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# GREENHOUSE-GROWN TOMATOES: MICROBIAL DISEASES AND THEIR CONTROL METHODS: A REVIEW

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**ABSTRACT**

The cultivation of tomatoes in greenhouse structures is becoming increasingly common as a crop production system. However, the environmental conditions inside a greenhouse favour the development of microbial diseases. These diseases have an adverse effect on the tomato yield and can lead to serious economic losses. This review will give an insight into the major diseases affecting greenhouse-grown tomatoes, the respective causal agents and recommended control strategies. Some of the major diseases are of a bacterial, fungal, viral or nematode origin and include bacterial spot and speck, bacterial canker, early blight, gray mold, leaf mold, powdery mildew, tomato mosaic virus, tomato big bug and root knot. For each disease, the symptoms and risk factors responsible for disease development are described. In addition, the different structural designs and set-ups of tomato greenhouses are covered along with their advantages and limitations, especially any adverse effects on yield and susceptibility to disease. Although conventional control measures are discussed, greater emphasis is laid on the use of alternative biocontrol measures that include Effective Microorganisms, natural antimicrobials and nanobiopesticides. Moreover, information gathered in this review is based on a combination of available literature and expert guidance. This compilation is hoped to be instructive for tomato growers opting for greenhouse farming and assist them in the application of timely and more effective control measures.

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## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is among one of the world's largest cultivated vegetable crops after potato and sweet potato and is well known for its nutritive benefits. It is planted in almost every country-not only in the open fields, but also in the greenhouses or net houses. Tomato is part of the *Solanaceae* family which consists of other members namely, potato, peppers, eggplant and tobacco (Gebhardt, 2016) and originates from the South American Andes (Naika *et al.*, 2019). Tomato was then introduced to Europe in the sixteenth century and later distributed into eastern and southern Asia, the Middle East and Africa (Naika *et al.*, 2019).

Tomato fruits are usually eaten fresh in salads or cooked during preparation of soups, sauces, fish or meat dishes. Tomatoes are also processed into juices and purees. They are an essential ingredient in Italian cuisines, especially in the making of pizzas. In India, tomatoes are a popular additive due to their tangy flavor. Moreover, products such as dried and canned tomatoes are also economically important as these products are affordable when the production is poor and during off-seasons.

Tomatoes are made up of 95% water, 4% carbohydrates and less than 1% of fats and protein (Bjarnadottir, 2019) and are an important part of a healthy and well-balanced diet. The fruit is rich in vitamins, especially vitamins B and C, sugars, essential amino acids, dietary fibres (Leonardi *et al.*, 2000) as well as minerals such as iron and phosphorus. Red tomatoes are also high in vitamin A and lycopene, an antioxidant molecule with known anticancer properties (Naika *et al.*, 2019). Both raw and cooked red tomatoes are an excellent source of lycopene. However, when tomatoes are cooked or processed, a larger amount of lycopene is bioavailable since the molecule is released from the fruit during the cooking process. Studies have demonstrated that consumption of tomato fruits and tomato-based products more than likely reduce the risk of getting several types of cancers including prostate, stomach, cervical and breast cancer (Lippi and Targher, 2011).

Tomato production is increasing exponentially in the world due to the concomitant rise in consumption (Khan, 2018). During the period of 2017- 2018, there was an increase of 3.5% in the global tomato production from 182M to 188M (Khan, 2018). In 2021, worldwide production of tomatoes attained 189,133,955,040 kg over 5,167,388,000 $m^2$ of cultivated area (FAO, 2021). It is expected that the tomato sector will continue to expand in the coming years (2020-2025) to reach USD 51930 per kg of tomatoes being produced globally (Branthôme, 2020). Because it is a short duration crop and produces significant yield, its cultivation area is expanding daily. Tomato production is a means whereby most semi-urban and rural producers in developing countries obtain their source of income. China, India, Turkey and the USA are among the top-listed countries that excel in the production of large quantities of tomatoes (Nag, 2020). According to a survey in 2017, China was found to be the highest rated country with a tomato consumption per capita of 59,626.9 kg/year followed by other countries (Nag, 2020). Moreover, China and India produced 676,367,248,40 kg and 2.1181 X $10^{10}$ kg of tomatoes respectively, in the year 2021 (FAO, 2021).

Tomato is an all-year-round crop that can attain a height of 1-3 metres. It is a sprawling herbaceous crop having a fragile woody stem, yellow flowers and fruits that differ in shapes based on the types of cultivars (Figure 1). Fruit size can range from 1-2 cm diameter for cherry tomatoes to at least 10cm for beefsteak tomatoes. The fruits can be either yellow or red although most cultivars produce red fruits during the ripening stage.


Figure 1. Tomato plant and morphology of fruits of few tomato varieties (Duford, 2022).

The optimum temperature for the growth of tomato plants ranges from 23 to 27°C. If the temperature falls below 15 °C or rises above 35°C, these conditions will have an adverse effect on tomato production (Naika *et al.*, 2019). Warm temperatures and abundant sunshine are ideal for the growth of tomatoes. A decrease in the level of light results in a reduction of fruit yield in winter. Well-drained soil is best to grow tomato plants where it can develop a well-spread and deep root system. The optimum pH of the soil is 6-7.5 (Naika *et al.*, 2019). It grows well during the dry season because it does not need a large amount of precipitation for germination.

The tomato crop can be cultivated under both outdoor and indoor conditions. In the last decade, tomato production in protected and controlled environments, for instance in high tunnels and greenhouses, has become a new trend in agriculture. The advantages of tomato production in greenhouses over open-field cultivation include: i) maintaining a warmer interior of the greenhouse, especially during winter seasons, ii) easier weed management, iii) higher yield, iv) greater weed control, v) easier tomato staking, vi) lower susceptibility to pests and diseases, vi) easier management of microbial diseases and viii) less labor, once the setup of the greenhouse is complete.

**Greenhouse Set-up for Tomato Cultivation**

Greenhouse-grown tomatoes are thought to be of the best quality (Toor *et al.*, 2006). A better shelf life and enhanced flavor are two major aspects that growers target when opting for the production of tomatoes in greenhouses. In fact, greenhouse tomatoes are more appealing to most markets as consumers prefer to savor fresh tomato fruits and they are prepared to pay a premium price.

Tomato crops can be planted in different types of greenhouses, as long as the structures are suitably high to support the growth of the plants vertically (Figure 2). Usually, inside greenhouses, the light intensity is reduced to about 65% of the outside light due to light being reflected off the greenhouses and also due to the interception of light by frames and covering materials of the greenhouses. A decrease by 1% in the light intensity can bring about a 1% decrease in the yield (Dannehl *et al.*, 2021). Thus, the level of transmitted light through the greenhouses should be high enough for proper development of the crops and should range between 70% and 81% (Nederhoff and Marcelis, 2010).

Figure 2. A high-structured greenhouse with an arch roof system that allows the vertical growth of the tomato plants (Copyright: (Mamode Ally *et al.*, 2021)).

The frames of the greenhouses are usually made of galvanized steel or aluminium with vertical sidewalls (3.5 – 6m) and an arched roof (Figure 2) (Von Zabeltitz, 2011). This type of design allows the frame to be covered with double-layered plastic and be attached to other greenhouses at the gutter to produce a larger well-spaced growing unit. With the implementation of high sidewalls, this facilitates the crop to grow taller and allows for climate control gadgets (fans, heaters, lights or screens regulating light intensity) to be fixed above the crops.

