al.* (2004). Actually, this type of specialized structure provides a site for nutrient uptake for pathogen also responsible for effector proteins delivery, which plays an important role in redirecting host metabolism and suppression of defense responses. Whisson *et al.* (2007). *P. cubensis* overwintering is retarded in areas among killing frost. It is also reported that a pathogen is an obligate parasite that needs living host tissue for its enduring. The pathogen survives and completes its life-cycle in southern regions, mostly in Florida on both commonly cultivated and Wild host species (Lebeda and Cohen, 2011). The mode of dispersal takes place through winds (anemochory) that can bring spores several hundred kilometers away towards a new host (Lebeda and Cohen, 2011).

Disease expression

The disease development is mainly characterized by the formation of small, faintly chlorotic to bright yellow areas on the lower surface of the plant leaves that later on change into necrotic and brown colors. These lesions are round in shape that is bordered by leaf veins. Sporangiospores turn into distinct colors on the dorsal side of the leaves by producing brown or colorless zoosporangia through the stomata to cause necrosis on the larger leaf area, which results in the death of the whole leaf. The disease mostly starts from lower leaves and spread toward younger leaves (Lebeda and Widrlechner, 2003). As the disease progresses, necrosis occurs very quickly, especially during dry and hot weather conditions; these chlorotic lesions turn into necrotic. Oerke *et al.* (2006). Low temperature may delay symptoms development but enhance colonization of pathogen on host tissue. Under hot and dry environmental conditions, pathogen goes challenging, especially in July, because the temperature at day time 35˚C and at night 25˚C. Also, wetting of leaves takes place simultaneously. Disease plays a vital role in yield reduction and fruits quality by leaves destruction and reduction in the foliar canopy. Dos-Santos *et al.*, (2005).

Climatic conditions conducive for Downy mildew of muskmelon

The Disease triangle is a three-way relation of the plants, the pathogen, and the environment and also the role of humans and time is considered to be potential (disease rectangle). In pathogen, duration of infection, the strain, and the time of infection can contribute to a certain extent, but environment plays a major role for both downy and powdery mildews are privileged by warm and humid weather conditions. Pitchaimuthu *et al.*, (2012). Thus, the epidemiology of the pathogen depends on environmental conditions greatly. Relative humidity and temperature are the important factors that affect variation in disease severity during the growing season of the crop. The life span of the sporangia is not more than 48 hours and during this short time period, sporangia locate on a susceptible host and start germination. The favorable temperature for germination is between 10-20 ºC, whereas the germination rate decreases at warmer temperatures (above 30ºC). *P. cubensis* and its other cucurbits host cannot survive at lower temperatures and mostly, it overwinters in a warm climate. Some other factors like wind, irrigation through the sprinkler system and rain splashes are also responsible for the dissemination of *P. cubensis*. Sporangia can travel long distances with the help of wind (Lebeda and Cohen, 2011).

Table 1. Summary about Downey mildew Pathogen and disease development.

Taxonomic Status	Kingdom	Phylum	Class	Order	Family	Genus	Species
Plant Pathogenic Fungus	Straminipila	Oomycota	Oomycetes	Peronosporales	Peronosporaceae	Pseudoperonospora	*Pseudoperonospora cubensis.*
Geographical Distribution of Pathogen	Eastern USA	North and South Hemispheres	Sweden	Poland	Germany	Western Europe	Central Europe
General characteristics of pathogen	Obligate Parasite or Biotroph	Mainly attack foliar parts of plants	Expression of disease symptoms occurs up to 4-12 days	Pathogen attacks on host plants at all developmental stages, which include seedlings, young and adult plants.	Optimum conditions for sporulation of fungus: Temperature up to 15 °C with the presence of 6 to 12 moisture cubensis	The pathogen has a wide host range. It can infect more than 20 different genera of cucurbits.	Five acknowledged species of *Pseudoperonospora cubensis:* *Ps. cubensis,* *Ps. humuli,* *Ps. cannabina,* *Ps. celtidis* *Ps. Urticae*

Biology of Pseudoperonospora cubensis

The primary and most infective unit for asexual reproduction is (conidiosporangium and zoosporangium) asexual spores. These Sporangia have elliptical or ovoid-shaped and about 15 to 25×20 to 35 µm. The mature sporangial color is almost light-grey to deep-purple. They can easily dislocate from sporangiophores then disseminate through rain droplets and by air. Sporangia need to be in contact with water, rain, or dew for its germination after attachment to the leaf surface of the host plant. Through multinucleate protoplast, germination starts indirectly and changes into a biflagellate zoospore (5 to 15 zoospores) having size 8-12µm, which originate from the papilum. Ojiambo *et al.* (2015). The zoospores swim actively toward the direction of stomatal apertures and patch up by losing their flagella and encyst (Lebeda, 2011). A germ tube consequently grows from the cyst and an appressorium is produced through which a penetrating hypha is formed and penetrates into the leaf tissue via cavity in substomata through stomatal aperture. This is the most common mechanism for the dispersion and penetration of *P.cubensis*. In a rare case, the direct or epidermal penetration may take place. Lebeda *et al.* (2006). The pathogen starts colonization very quickly in host tissue and sporangiophores came forward from stomata in 5-7 days at a susceptible host under favorable environmental conditions, mostly on the lower side of leaves where stomata are more recurrent (Lebeda, 2010). *P. cubensis* has polycyclic disease cycle. New infection on a susceptible host occurs within 7-14 days in favorable environmental conditions (Lebeda and Widrlechner, 2006). Up till now, there is no report for sexual reproduction confirmation in all countries where *P. cubensis* exists. However, sexual reproduction is present in some species, same as Peronosporaceae members, which produce oospores. It takes place mostly at the end of the growing season because infected tissues of floral parts change into necrotic. Form the Austria, unequivocally occurrence of oospores was observed. Also, some record have come from Israel. Cohen *et al.*, (2003). The experiments have no significant results, which were conducted to check oospores on some cucurbits cultivars in the Czech Republic (Lebeda and Widrlechner, 2006). Similar experiments were also conducted in the USA but were unsuccessful. Therefore, it is still not clear whether this pathogen is present by the oospores in Central Europe or the USA (Lebeda *et al.*, 2004).

