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Etiology and management of citrus Melanose disease in Pakistan: A review

Muhammad Ateeq[1], Mustansar Mubeen[2], Sonum Bashir[2], Rabia Tahir Bajwa[3,*], Hafiz M. Imran Arshad[4], Aqleem Abbas[5] and Maria del Carmen Zuñiga Romano[6]

[1]College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan, P.R. China; [2]Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, Pakistan; [3]Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Bahawalpur, Pakistan;[4]Plant Protection Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan; [5]College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, P.R. China; [6]Fitosanidad-Fitopatología, Colegio de Postgraduados, Campus Montecillo, Texcoco, México
*Corresponding Author's e-mail: rabia.kpr@gmail.com

Diaporthe species are important pathogens of many hosts, including economically important crops. They cause severe melanose disease, stem-end rot of fruits, and gummosis of perennial plants worldwide. Among the *Diaporthe* species, *Diaporthe citri* is responsible for causing melanose disease. Melanose disease harms fruit, leaves, twigs, branches, and in certain cases, the main stem of trees of any age. The melanose infection can affect practically all citrus cultivars. Citrus melanose disease is a persistent and incurable disease leading to a massive loss of output in the citrus industry. Due to the lack of resistant cultivars, the failure of chemical treatments, and the dangers to environmental health, large-scale research is being conducted to develop a long-term remedy. Recently, biological control agents (BCAs) have been used as an alternative to fungicides to manage citrus melanose disease. In this review, we highlighted some significant aspects of melanose disease (*Diaporthe citri*) in citrus, such as its history, morphology, disease cycle, pathogenicity symptoms, and impact on the economy of the citrus industry. The current literature assists the researchers in averting melanose disease in citrus, developing genetically resistant cultivars, and ecofriendly management against *Diaporthe citri*.

Keywords: *Diaporthe citri*, Melanose, Symptoms, Transmission, Disease cycle, Control management

INTRODUCTION

Citrus is the second-fastest-growing fruit with the largest worldwide market (Lee *et al.,* 2019). One of the most important fruit crops in the *Rutaceae* family is citrus has 130 genera and seven subfamilies (Khan *et al.*, 2015). It is widely grown in 140 countries, primarily in tropical and subtropical regions (Sanofer, 2014). Citrus originated from China and other eastern regions like India, Thailand, and Malaysia. In the fourth century, India introduced citrus in the Middle East, Europe, North Africa, Turkey and Greece. Due to lush green trees, beautiful flowers and delicious fruit, citrus occupy all continents, societies and cultures.

Nowadays, 115 million tons of citrus are produced annually worldwide. Brazil is at the top in citrus production, with 20 million tons, followed by China, with 19.6 million tons. The United States are the third-largest producer with 10 million tons of citrus production. Other important citrus-producing countries are Nigeria, Iran, Mexico, India, Spain and Turkey. Oranges, mandarins, lime and grapefruits are the central grown varieties of citrus. Several citrus cultivars are available to grow, but it is mainly classified into oranges, tangerines, mandarins, grapefruit, citron, limes, sour oranges, pummelos and lemons (Turner and Burri, 2013). Citrus is filled with nutritional advantages. The vitamin C in citrus aids in absorbing iron, zinc, and other nutrients. Compared to other fruits, higher antioxidants are present in citrus, catalyzing our immune system and protecting us from cancer and heart diseases. A good quantity of dietary fiber is present in citrus fruit, which helps digestion and prevents constipation. Low sodium and high potassium content are good for maintaining normal blood pressure. In addition, citrus contains enough nutrients, including carbohydrates, thiamine, calcium, copper, folate, phosphorus, potassium, magnesium, niacin vitamin B and enriches the number of phytochemicals. The citrus market has a size of around 1 trillion won and accounts for the

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majority of agricultural income (Lee *et al.,* 2019). Citrus melanose disease has severely infected citrus worldwide. To manage citrus melanose disease, fungicides are widely used in most orchards. Mancozeb, for instance, has frequently been utilised . Nevertheless, other systemic fungicides, such as strobilurins, have been used as an alternative because when Mancozeb is sprayed on citrus orchards, the total number of helpful insects dropped, and the quality of citrus fruit is diminished since the fungicide damages the fruit peel (Miles *et al*., 2004; Bushong and Timmer, 2000). An excessive amount of fungicide residue in the agricultural product may result from the careless application of fungicides. Thus, it is necessary to design a new protection method that poses less of a danger of systemic fungicide overuse. Recently, several environment-friendly techniques such as natural substances, organic resources and biocontrol agents have been used to control citrus melanose disease. This review summarizes the major disease of citrus in Pakistan, focusing on citrus melanose and its management strategies.