Light penetration inside the greenhouse structure depends on its covering material which can be of different types namely rigid plastics, glass and polyethylene plastic film. The plastic film coverings may either be single or double-layered. In cold areas, to conserve maximum energy inside the greenhouse, an insulating layer of air (approximately 10cm thick) is maintained between the double layers of the plastics (Kubota *et al.*, 2018). However, extremes in temperature, air pollutants or ultraviolet radiation can decrease the longevity of all kinds of plastics. Normally, the replacement of polyethylene coverings takes place every 2 to 4 years in order to continue having a good light transmission. However, there are some technologies that are available to modify the properties of plastics so as to increase their durability. Newly manufactured plastics can now minimize 20% of the heat loss that takes place (Bartok, 2013). Wavelength-selective plastics are also manufactured with the purpose to decrease insect pressure, reduce disease incidence or to control the height of the plant (Kubota *et al.*, 2018).

Rigid covering plastic materials, for instance, acrylic (polymethylmethacrylate), polycarbonate or polyvinyl chloride have an efficient light transmission that lasts for at least 10 years and also helps in energy conservation (Teitel *et al.*, 2017). Rigid plastics cost more than polyethylene films (Teitel *et al.*, 2017) but are as strong as glass coverings and can be fixed as large panels to decrease the shading caused by support structures. In countries such as the USA, Mexico and Canada, the covering of greenhouses is mostly with double-layered plastic film rather than with rigid plastic or glass (Teitel *et al.*, 2017). Greenhouses made of glass, on the other hand, allow more light transmission (Holsteens *et al.*, 2020) and need only to be regularly cleaned. However, glass is more expensive than the polyethylene plastic film but is less costly than the use of rigid plastics (Dalai *et al.*, 2020). In Europe (north-west region), glass is usually used to cover greenhouses due to the economic advantage of light transmission (Karanisa *et al.*, 2022).

In greenhouses, tomatoes can be cultivated either in soil or without soil media. The soilless technique is known as hydroponics and makes use of nutrients dissolved in an aqueous solvent. Greenhouses with concrete flooring enable extensive cleaning of the floor using hot water to kill any pests and diseases remaining between crop rotations. In cases where concrete floors are not affordable, the ground can be covered with a black cloth to suppress the growth of weeds followed by the addition of a white layer to enhance the reflection of light inside the structure (Meuldijk, 2020).

Occasionally, the plants are cultivated in net houses. There are two types of net houses: i) insect-proof houses that comprise of 50-mesh net that prevents the entry of insects and provides 17 to 20% shade and ii) common black or white netting that provides about 25-35% shade (Maraveas, 2020). These two types of greenhouse nets protect the tomato plants from wind, sand, extreme sunlight, hail and insects.

Currently, there is significant effort to efficiently reuse the wastewater generated in most greenhouses to reduce the application of pesticides and for irrigation purposes. These measures are thought to lead to significant cost savings in the long term. In greenhouses where hydroponic farming is carried out, bacteria and fungi can likely grow in pipes that carry the nutrients and water to the crops (Mellifiq, 2020). Thus, it is crucial to have a suitable technology that also permits effective disinfection of the water before it is reused. Ozone ($O_3$) is a novel technology that can ensure large scale disinfection and treat the wastewater that has been contaminated with a high level of pesticide residues (Figure 3) (Mellifiq, 2020). The use of ozone has proven to be highly beneficial due to its potent disinfection capacity and its residue-free characteristics (Mellifiq, 2020). Besides the disinfection of irrigation water, breakdown of residual ozone produces oxygen which enhances growth of crops (Mellifiq, 2020).

**Diseases Affecting Greenhouse-Grown Tomatoes**

Tomato crop is susceptible to over 200 known diseases during cultivation or at the post-harvest stage (Singh *et al.*, 2017). In fact, plant diseases are the main limiting factor for the production of tomatoes and lead to significant economic losses (Singh *et al.*, 2017). These plant diseases can be categorized into two groups. The first group includes those diseases that are caused by pathogenic microorganisms, namely, bacteria, fungi, viruses and nematodes. When the prevailing environmental conditions are optimal, these microbial pathogens are infectious and can spread rapidly from one plant to another (West *et al.*, 2012). The second group consists of those caused by non-contagious chemical or physical factors, for instance, extreme weather conditions, physiological or nutritional disorders and herbicide damage. The occurrence of these two diseases categories is often linked to the impacts of climate change. A rise in the level of carbon dioxide, higher temperature and changes in the rainfall pattern can have an adverse effect on crop diseases and their control, resulting in more frequent and severe incidence of diseases (West *et al.*, 2012). Microbial diseases affecting greenhouse-grown tomatoes will be discussed in greater detail while non-microbial diseases of greenhouse-grown tomatoes fall outside the scope of this review.