Management of Pseudoperonospora cubensis

The epidemics of *P. cubensis* have occurred over the last several years, which cause an 80% yield reduction annually. That's why this disease is considered to be the most destructive for cucurbits in Europe (Lebeda and Urban, 2005). Both systemic products and protectants can be applied for the management of the disease. Successful management of *P.cubensis* needs the use of resistant cultivars along with cultural practices and fungicide applications. The cultivar of muskmelon "pusa Mashuras" is tolerant against downy mildew disease (Thamburaj and Singh, 2005). *Trichoderma harzianum* and *Trichoderma hamatum* have seemed the most successful antagonists for the management of downy mildew of *P.cubensis*. Abd-El-Moity *et al.*, (2003). For the biological control against downy mildew, there is only a single report that shows treatment of cucumber seeds by spraying the leaves with the mycoparasitic fungus "*Pythium oligandrum*" delayed the primary infection of the leaves and leaves become durable for a longer time period (Lebeda and Cohen, 2011). In the future, it seems that both biologically mediated systemic resistance and chemicals in cucurbits against *P. cubensis* and some other fungal pathogens will be an inventive part of Integrated Pest Management (IPM) which is currently implemented for the control of plant disease to decrease the application of fungicides (Urban and Lebeda, 2006). Extracts prepared from the dry leaves of a plant *Inula viscosa* was seemed to be efficient against many fungal pathogens (which mainly affect floral plant parts), as well as *P.cubensis*. Wang *et al.*, (2004). These extracts actually inhibit zoospore spread, antifungal, and also control the germination of cystospore (Cohen, unpublished). Registration of these extracts for organic farming is in the procedure. Also, a volatile compound and an antimicrobial material named allicin (diallylthiosulphinate) obtained from garlic extracts (Allium sativum), at the concentration of 50–1000 µgml-1, reduced the disease severity of *P. cubensis* on cucumber by just about 50–100% (Portz *et al.*, 2008). An integrated fungicide program is needed to avoid yield reduction under favorable environmental conditions for the development of disease in order for the protection of crops against sudden outbreaks Savory *et al.*, (2011). Seed treatment with the mixture of Agrosan GN or Emisan at the rate of 2.5g/kg before sowing is also effective against pathogens (Khare *et al.*, 2015).

The fungicide mixture of (metalaxyl + mancozeb) was seemed to be highly effective for control of downy mildew both in the rainy and dry season, while the mixture of (Chlorothalonil and methyl hyphenate) seemed to be very effective only during the dry season. Santos *et al.*, (2004). The mixture contains (0.3 % copper oxychloride and 0.3 % wettable sulfur) spray after every 10-day interval seemed to be very efficient for the control of downy mildew caused by *P. cubensis* (EI-Syed *et al.,* 2020). Fungicides show the most efficient results only when they are applied earlier to infection and repeat sprays at 5 to 7day intervals. Some products have been proved to be the most efficient fungicides for controlling cucumber downy mildew during a trials experiment in North.

Some of the products, including Carolina during the years 2004-2006, Tanos (Dupont, cymoxanil+fenamidpne), Ranman or cyazofamid by FMC, Previcur Flex (propamocarb, Bayer), and Gavel (zoxamide + mancozeb, Dow by Agro Sciences), can be applied to avoid pathogenic resistance (for example, rotated with fungicides having a different mode of action). Protectant fungicides, i.e., chlorothalonil and mancozeb, can also be used as a mixing partner (Brzozowsk,2016). Ridomil Gold (2.5gm in per liter water) gives 92.8% control of disease during a trial experiment at Ayub agriculture research center Faisalabad, Pakistan Ihsan *at el*., (2010). The application of non-proteinic amino acids like BABA (β-aminibutyric acid) induces the resistance of the host plant against downy mildew diseases for *P. cubensis* in many species of cucurbataece. This non-protein amino acid has not had any direct effect on this pathogen, but it can activate the host defense to overcome diseases. BABA was also seemed to be very effective when it was mixed with mancozeb and used for the control of *P. cubensis* in cucumber (Walz and Simon, 2009). Due to high genetic diversity in the pathogen, diverse environmental conditions and wide host range, significant control through any nutritional or plant extract is still unknown. Previous research investigated some aspects for basic control but needs some additional research to clarify further management strategies through any nutritional or biological control. An integrated research approach that includes all important factors for the development of disease will be helpful for the investigation of control measures by biological agents.

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