***Major disease of citrus in Pakistan*:** The major cause of poor production is citrus illnesses brought on by fungus, bacteria, viruses, nematodes, and Spiroplasma (Tennant *et al.,* 2009). Spiroplasma causes a citrus stubborn disease; a plant infected with stubborn disease produces 25-32% less fruit than a non-infected plant (Mello *et al.,* 2010). Approximately 30 viral diseases of citrus are known to occur; among these diseases, citrus tristeza virus, yellow vein clearing virus, and citrus ringspot virus are the most important (Ahlawat and Pant, 2003). Three important bacterial diseases of citrus affect citrus yield, citrus canker caused by Xanthomonas axonopodis, citrus variegated chlorosis caused by *Xyllela fastidiosa*, and citrus greening disease caused by *Candidatus liberibacter* (Abutineh *et al.,* 2021). More than forty nematode species are known to be pathogenic to citrus trees; among these, *Tylenchulus semipenetrans* is the most important which causes citrus slow decline. Numerous fungal diseases result in low citrus yield per hectare. Fusarium dry rot caused by *Fusarium solani* is an important soil-borne fungal disease*;* this disease affects the plant's root system, reducing the plant's nutrition uptake ability (Ezrari *et al.,* 2021). This fungus also produces a toxin that moves in the xylem system of the plant and causes vessel plugging. Other fungal pathogens involved in soil-borne diseases are *Diplodia metalensis*, *Armillaria mella*, *Pythium* spp. and *Thielaviopsis basicola*, having a negative effect on yield (Aćimović *et al.,* 2019). Mal secco, a vascular disease caused by the fungi *Phoma tracheiphila*, was first observed in Greece in the 1880s (Tennant *et al.,* 2009). Systemically it enters into the stomata and occupies the xylem; as a result, wilting starts; other symptoms include discoloration of wood, venial chlorosis and twigs di-back. Brown spot of citrus, black rot of citrus and stem-end rot are well-known citrus diseases. Among these diseases, black rot caused by *Alternaria citri* produces brown to black spots on fruit (Mojerlou and Safaie, 2012). Several species of *Colletotricum* are known to cause important diseases of citrus; the most important disease caused by this pathogen is citrus anthracnose, and the characteristic symptom is the withering of twigs and lesions on fruits and leaves (Guarnaccia *et al.,* 2017). Citrus scab, caused by *Elsieno fawcettii* and *Elsieno australis*, severely affects citrus fruit, leaves and shoots under warm and humid weather (Gopal *et al.,* 2014). In 1870, the genus *Diaporthe* was introduced by Nitschke, with *Diaporthe eres* as the type species. It belongs to *Sordariomycetes*, the family *Diaporhaceae*, and the order *Diaporthales* (Maharachchikumbura *et al.,* 2016). Diaporthe species are not host-specific based on pathogen connection with the host, and even a single species can be found on several hosts (Rehner and Uecker, 1994). Plant pathogens depend on Diaporthe sp. Every year, Diaporthe sp. causes billions of dollars in damages. They are also some of the most prevalent pathogens, causing a variety of diseases in economically significant plants all over the world, including citrus melanose, grapevine trunk canker, sunflower canker, apple, pear, and plum rootstock diseases, soya bean stem canker, and canker of the grapevine trunk (Gopal *et al.,* 2014). In 1940 *Diaporthe phaseolorum* var. *batatatis* was found to cause stem canker of soybean (Mena *et al.,* 2020). Symptoms begin as brick-red lesions on the stems and the nodes. As the disease progresses, the lesions become elongated and darken, often griddling the stem. On leaves, interveinal chlorosis and necrosis are evident (Chaisiri *et al.,* 2020). *D. ambigua* was identified as the causal agent of apple canker disease in South Africa (Merwe *et al.,* 2021). The disease initially appears as sunken bark areas around buds, shoot base and leaf scars. With the disease development, the scars become cankers and the bark flakes off (Dissanayake *et al.,* 2017). Sometimes, fruiting bodies of red or white color can also be seen. *D. ambigua* also causes pear canker disease that produces blister-like symptoms on ground level (Smith *et al.,* 1990). In plum rootstocks it also causes canker, symptoms produced by this disease are sunken and pointed lesions of light brown to dark brown colour along with the marginal longitudinal cracks. Fruit begins to show brown centers with water-soaked lesions 3 to 5 days after infection, and the lesions spread to cover the entire fruit. If significant humidity is present, white mycelium will develop behind the lesion. In Texas, new diseases of sunflower i.e., stem canker and leaf spot caused by *Diaporthe helianthi* were found. This disease is characterized by light brown stem lesions with dark brown borders that can vary in size and form. In the afflicted locations, pycnidia were generated. Moreover, petioles, leaves, and leaf scars all developed lesions. The major diseases of citrus are listed in Figure 1. Among these diseases, citrus melanose severely infects the citrus trees in Pakistan.