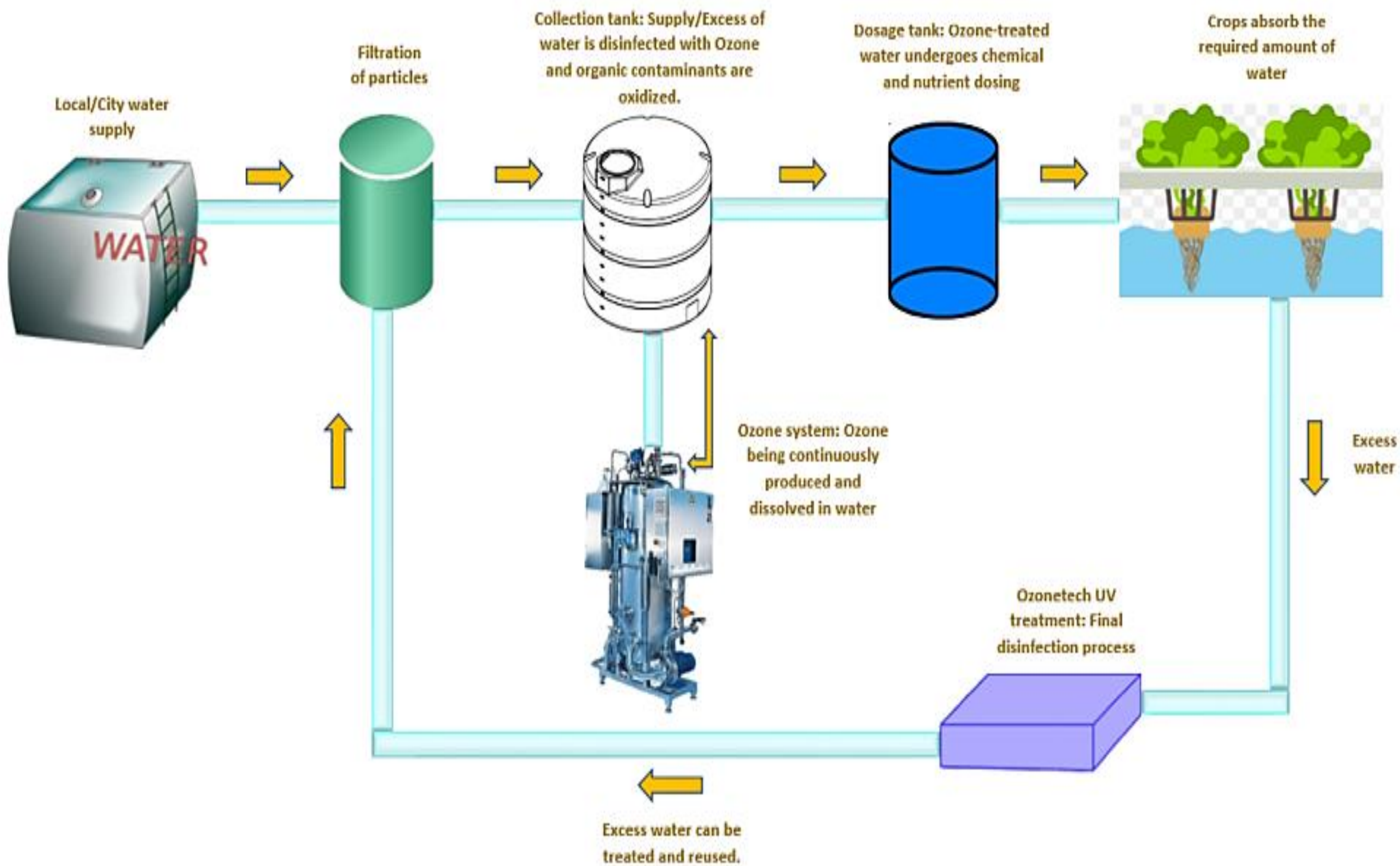


Figure 3. Illustration depicting the setup of the Ozone water treatment system that can be adopted in a greenhouse facility (Adapted from Mellifiq (2020)).

**Bacterial Diseases**

Tomato diseases due to bacteria can have severe and destructive consequences affecting both greenhouse and field-grown crops (Kolomiets *et al.*, 2017). In greenhouses, under moist conditions, total crop losses due to bacterial diseases can take place (Kolomiets *et al.*, 2017). The major tomato diseases occurring in greenhouses are bacterial canker, bacterial speck and spot and tomato pith necrosis.

**Bacterial Canker**

Bacterial canker is a destructive disease that has a worldwide distribution. Its incidence is usually sporadic; however, it can result in significant crop losses if the disease is not properly managed. It is known to infect both tomato crops and their seedlings in the greenhouse (Sabaratnam, 2018). Bacterial canker was first spotted in 1909 by E. F. Smith in Michigan and was originally known as the Grand Rapids disease (Jones *et al.*, 2016).

Bacterial canker is caused by *Clavibacter michiganensis* subsp. *Michiganensis.* It is a Gram positive, coccoid to rod-shaped, non-spore forming, aerobic, non-motile and non-acid fast bacterium. It forms yellow and smooth colonies that have entire margins on nutrient agar and can reach a diameter of 2-3mm after 5 days post-inoculation.

The main symptom is the wilting of the tomato plant. Initially, cream-colored spots having a diameter of 1-2 mm can sometimes be observed on leaflets. As the level of infection progresses and the plant becomes more mature, wilting can be observed of the leaflets, eventually displaying greenish-gray patches on the collapsed tissue. Secondary infection arises when necrosis at the edge of the leaves occurs and makes its way inward (Figure 4A). There can also be the development of adventitious roots. Afterwards, yellow-brown cankers may form on the stems and petioles. If the diseased stem is cut from the end, yellow bacterial ooze can be seen emerging from it upon squeezing. The vascular tissue also acquires a dark-brown colour (Figure 4B) and small white spots can be observed on the tomato fruits (Figure 4C). These spots then enlarge and change into a light-brown colour which become enclosed by a white halo and elevated in the center, giving rise to the characteristic "bird's eye" spots. These lesions can attain a diameter of 3-6mm (Figure 4D). In some instances, infected greenhouse-grown tomato fruits may also display a netted look.


Figure 4. (A) Foliar lesions start to develop eventually leading to wilting of the plant (B) Discoloration of the vascular tissue (C and D) Bird-eye symptoms on fruits (Grabowski, 2018; McGrath, 2021).

Bacterial canker is a seed-borne disease that can occur under prevailing temperatures of 18-24°C and relative humidity greater than 80% **(Suresh *et al.*, 2017)**. Low intensity of light, high levels of nutrients specifically nitrogen, sandy soil and optimum soil moisture content are all risk factors for the disease. The bacterium can also thrive on plant debris for extended periods and can live on weeds outside the greenhouse structure, tomato seeds and plants and on greenhouse equipment **(Suresh *et al.*, 2017)**. Generally, this pathogen enters and grows in the xylem tissues through injuries or other natural openings (Nandi *et al.*, 2018). This hinders the water transport system inside the vascular vessels and thus leads to wilting of the tomato plant. The crops can survive the disease and may even yield commercial fruits, however, they remain a prominent source of infection for the following growing season (Nandi *et al.*, 2018).

**Bacterial spot**

Bacterial spot is ubiquitous in areas of tomato cultivation. However, the disease is more damaging in subtropical and tropical areas where there is presence of a high level of precipitation (Blancard, 2012). Bacterial spot was first detected in the United States (1912) and later in South Africa (1914) (Jones *et al.*, 2016) and was identified by E. M. Doidge in 1921. The disease causes defoliation with ensuing reduction in yield (Jones *et al.*, 2010). Bacterial spot is also manifested by the formation of circular, brown and water-soaked spots, of at least 3mm in diameter, on the stems, leaves and fruits (Figure 5). Leaflets with lots of lesions on their surface can become yellow (Figure 5A). Fruit spots start as small, slightly elevated blisters. As the disease progresses, the spot on the fruits enlarges, becomes scab-like, slightly elevated and brown in colour (Figure 5B, C).

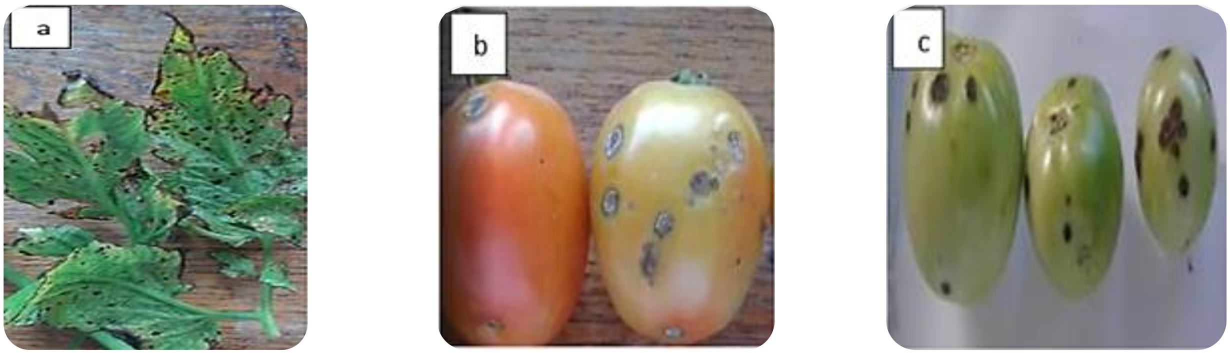


Figure 5. Tomato leaves and fruits infected by bacterial spot (A) Brown circular spots are observed on tomato leaves (B, C) Water-soaked and raised spots developed on the surfaces of the tomato fruits (Copyright: (Mamode Ally *et al.*, 2021)).

*Xanthomonas* species are responsible for bacterial spot and include *X. euvesicatoria* (race 1), *X. vesicatoria* (race 2), *X. perforans* (race 3 & 4) and *X. gardneri* **(Suresh *et al.*, 2017)**. *Xanthomonas* is a Gram negative, rod-shaped (0.7-1.0 X 2.0-2.4 μm), strictly aerobic and motile bacterium. The organism grows slowly on nutrient agar and produces circular, shiny, wet, entire and yellow colonies due to elaboration of xanthomonadin pigment.

Optimum temperatures of 24-30°C or overhead irrigation are ideal for the spread of the pathogen (Blancard, 2012). The bacterium can survive in tomato seeds, plant debris, weeds and can be found on tomato transplants that show no sign of infection **(Suresh *et al.*, 2017)**.

**Bacterial speck**

Bacterial speck and its causal agent were first observed and identified in Taiwan and the United States (Jones *et al.*, 2016) in 1933. Two races of the pathogen have been discovered; race 0 having a worldwide distribution and Race 1 only observed in Canada, Bulgaria, Serbia, United States and Italy **(Suresh *et al.*, 2017)**.

The seed-borne agent, *Pseudomonas syringae* pv. *tomato,* responsible for the disease, is a Gram-negative, rod-shaped (0.69-0.97 X 1.8-2.8 μm), oxidase-negative and strictly aerobic organism. Upon culturing on King's B media (King *et al.*, 1954) it produces a green pigment that can be seen under ultraviolet light. Disease onset is characterized by formation of round, dark brown or black lesions on the leaflets. With time, a halo surrounding these lesions begins to develop (Ahmed, 2022). The whole leaf subsequently becomes affected and symptoms are more prominent on the abaxial surface. Shrinking of these spots causes necrosis of the tissue (Figure 6A). The disease also affects the stems, petioles and sepals with the appearance of oval and elongated lesions on these plant regions. On the fruit, tiny dark, elevated or sunken lesions (speck) of at least 1 mm in diameter, can be seen surrounded by a dark green halo (Figure 6B, C).

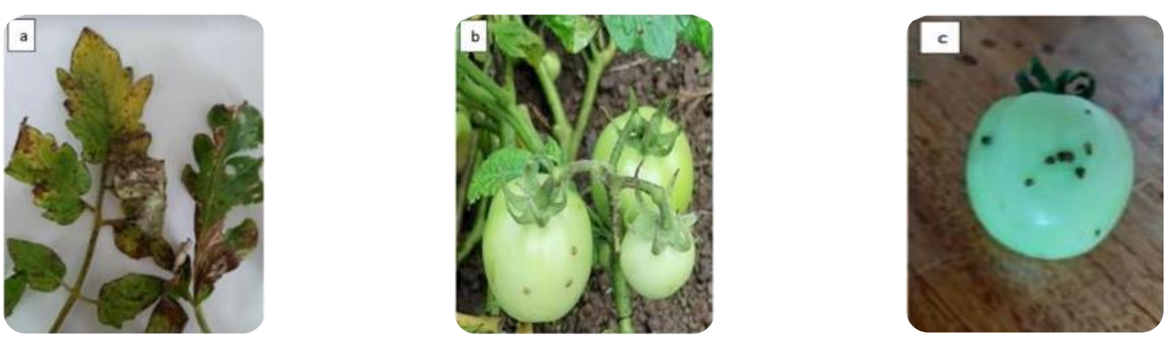


Figure 6. (A) Dark brown specks are formed and yellowing of the leaves starts to occur (B, C) Tiny raised lesions formed on tomato fruits (Copyright: (Mamode Ally *et al.*, 2021))

Bacterial speck is frequently observed in greenhouses where a cold environment prevails due to absence of heating systems and condensation of water vapor occurs. Low temperatures (18-24°C) and high humidity are generally favorable for the growth of the bacterium (Blancard, 2012). Moreover, the pathogen is seed-borne and can survive on various crops, weeds and untreated soil (Blancard, 2012). It can also be spread via infected tomato transplants.

**Tomato Pith Necrosis**

High incidence of Tomato Pith Necrosis usually occurs in traditional greenhouses and tunnels although it can also occur in field-grown tomatoes. This disease was first observed in 1978 in the UK and since then, it has spread to various parts of the world (Blancard, 2012).

*Pseudomonas corrugata* is mainly responsible for this disease. It is a Gram negative, rod-shaped, nonfluorescent, strictly aerobic, oxidase positive bacterium and contains one or more polar flagella. It forms yellow, round and raised colonies with sometimes a greenish coloured centre and produces a diffusible yellow-green pigment on nutrient-dextrose agar after 48 hours. The appearance of the colonies is normally wrinkled and the maximum temperature needed for growth is 37°C.

Disease symptoms include wilting and chlorosis at the top part of the plant with necrosis occurring on the lower stem (Figure 7A). Gray or brown lesions can also be seen on the surface of the infected stem. The appearance of the diseased parts of the stems can look rigid but when cuts are made longitudinally inside the stem, a hollow pith can be observed. In some cases, plants may not exhibit any overt lesions but a brown discoloration of the vascular tissues may be observed upon cutting (Figure 7B). Development of abundant adventitious roots may also occur in areas where the pith is infected (Figure 7C) and death of the plant is possible if the lower stem is infected **(Suresh *et al.*, 2017).**


Figure 7. (A) Wilting occurred at the top of the plant (B) Browning of the stem's vascular tissues and (C) Numerous adventitious roots are developed in response to the disease stress caused by the bacterium responsible for pith necrosis (Copyright: Mamode Ally, 2021).

Factors encouraging disease development include low night temperatures, high humidity and high nitrogen level (Jones *et al.*, 2016). Disease usually starts soon after fruit set when tomatoes are close to obtaining their green colour **(Suresh *et al.*, 2017).**

**Fungal and Fungal-like Diseases**

Greenhouse structures generally promote a high humidity level in the interior where the circulation of air is poor (Scherer and Meadows, 2020). Thus, this increases the likelihood of fungal diseases that are not often seen in open-field production, for example, powdery mildew and leaf mold (Scherer and Meadows, 2020). *Botrytis* gray mold is another common tomato disease in greenhouses and high tunnels where cool temperatures prevail and favor its development (Liao *et al.*, 2019). This section outlines fungal diseases that can be often observed on greenhouse-grown tomato plants.

**Leaf mold**

Leaf mold is a fungal disease primarily affecting greenhouse-grown tomatoes. It is highly destructive since tomato plants are exposed to high humidity although field-grown tomatoes are also susceptible. Tomato leaf mold was discovered in 1883 in North Carolina by Cooke (Jones *et al.*, 2016) and was originally observed in South and Central America. It has a worldwide distribution and is now a major cause of tomato crop losses.

*Passalora fulva*, previously referred to as *Cladosporium fulvum* is the pathogenic fungus responsible for leaf mold. The conidiophores of the microorganism are unbranched, constricted near the base and larger at the tip with conidia size of 12-47 µm long x 4-10 µm wide. Moreover, they typically have 0-3 septate that are pale to dark brown in colour with a thick hilum (Jones *et al.*, 2016). The fungus can be recognized by its characteristic velvety appearance and is sandy to brown-colored colonies on potato sucrose agar medium.

The leaf is usually the main part of the plant that is affected. However, infection of the stem, blossom and fruit can also occur. Older leaves are affected first with the appearance of yellowish, light-green spots on their upper surfaces (Figure 8B). Simultaneously, conidia that are olive-green in colour can form masses on the lower surface of the leaves (Figure 8A). At a later stage, the lower leaves become yellow and fall to the ground. Moreover, black leather-like rot can be seen on the calyx end of diseased fruit.