***Citrus melanose disease*:** D. citri produces two fatal diseases in citrus fruit, including melanose disease, which is brought on by the fruit's ideal stage. A wound on leaves and fruits is classed as melanose disease. Whiteside claims that the asexual stage of this fungus results in "decaying of the stem," a postharvest illness (Whiteside, 1980). First discovered in 1892, melanose was originally discovered near Citra, Florida (Bach and Wolf, 1929). Nevertheless, the diseases weren't verified until 1928, when Koch's postulates were used and D. citri was shown to be the disease's causative agent (Bach and Wolf, 1929). Phomopsis citri, according to Fawcett, is the cause of citrus stem-end rot (Fawcett, 1912). Diaporthe sp. is responsible for several serious citrus diseases. Citrus melanose is one of the most important fruit and foliar disease of citrus caused by *D. citri*. The fruit pulp is unaffected by this disease, but the market value is decreased (Gopal *et al*., 2014).

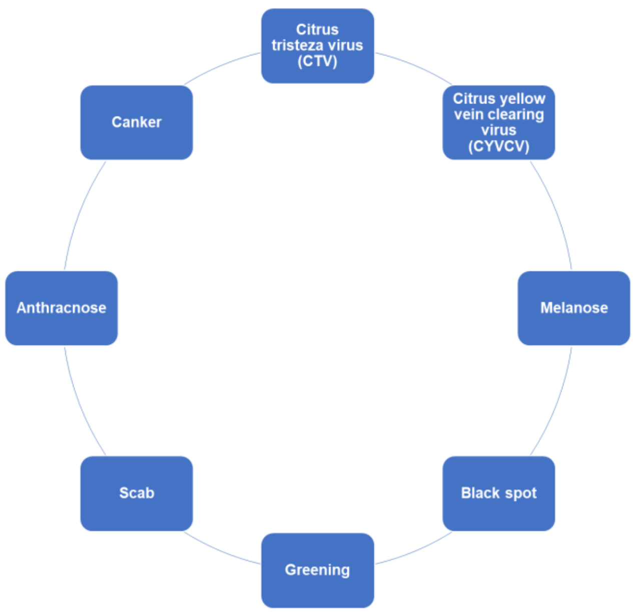

Figure 1. Major diseases affecting citrus trees in Pakistan

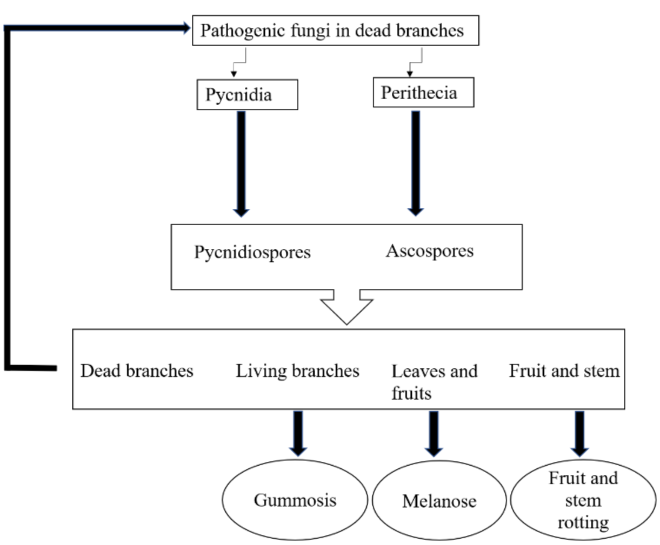

Figure 2. *Diaporthe* spp. are one of the major diseases of citrus, causing gummosis, melanose and fruit stem rotting

***Symptoms*:** The most vulnerable parts of leaves and twigs are their juvenile tissues to melanose disease (Dewdney, 2021). The first symptoms will show four to seven days after infection if the temperature is between 24 and 28 degrees Celsius. When the temperature is lower, the symptoms take longer to manifest. The lesions first look like little water-soaked dots with a translucency yellow halo that gradually vanishes. The rupturing of the cuticle causes the release of gooey material. On the surface of leaves and twigs, this gooey material solidifies, becomes dark, and takes on the roughness of sandpaper. The lesions might be streaked, grouped, or diffused. In extreme cases, the leaves gradually fall off and either stay pale green or turn yellow. Only dead twigs that have either been infected while alive or colonized after death