Figure 8. (A) Olive-green masses of conidia are formed on the abaxial surface of the leaf and (B) Yellow spots can be observed on the adaxial leaf surfaces (Copyright: Mamode Ally, 2019).

High temperature and high relative humidity (at least 85%) are ideal conditions for the proliferation of the disease (Blancard, 2012). Optimum temperature for disease development ranges from 22 to 24°C but it can occur at a wider range of 4-32°C, although temperatures under 10.5°C the incidence of the disease is low (Blancard, 2012). *P. fulva* thrives on plant debris as a saprophyte and as conidia in the soil. The conidia may survive for at least one year.

**Gray Mold**

Gray mold occurs frequently wherever tomato crops are planted. It affects greenhouse as well as field-grown tomatoes, resulting in fruit rot post-harvest eventually resulting in a decrease in the market quality.

The fungus *Botrytis cinerea* responsible for the disease grows efficiently on various types of culturable media such as Czapek –agar, Potato Dextrose Agar and Malt Agar. The spores form a cluster that is seen as a bunch of grapes under the microscope. Conidia are single-celled, colourless and elliptical and range from 9.7-11.1 x 7.3-8.0 µm in size **(Suresh *et al.*, 2017)**. Formation of dark brown or black, irregular and flat sclerotia can form on the plant tissues. In fact, the sclerotia allow *B. cinerea* to survive for extended periods on crop debris and in soil for a long span of time (Li, 2020). It can also grow on other hosts such as potato, brinjal, cucumber, grape, pumpkin, strawberry and zucchini planted in the same greenhouse and on weeds found inside as well as outside the structure

(Droby and Lichter, 2007; Ahmed *et al.*, 2018).

Initially, ovoid water-soaked lesions having a concentric pattern appear on stems. Foliar infection occurs at the source of an injury and formation of v-shaped lesions starts to occur. These lesions are submerged with gray fungal spores (Figure 9A). The fungus also attacks the calyx end of the tomato fruit where grayish-brown lesions are formed that later turn into a watery rot (Figure 9C). Another fruit symptom is the characteristic ghost spot which is defined by small, white, green or pale-yellow rings that form on green or ripe fruit (Figure 9B). High humidity can accelerate the development of these lesions into a moldy, gray growth that ultimately leads to plant death.


Figure 9. (A) Brown lesions with gray fungal spores developing on the leaf surface (Mamode Ally *et al.*, 2021) (B) Several white rings defined as the ghost spot symptoms appeared on the green tomato fruits (Credit: F. Maudarbaccus) and (C) An advanced stage of a rotted tomato fruit being infected with Gray mold disease in a greenhouse (Mamode Ally *et al.*, 2021).

A cool and humid environment in greenhouses and high tunnels also exacerbates the formation of masses of gray fungal spores. Inadequate ventilation and limited crop spacing are also risk factors. The fungus grows well at temperatures of 18-23°C **(Suresh *et al.*, 2017)**. As the temperature rises above 24°C, there is a rapid decrease in the germination of the conidia and no growth takes place at a temperature greater than 31°C (Jones *et al.*, 2016). Further damage can be prevented by exposure of the fruit to high temperatures and sunlight.

**Powdery Mildew**

First identification of the disease in greenhouse-grown tomatoes was in 1995 in Connecticut **(Suresh *et al.*, 2017)**. Powdery mildew is caused by the fungi *Leveillula taurica*, *Oidium neolycopersici* and *Oidium lycopersici*. Isolates of *L. taurica* vary greatly in terms of the conidia size. These range in size from 44.6 - 65.2 µm long x 16.2 – 22.7 µm wide and 49.7 – 71.4 µm long x 16.6 – 24.1 µm wide for cylindrical and pyriform conidia, respectively (Jones *et al.*, 2016). The *Oidium* species produce hyaline conidia, ellipsoidal to ovoid shape, and measure 30 µm long x 15 µm wide (Jones *et al.*, 2016; Jones *et al.*, 2000).

Disease symptoms by *L. taurica* include the formation of pale green to bright yellow lesions on the upper surface of the leaf (Figure 10A). Additionally, spots that are necrotic and have concentric rings can also be found in the centre of the leaves. The abaxial leaf part is often covered by a light powder (Figure 10A). When conditions are optimum for disease proliferation, abundant formation of conidia and conidiophores can occur on the adaxial or abaxial leaf surfaces. As a result, severely infected leves die or some just drop from the plant. With regard to *O. neolycopersici* and *O. lycopersici*, the main symptoms on the upper surface of the leaves include circular small regions of whitish mycelial growth (Figure 10B). As the lesions continue to sporulate and enlarge, the underlying tissue becomes yellow to brown in colour and wrinkled. When conditions are optimum for disease proliferation, abundant formation of conidia and conidiophores occurs on the adaxial leaf surfaces which differentiate *Oidium* species from *Leveillula* that normally sporulates on the abaxial part of the leaf. Severe infection results in the production of copious powdery conidia that cover the whole leaf surfaces, the petioles and calyxes, leaving the fruit unaffected.

For *L. taurica*, germination of the conidia takes place at temperatures ranging from 10 to 35°C and at a relative humidity of 52-75% **(Suresh *et al.,* 2017)**. In greenhouses, initial development of the disease occurs at temperatures below 30°C. Once a tomato leaf becomes infected, temperatures exceeding 30°C can then favor the progression of the disease and cause necrosis of the leaf tissue (Suresh *et al*., 2017). In regions where there is prevailing high temperature during the day, cool nights are enough for the onset of the disease. *O. neolycopersici* and *O. lycopersici* require optimum temperatures of 20-27°C, low light intensity and high relative humidity (85-95%) for optimal growth (Suresh *et al*., 2017).


Figure 10. (A) Leaf infected by *Leveillula Taurica* that caused chlorosis on the upper leaf surfaces and formation of white spores on the lower leaf surface **(Suresh *et al*., 2017)** and (B) Leaves infected with *Oidium* spp. on the upper leaf surfaces, displaying white patches of fungal spores (Copyright: (Mamode Ally *et al.*, 2021).

**Early Blight**

The presence of Early Blight occurs frequently wherever tomato crops are planted. It develops rapidly in humid or semiarid climates. If it is not properly managed in greenhouse production, it can cause defoliation followed by a decrease in the size and number of fruits, thus causing severe crop losses.

The fungi *Alternaria tomatophila* and *Alternaria solani* are responsible for early blight. For *A. solani*, its mycelium is branched and septate and darkens with time. The conidia measuring 12-20 µm wide x 120-296 µm long can be single-borne or occur in chains of two (in culture), beaked, muriform and dark (Gannibal *et al.*, 2014). It can grow easily on artificial media and produce a lot of yellow to red pigment that diffuses through the substrate. Rate of sporulation can be increased by the exposure of the cultures to fluorescent light. *A. tomatophila*, thought to be more virulent than *A. solani* (Gannibal *et al.,* 2014), has a septate mycelium that melanizes with time. The conidia *of A. tomatophila* are multicellular, brown and much elongated, with the presence of a long hyaline beak that measures 120-300 µm in length (Blancard, 2012).

The disease can cause significant damage during the different stages of plant development. Stem, leaves and fruits are affected. Firstly, the older leaves display small brownish-black spots. Yellowing of the tissue surrounding each lesion occurs and when there is abundant spotting, the whole leaf turns yellow (Bashir *et al.*, 2020). The spots then enlarge and reach at least 6mm in diameter. A distinct feature of this disease is the presence of concentric rings in the dark brown area of the spot (Figure 11A). On the stem, spots are a little sunken, small and dark. They will then enlarge to form elongated or circular lesions associated with concentric rings with light centres. Spots with concentric rings are also observed on fruits (Figure 11B). The infected portions have a leathery appearance and velvety-like mass of black fungal spores (Figure 11B). The fruits then drop and cause losses of 30-50% of the immature tomato crop **(Suresh *et al*., 2017)**.


Figure 11. (A) Concentric ring spots on tomato leaves (B) Black and leathery fruit spots caused by Early Blight (Copyright: (Mamode Ally *et al.*, 2021)).

The fungi can continue to thrive on infected crop debris and in the soil, thus representing a perpetual source of inoculum. The onset of infection, sporulation and rapid spread of the pathogen usually take place when conditions are optimal i.e., a temperature range of 24-29°C and in humid conditions. Rapid spread of the disease occurs when the optimum environment is present.

**Late blight**

Late blight is a potentially destructive disease infecting both potato and tomato. It was first observed in 1845 on potato and in 1847 on tomato (Blancard, 2012). Its origin is thought to be in Central America. The Irish potato famine was caused by this disease in the 1840s (Blancard, 2012). It is responsible for many epidemics on potatoes and tomatoes. Usually, if the weather is persistently cool and rainy, serious damage by the Late blight disease can occur on tomatoes. The disease can be prevalent in greenhouses where the roofs are broken and leaking during a period of heavy rainfall.

It is caused by the oomycete *Phytophthora infestans*, best known for its distinct lemon-shaped and hyaline sporangia that protrude through the stomata. Germination of the sporangia can occur directly in cool and humid conditions. They measure 21-38 x 12 - 23 µm in size and each sporangium can release at least eight zoospores (Nowicki *et al.*, 2012). These zoospores can cause new rounds of disease infection with the help of two flagella in the presence of a film of water on the surface of the plant.

According to **Suresh *et al.* (2017)**, water-soaked spots that are indefinite and brown in colour can enlarge quickly and cover large parts of the leaf (Figure 12A). In moist conditions, presence of white-gray mold growth can be seen on the undersides of the lesions (Figure 12B). As the disease progresses, the leaves turn brown, wilt and die. The petioles and stems are infected in the same way and eventually, the whole plant perishes. Spots that are dark, greasy and olive-green start to develop on fruits (Figure 12C) until they evade the whole fruit causing soft rot. Presence of a thin layer of white mycelium can cover the lesions formed on the fruits.


Figure 12. (A) Brown water-soaked lesions caused by Late blight on tomato leaf (B) Gray-white fungal growth underneath the infected leaf and (C) Greasy olive-green lesions on infected tomato fruits (Copyright: Mamode Ally, 2021).

*Phytophthora infestans* can live between the cropping seasons in plant debris. Warm days and cool nights are favorable for the development of the disease. Formation of sporangia takes place at temperatures of 12-26°C (optimum at 18-22°C) and relative humidity of 91-100% (Nowicki *et al.*, 2012) while the development of late blight is hindered at temperatures above 30°C although the fungus can survive high temperatures (Nowicki *et al.*, 2012). The presence of water and cool environment prevailing at night (19-21°C) likely favor disease progression.

**Target spot**

Target spot can be a serious disease both in greenhouses and in fields. It was first observed in 1972, in Florida (Mackenzie *et al.*, 2018). This disease has caused significant damage to tomato leaves and fruits in the recent few decades and its causative agent is usually found worldwide in the subtropics, tropics and greenhouse production (Mackenzie *et al.*, 2018).

The fungus *Corynespora cassicola* is responsible for target spot. Conidia are single-borne or occur in chains (up to six chains). The conidia (40-220 X 9-22 µm) are curved or straight, cylindrical, smooth, subhyaline to pale olive-brown, pronounced hilum towards the base and having 4-20 pseudosepta (Jones *et al.*, 2016).

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Nearly all parts of the plant including the stems, leaves, petioles and fruits are affected. Fungal sporulation occurs more on the abaxial leaf surface than the adaxial leaf surface. Initially, small, water-soaked, circular, light-brown spots can be seen on leaflets. Later, the lesions have a target-like appearance and eventually become surrounded with yellow halos (Figure 13A). Same types of lesions form on the stems and petioles but are elongated in size. These lesions may engulf the petioles and the stems resulting in foliar death. On newly-formed fruits, pinpoint, brown and sunken spots can be seen while on mature and ripe fruits, circular lesions having brown centres develop, eventually forming cracks in the skin (Figure 13B).

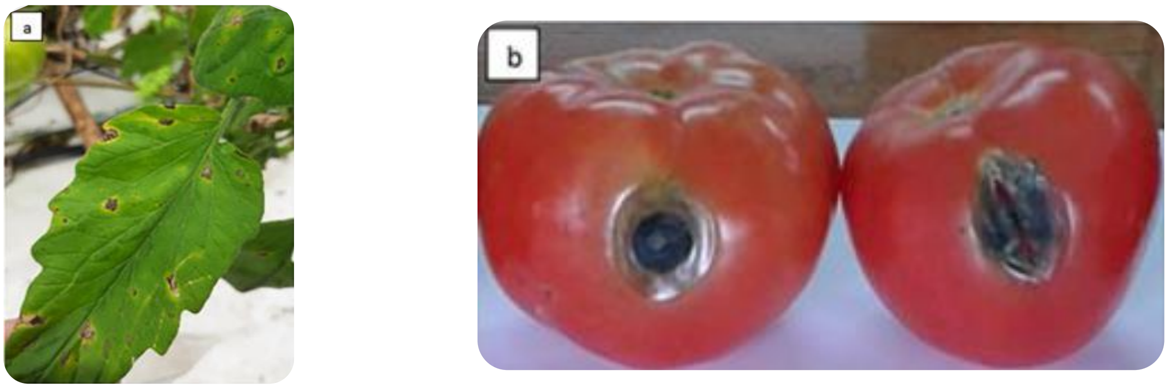

Figure 13. (A) Brown spots with yellow halos developed on the leaf surface and (B) Lesions on ripe tomato fruits having dark to brown centres that form cracks in the skin afterward (Mamode Ally *et al.*, 2021).

**Viral Diseases**

Viruses need a host to be able to replicate and complete their life cycle. A vector is also important for plant viruses as they are capable of spreading the pathogen between plants. The main viral diseases affecting greenhouse-grown tomato crops are Tobacco and Tomato mosaic virus (TMV and ToMMV), Tomato mottle mosaic virus (ToMMV), cucumber mosaic virus (CMV) and Tomato spotted wilt virus (TSWV).

**Tobacco and tomato mosaic virus**

Tobacco and Tomato mosaic viruses belong to the genus *Tobamovirus.* They can be found in leaf and root debris or dry soil for a duration of two years. However, if the soil is well-drained, these viruses survive for only one month (Fillhart *et al.*, 1998).

Growth of plants that become infected by either one of these viruses is hampered and yellow mosaic patterns can be seen between the foliar veins (Figure 14A, B). The leaves can also roll upwards, become smaller in size and show signs of necrosis (Figure 14A, B). Brown spots can develop on fruits (Figure 14C) leading to a reduction in the yield and in some cases, the death of the plant.