can produce inoculum from structures embedded in the wood. Seldom does the spring growth flush experience significant melanose. The pustules are often small and there is little to no leaf drop when it happens on this flush. In summer, the melanose could be dreadful as it may give rise to the widespread loss, especially in frost-induced twig die-back season (Hanna, 2009). Young green twigs are more susceptible to infection than mature ones.

It might be challenging to treat and remove melanose disease on fruit and foliage. Citrus sinensis sweet oranges, Citrus paradisi grapefruit, and Pumelo are typically affected by the illness (C. grandis) (Turner and Burri, 2013). Although the disease does not affect the edibility of fruit but disease decreases the aesthetic value of fresh fruit in the marketplace. *Phomopis citri* is also known as the causal agent of citrus melanose disease as it produces melanose-like blemishes on Satsuma mandarin fruit (Yamato, 1976). Little dark brown to black patches on fruit and foliage are a few signs. The patches have mud cake and tear-stained patterns and range in diameter from 0.1 to 0.5 mm. The fungus is also known to cause stem end rot in some citrus varieties.

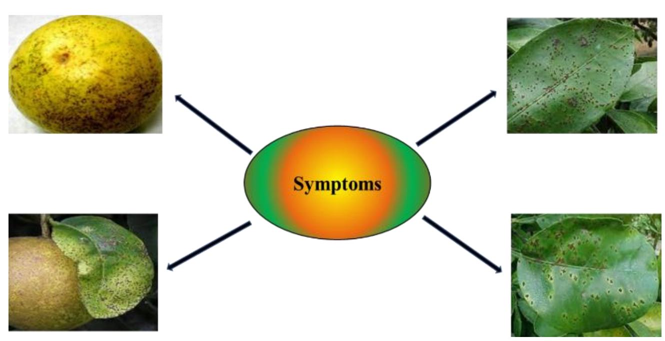

Figure 3. Symptoms on citrus leaves and fruits caused by citrus melanose disease

On the fruit, scattered specks are produced by the light infestation. Young diseased fruit may be tiny and prematurely abscise. Disease progress produces flatter pustules (Gopal *et al.,* 2014). Solid patches of blemish are produced under severe conditions. The disease is called Mudcake when the fruit surface produces rough conditioned cracks. If the infection occurs soon after the petal fall, the Mudcake melanose will occur (Mondal *et al.,* 2007b; Udayanga *et al.,* 2014). Sometimes, copper damages the leaves if copper is applied late during the warm season. There is no relationship between copper damage and melanose disease, but the symptoms are similar. Copper injury causes a corky appearance of tissues and gives a darker appearance than normal fruit.

Spread and etiology of the pathogen**:** Fruit is susceptible to the disease from late June to early July. The symptoms of melanose vary from mud cake melanose, a solid infected area that can cover most of the fruit surface, to small isolated spots. Especially fruit lesions are tear-stain-shaped. The lesions are sandpaper-like on leaves and twigs, distinguishing from rust mite damage and greasy spot. On green fruit, the lesions tend to be a reddish color. The fruit can be stunted if infected early and may drop (Aguilera-cogley and Vicent, 2019). D. citri affects most citrus varieties, although lemon and grapefruit diseases are particularly severe (Mondal *et al.,* 2007a). Melanose is also known as Wolf disorder in all citrus-producing regions worldwide. *D. citri* is reported in seven citrus-producing countries out of the top ten (FAO, 2020). The fruit affected by melanose disease does not remain marketable. Almost 1/5 market value is decreased due to this disease compared to non-infected fresh fruit having no blemishes. The disease significantly impacts farmers and exporters due to quality reduction. In all citrus-growing regions of the world, several diseases are present. The disease becomes more destructive in sub-tropical areas under moisture conditions and has a comparatively lower incidence in the misty tropic; however, it has the least incidence in the dry area. Although the growth of trees and fruit yield are not affected by diseases, it is the aesthetic quality of fruit. The fungal diseases, specially melanose, are responsible for the losses of thousands of dollars per hectare by reducing 10% of fresh-market in Florida (Timmer *et al*., 1998). Any life cycle stage, such as teleomorph or anamorph of *D. citri* and *P*. *citri* Fawc, severely affects citrus, respectively (Udayanga *et al.,* 2014). Generally, the fruit and the leaves are more susceptible to the pathogen. The *P*. *citri* also causes stem-end rot in citrus and has a saprophytic characteristic; it infests dead twigs of the tree (Dewdney, 2021). Wolf fungi have two-celled hyaline ascospores; each cell consists of two oil droplets or guttulae. At the septum, the ascospores are slightly constricted and the size of ascospores ranges from 11.5-14.2 microns by 3.2-4.5 microns (Gopal *et al.,* 2014). Inside the perithecium, the ascospores are developed in a flask-shaped body. The shape of the perithecia is round, compressed in the bottom with long thin beaks structure which comes out. Beaks size area ranges from 200 to 800 micrometers in length and 40-60 micrometers in width, while perithecium size area ranges from 125-160 micrometers in diameter (Whiteside, 1977, 1980; Wolf, 1926). The stromata of perithecia widely spread inside the bark; the perithecia obtrude from bark and can be seen easily in the microscope. The ascospores are windborne and, therefore, responsible for the pathogen's long-distance spread; the ascospores are forcibly discharged from the asci (Wolf, 1926). By finding an appropriate place, one or both spores can be sprout by making hyphae which rapidly become septate mycelium (Whiteside, 1988). The mycelium is of fan-shape in culture media having white color (Timmer *et al.,* 2004).

Disease cycle**:** The conidia of fungus play the most important role in the disease cycle. Wolf fungi make two kinds of conidia: alpha conidial and beta conidial (Gopal *et al.,* 2014). The alpha conidial spore has properties like single-celled, functional and two guttulae with hyaline color having size ranges from 5-9 micrometers by 2.5-4 micrometers. The beta conidial spore developed in mature pycnidia compared to the alpha conidial spore and the beta conidial spores are longer in size, slim tubular-shaped and cannot grow in one end. Rain splashes spread the conidial spores to near substrates with great efficiency and the conidial spore submerged into sticky material in erumpent pycnidium. Then the conidial spores germinate, forming a mycelial body on the substrate and starting a new cycle (Whiteside, 1988; Wolf, 1926).

Management**:** present in the field. Preventive measurement against melanose disease is better than cure; hence sanitation and elimination of dead wood from disease parts, especially in older trees, are required. Melanose management is not typically required in small groups of trees, less than 10-12 years mature citrus plants, or in those where the fruit is intended for processing. All citrus varieties are susceptible to melanose disease, but grapefruit is more vulnerable and quickly affected.