Figure 14. (A) Tomato mosaic virus is responsible for the yellowing of the leaves (B) Tobacco mosaic virus on leaves that roll upwards and display yellow mosaic patterns that will result in a stunted growth of the plant and (C) brown lesions on infected fruits (Chin and Miller, 2010; Marks, 2010; Gardening Channel, 2021)

These viruses have a broad host range including many ornamental plants and weeds. Transmission occurs due to accidental mechanical injury mainly or the viruses can be seed-borne. During routine activities such as pruning and harvesting, the spreading of the virus can take place from infected plants and contaminate the worker's clothes, hands and equipment. These two diseases manifest rapidly in low light intensity, for instance, in greenhouses that do not have any light systems or in high tunnels.

**Tomato mottle mosaic virus**

Tomato mottle mosaic virus (ToMMV) is the third *Tobamovirus* known to infect tomato crops and it was first detected in Mexico (Li *et al.*, 2013). Infection by this virus has been reported on both pepper and tomato plants grown in both outdoor fields and greenhouses **(Suresh *et al.*, 2017)**.

The diseased tomato plants become stunted and show serious symptoms that include mosaic mottling, abnormal foliar growth and necrosis (Figure 15). This virus is highly transmissible and is either spread mechanically from one plant to another through regular traditional practices or the virus affects the seed coat of the host plants. Bumblebees can also be vectors of the ToMMV and facilitate the contamination of tomato plants if they have been introduced in greenhouses for natural pollination.

Figure 15. The tomato crop has stunted growth after having been infected with ToMMV, the shapes of the leaves start to become deformed and the fruits display symptoms of mottling (Maudarbaccus *et al.*, 2021).

**Cucumber mosaic virus**

Cucumber mosaic virus was first observed on cucumber and other cucurbits in 1916 and they now affect many other edible and ornamental plants in temperate and tropical climates (Zitter and Murphy, 2009). It is transmitted by aphid species such as *Myzus persicae* (the green peach aphid) and *Aphis gossypii* (cotton or melon aphid).

Infected tomato leaves and fruits do not develop well and become malformed. Symptoms such as green mottling, chlorosis or necrosis can be seen on the leaves. Some strains of the virus cause also severe suppression of the leaflet's blade causing leaves to become spindly-like shaped (Figure 16).

Figure 16. Leaf having a spindle-like shape caused by CMV (Zitter and Murphy, 2009).

The virus has a host range of about 800 species and is transmitted rapidly in greenhouses from one plant to another by aphids **(Suresh *et al.*, 2017)**. The pathogen can also be spread mechanically, but because this type of virus particle is known to be unstable, the probability of viral transmission by workers and their equipment is less compared to that of TMV and ToMMV.

Tomato spotted wilt virus

Tomato spotted wilt virus is insect-borne and thus transmitted by different kinds of thrip species such as *Frankliniella occidentalis* and *Thrips tabaci*. These vectors are also infected by the virus. When the nymphs feed on diseased plants, they become infected with the virus in their body and can transmit the virus for the rest of their lives. However, TSWV cannot be transmitted by infected females to their eggs and cannot be transmitted by seeds.

Young diseased leaves exhibit small, dark spots (Figure 17A), followed by the formation of dark streaks on the stem while yellow to red mottling can be observed on ripening fruits (Figure 17B).

The virus has a wide host range that includes weeds, crops and ornamental plants. Temperatures above 22°C speed up hatching of larvae eggs, thus resulting in a destructive spread of the vector and the agent **(Suresh *et al.*, 2017)**.


Figure 17. (A) Dark coloration on leaves infected with TSWV and (B) Yellow-red mottling on a ripe tomato fruit (Burrows, 2019).

**Phytoplasma, Nematode and Viroid Causing Diseases**

**Phytoplasma**

Phytoplasmas are obligate bacteria that do not possess cell walls. They survive and move inside plant tissue by means of the phloem vessels. One potential tomato disease caused by a phytoplasma is the Tomato Big Bud.

**Tomato big bud**

Phyoplasmas causing Tomato Big Bud include *Candidatus* Phytoplasma asteris, *Candidatus* Phytoplasma trifoli and *Candidatus* Phytoplasma aurantifolia. Different types of leafhopper species are responsible for the pathogen transmission such as *Orosius argentatus* and *Macrosteles quadrilineatus*. Flower buds grow abnormally large, after which this disease is named. Sometimes, the sepals cannot separate as the flower is opening and the buds are green and swollen (Figure 18B). The stem thickens and formation of adventitious roots or small side shoots occur (Figure 18A).

The internodes can be shortened and the whole plant may turn yellow. The fruits become deformed and shrink in size (Figure 18C).


Figure 18. (A) and (B) The thickened plant and sepals being affected with phytoplasma and (c) Infected tomato fruits became deformed (Sherf, 2019; Jackson, 2017; Seethapathy, 2018).

The phytoplasmas can live in a wide range of hosts such as eggplant, chilli pepper, lettuce, potato and even weeds and are then transmitted to the tomato plants by the common brown leafhopper. Infection occurs when the leafhoppers transporting the phytoplasma feed on these tomato plants.

**Nematodes**

Nematodes are microscopic worms that reside in soils. A common tomato disease caused by nematodes is Root-knot. Different species of root-knot nematodes including *Meloidogyne hapla*, *M. arenaria*, *M. incognita*, *M. enterolobbii*, *M. javanica* and other *Meloidogyne* species are responsible for this disease. The infected plants become wilted and stunted. Chlorosis occurs and symptoms similar to those that occur when the plant lacks nutrients start to appear such as the lower surface of the leaf turns purple indicating a deficiency of phosphorus. The roots become swollen and knots or galls can be observed (Figure 19). Galls caused by southern root-knot nematode (*Meloidogyne incognita*) are more irregular and larger compared to the northern root-knot galls (*Meloidogyne hapla*) which are smaller and uniform with the development of lateral roots beside the galls.

Figure 19. Formation of galls on infected roots (Guan, 2017).

Root-knot nematodes have a great range of hosts upon which they are dependent for survival and that includes many agricultural plants and also weeds. The disease is more destructive in regions where the growing season is long and has no winter season. When the soil temperature is warm (27°C), proliferation of the disease by *M. incognita*, *M. javanica* and *M. arenaria* occurs more rapidly (Tariq-Khan *et al.*, 2020) whereas cool prevailing soil temperatures (16-20°C) enhance the development of the disease by *M. hapla* **(Suresh *et al.*, 2017)**. Disease incidence by *M. incognita* occurs in most parts of the world **(Suresh *et al.*, 2017)**.

**Viroids**

Viroids are known to be the smallest causative agent of plant diseases. They have an unknown origin and contain a single-stranded RNA. All the tomato viroids are transmitted mechanically. Ornamental plants such as *Vinca minor*, *Verbena* spp. and *Physalis peruviana* are known to be the hosts of viroids that can also infect tomato plants.

Tomato apical stunt viroid (TASVd) and Tomato chlorotic dwarf viroid (TCDVd) are two viroids that can severely affect greenhouse-grown tomatoes. The infected tomato plants display symptoms of chlorosis, young terminal shoots, stunted growth, bending of the leaves, stem and leaf necrosis and abnormal growth of the fruit (Figure 20) which eventually lead to the death of the plant. These symptoms are similar to those caused by viral diseases, thus molecular testing is the only reliable method to identify the causal viroid agent.