Chemical control**:** Chemically controlling citrus diseases using copper, strobilurin and other fungicides is common in several citrus-growing regions (Bushong and Timmer, 2000). A supplement of potassium and phosphorus is also employed to activate and boost the natural disease tolerance in plants (Table 1). In general, many fungicidal sprays are used to minimize the hazardous effects of *D. citri*, such as; disodium ethylene bisdithiocarbamate, di-chloro naphthoquinone, 2-2, dihydoxy-5-5 di-chloro dimethyl methane, 2-heptadecyle glioxaldine, hydroxyethyl a-heptadecyle glioxalidine, ferric dimethyl dithiocarbamate, 8-hydroxyquinoline sulfate and polyethylene polysulfide Suit, 1948).

Copper Fungicides**:** As discussed above, young leaves and fruits are most susceptible to melanose disease, so fungicide application at an early stage may be necessary for control measures. Copper pesticides act as fungicides with compounds such as copper oxychloride, copper sulfate and copper hydroxide, controlling citrus diseases Sahi et al., 2012). Due to disease severity, regular and frequent fungicide applications may be required. Copper fungicides are widely used to control citrus melanose. After applying chemical sprays, especially copper spray, to citrus leaves and fruits, star melanose symptoms may appear that differ from those discussed on unsprayed leaves and fruits (Giesler, 2000). Postharvest treatments and storage conditions of fruits are ineffective in reducing melanose disease damage to citrus rinds. Copper fungicides are inexpensive and more suitable for citrus growers to control melanose disease. However, in high temperatures and rainy season, copper fungicides can change fruit texture, so the use of strobilurins (Do not use more than four times of the strobilurin-containing fungicides/season) at that time will avoid any damaging effects on fruit and control fungal diseases (Miles *et al.*, 2004).

Table 1. Application of fungicides against citrus melanose disease (Dewdney, 2021

Pesticides	FRAC/MOA	Mature tree/Acre
Copper fungicide	M1	Use as on label
Pristine	11/7	Do not apply more than 74 oz/acre/season for all uses.
Gem 500 SC	11	Do not sue more than 15.2 fl oz/acre/season for all uses.
Gem 25WG	11	Do not apply more than 32 oz/acre/season for all uses.
Quadris Top	11/3	Do not apply more than 1.5 lb ai/acre/season azoxystrobin
Headline	11	Do not apply more than 54 fl oz/acre/season for all uses.
Abound 2.08F	11	Do not use more than 92.3 fl oz/acre/season for all claims.

***Strobilurins fungicides*:** The metabolic process, especially respiration, is very important in living organisms. That's why Strobilurin fungicides are frequently used to hamper the respiration mechanisms in fungi. Therefore, strobilurin fungicides become a treasurable tool to control fungal diseases like greasy spots, black spots, scabs and melanose (Miles *et al.*, 2004). Some strobilurin, viz. azoxystrobin, pyraclostrobin and trifloxystrobin, are commercially used against fungal infection according to label instructions with seasonal limitations (Dewdney, 2021).

Fenbuconazole fungicides: Some triazole fungicides that hinder sterols composition are fenbuconazole, fenbuconazole and fenethanil (Gopal *et al.*, 2014). It has been reported that in Florida since 1999, fenbuconazole fungicides are frequently used when copper fungicides do not work properly (Facts and Regions, 2011). Another study showed that citrus growers applied fenbuconazole to control melanose and other fungal diseases. (Yesmin *et al.*, 2017).

***Other fungicides*:** The strobilurin-containing fungicides can be used to avoid phytotoxicity, but the same restrictions should be followed as in the case of greasy spots (Table 2). The residual activity of strobilurins is shorter than copper, so more frequent applications are needed. Applying the strobilurin chemical class, such as pyraclostrobin, to citrus plants significantly controls fungal disease in spring (Mondal *et al.*, 2007b). Some non-systemic agricultural fungicides like mancozeb are also useful (Chen *et al.*, 2010; Jiang *et al.*, 2012). It has been reported that strobilurin can be used in any season for melanose but are less effective than copper fungicides (Timmer *et al.*, 2011). Literature has investigated that strobilurin fungicides were unsuccessful during the rainy season due to fungal resistance (Jiang *et al.*, 2012).

Table 2. Fungicides are being used to control citrus melanose disease (Dewdney, 2021).

Fungicide	Mode of Action
Enable 2F (fenbuconazole)	3
Petroleum oil	—
Priaxor (pyraclostrobin + fluxapyroxad)	11 + 7
Amistar Top (azoxystrobin+ difenoconazole)	11 + 3
Ferbam (ferbam)	M03
Blockade 50WG (acibenzolar-S-methyl)	21
Luna Sensation (trifloxystrobin + fluopyram)	11+7

Non-chemical control

***Cultural practices and host resistance*:** Non-chemical pest management strategies are also potential methods to reduce disease impacts. Numerous tactics include planting virus-free stocks of citrus; sowing disease-tolerant cultivars and varieties; sustaining actual deployment of herbicide strips; minimizing or abolishing sprinkler irrigation; and proper drainage and irrigation management (Tennant *et al.*, 2009). It has been previously reported that some bio-based materials such as extract hydrolysate from brewer's yeast, strain KRL-AG2 of *Trichoderma harzianum*, QST 713 strain of *Bacillus subtilis*, GI-21 strain of *Gliocladium virens* and extract of *Reynoutria sachalinensis* are also effective in controlling fungal diseases (Sultana and Ghaffar, 2013). There are many non-chemical cultural ways to minimize the fungal attack in citrus (Facts and Regions, 2011; Tennant *et al.*, 2009; Tjosvold *et al.*, 2008). Some practices to control citrus melanose disease using non-chemical methods are as follows;

1. Sanitation: Periodically prune away dead branches. This will reduce pathogen survival, increase air circulation to dry out the canopy, and allow for more effective fungicide penetration and foliage coverage. Pick up and destroy plant materials that have fallen from the citrus canopy.
2. Citrus varieties: Avoid planting extremely susceptible citrus varieties or species (sweet orange, grapefruit) in high-rainfall areas.
3. Choice of planting location: Plant citrus in regions with sunny environments and low rainfall.

4. Cropping system: Interplant citrus with non-susceptible hosts (avoid mono-crops).
5. Seed germination of different weed species can keep down by using sanitation practices.

In addition, there are many traditional ways to eliminate annual weeds effectively. Seed production during plant and crop weeds' life cycle must be ruined with mechanical cultivation methods. Even so, during the cultivation process, some seeds of weeds get mixed with the soil and germinate with the crops. During this process, seeds of weeds can spread in the soil and germinate routinely. Sometimes cultivation can be the short-term control method as the weeds seeds get dispersed in the soil, but it rejuvenates the perennial weeds by increasing the number of buried seeds and frequent dissemination. Continuous cultivation also demolishes the fibrous roots of citrus and grows unevenly in the soil. Trimming or mowing practices in citrus farms can also eliminate weeds but is very expensive. Hence, all mechanical strategies must be performed before seedhead formation to reduce seed dispersion and re-infestation.

***Biological control*:** Chemical fungicides are widely used to control citrus melanose disease. However, increased use of chemical pesticides to control such diseases causes several negative effects, such as developing pathogen resistance, deterioration of human health, and environmental risks Tennant et al., 2009). Therefore, biological control agents (BCAs) are considered suitable Various microorganisms, such as plant growth-promoting rhizobacteria (PGPR), have many potential uses as biocontrol agents to enhance plant growth and manage citrus melanose disease. PGPR which is used as biocontrol agent, produces cell wall degrading enzymes, including lytic activity and antibiotics to restrict the pathogen growth. Their mechanisms also include competition for space and nutrients and induction of systemic resistance. Although most studies have concentrated on the effectiveness of biocontrol, field application has not yet been researched.

***Conclusion and future aspects*:** In conclusion, it is confirmed that melanose is a severe problem in citrus-growing areas of Pakistan, and it should be adequately controlled on an emergency basis. If it remains uncontrolled, citrus production gets severely affected in a few upcoming years, leading to huge economic losses. Alternatives to chemicals, such as eco-friendly strategies effectively control citrus melanose disease and protect the environment. Plant biochemicals and allelochemicals can also be used to control citrus melanose disease. However, before chemical application, the chemical's toxicity in the agricultural environment or their influence on human health should be properly checked. In addition, new tools such as sensors should be installed in the citrus orchards to monitor and detect citrus melanose disease timely to prevent future outbreaks.

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