Figure 20. (A) Tomato apical stunt viroid causing bending of the leaves and abnormal fruit development inside a greenhouse and (B) Tomato chlorotic dwarf viroid infecting tomato plant that result in chlorosis of the leaves and stunted growth (Olmedo-Velarde *et al.*, 2017; NPPO of the Netherlands, 2021).

**Causes of Diseases in Greenhouses**

Microbial diseases are introduced in protected facilities by a number of ways that include: i) use of contaminated equipment, tools, trays and clothing ii) use of infected tomato seeds, iii) contaminated hands of handlers, iv) contaminated irrigation water, v) fungal spores that enter the greenhouse from outdoor infected plants and are dispersed by air currents or activities carried out by workers, v) spores and bacteria that are spread through water droplets when overhead irrigation is used, vi) high humidity and poor circulation that favor the spread of certain diseases, vii) plants that come into contact with infected plant debris and soil, viii) the action of clipping transplants, ix) plant wounds created by mechanical means or punctures by insects, x) short plant spacing facilitating the spread of the disease from one plant to another and xi) tomatoes planted in-ground in greenhouses do not usually undergo rotation with another crops which increases the probability of being infected with pathogens.

However, the main advantage of crop cultivation inside greenhouses as opposed to open-field systems, is that it is possible to control the environment so as to optimize the disease pressure by reducing the impacts of abiotic and biotic factors (Simko *et al.*, 2007). The possibility to maintain appropriate interior climatic conditions that will help to reduce the severity of these common greenhouse diseases and the application of a more efficient control against different types of disease-causing agents have resulted in obtaining higher crop yields in greenhouses (Iddio *et al.*, 2020). A study demonstrated that the mean tomato yield was 13.1 kg $m^{-2}$ when cultivation inside greenhouses was adopted and 1.8 kg $m^{-2}$ for harvested open-field grown tomatoes (Idris, 2006). The estimated cost of production was recorded to be SD 960 per $m^2$ in greenhouses and SD 50 per $m^2$ in open fields (Idris, 2006).

## Traditional and Novel Approaches for Disease Management of Greenhouse-Grown Tomatoes

### Routine/conventional control methods

Some traditional management strategies that can be adopted to control the occurrence and severity of greenhouse-grown tomato diseases are listed below in Table 1, together with their associated pros and cons.

### Novel/innovative solutions

New approaches for crop protection are being widely investigated in the agricultural sector, including the use of novel cultural techniques, biocontrol agents or exploiting the natural defence mechanisms exhibited by plants. The following section of this review will elaborate on certain novel approaches that are used to control plant diseases without the need to use chemical products.

### Biocontrol Methods

Controlling plant diseases through the biological approach, i.e., the use of composts and biological agents has become common. It is an alternative to the rigorous utilization of chemical products. Biological control is considered to be safer, leaving no toxic residue in the food chain, is cheaper and has a minimal effect on the environment (Bale *et al.*, 2008). Greenhouse growers commonly apply biological control agents. This is because of the environmental conditions within the greenhouse that can be controlled, allowing the biological agents to perform efficiently. Moreover, the reduced population of pests in greenhouses makes the application of these products manageable (Pilkington *et al.*, 2010). In addition, the high value of fresh greenhouse produce makes implementation of biological controls cost-effective to control plant diseases (Pilkington *et al.*, 2010).

Effective microorganisms (EM) such as bacteria, fungi and actinomycetes are used as biological agents against crop diseases (Shakoor *et al.*, 2015). They can exist as a formulation consisting of all the beneficial microbes; i.e., photosynthetic bacteria, yeasts and fermentative lactic acid bacteria mixed together. The use of EM is safe for the environment and is organic (Joshi *et al.*, 2019). Their application into the soil can be in the form of a liquid inoculant, or impregnated onto fermented organic matter and this is known as 'Bokashi' (Figure 21). EM-treated soil was observed to suppress the growth of certain fungal pathogens such as *Sclerotina sclerotium*, *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani*, *Phytophthora* spp. and *Sclerotium rolfsii* (Tokeshi *et al.*, 1998). The plants are at an advantage as they have access to additional nutrients due to the decomposition of organic residues in the soil by the beneficial microorganisms (Joshi *et al.*, 2019).

Another promising fungal biocontrol agent is *Trichoderma. Trichoderma* can provide protection to tomatoes against the fungus *Botrytis cinerea* by preventing the colonization of the pathogen in wounded tissues (Elad *et al.*, 2007). Khalifa (1991) investigated *T. harzianum* acting as a control against the fungus responsible for *Fusarium* wilt *(F. oxysporum* f.sp. *lycopersici*). The microorganism prevented the growth of the pathogenic fungi, decreased its population in the rhizosphere and the population of the potential biocontrol agent increased after 4 weeks of transplanting.

Plant growth-promoting rhizobacteria (PGPR) such as *Bacillus* spp. and *Pseudomonas* spp. have also been able to control many bacterial and fungal diseases such as tomato wilt and canker *in vitro* or under greenhouse conditions and enhance the growth of these treated plants (Abo-Elyousr *et al.*, 2019). Soil-borne and some leaf infections are also suppressed by fluorescent pseudomonads (Abo-Elyousr *et al.,* 2019).

Table 1. Conventional control methods against tomato diseases in greenhouse structures.

| | Methods | Description | Advantages | Disadvantages |
|---|---|---|---|---|
| 1) | Crop Rotation | Alternating tomato and non-solanaceous plants every 3-4 years | Most microbial diseases can be reduced. | Can be economically unfeasible for some producers to rotate out of tomatoes. |
| 2) | Reduction of plant residues by cloth covering | A woven cloth covering the ground between each planted row is used to prevent the accumulation of crop residue near the soil between the tomato plants | Cloth can be washed, sanitized and then re-used.<br>Cover aids in weed elimination and thus the structure can be swept easily.<br>White cover may cause light to reflect into the canopy and trap maximum radiation from the sun. | Black cloth cover is usually not re-useable. |
| 3) | Improvement in the greenhouse air circulation | Careful pruning of tomato plants<br>Use of fans<br>Spacing of plants at a distance of 20-24 inches in the row and the rows should be at least 5 feet apart to allow proper circulation of the air current. | Effectively reduces humidity and moisture from being trapped inside the greenhouse | Improper pruning or use of contaminated pruning equipment may wound the plants, allow the entry of bacteria inside the plant tissues and cause disease spread from one plant to another.<br>Systems of ventilation can be expensive to operate. |
| 4) | Use of disease-free seeds | Hot water seed treatment for seed decontamination<br>Use of seeds from disease-free crops<br>Use of resistant tomato varieties | Reduced risk of pathogen contamination of seeds | i) Variable efficacy depending on the quality of the seeds<br>ii) Can affect the viability and germination rate of seeds |
| 5) | Use of drip irrigation | The crop is watered directly near its root area | i) Avoids the spread of diseases through the splashing of water<br>ii) Halts the growth of weeds<br>iii) Has a positive effect on the germination of seeds and crop yield<br>iv) Low operational expenses due to lesser demand for labour and lower energy consumption | i) The initial investments to implement drip irrigation are high.<br>ii) The emitters installed to perform drip irrigation are susceptible to clogging and impaired functioning<br>iii) Inspection for diseases should be carried out on a routine basis |
| 5) | Hygiene | Proper cleaning and disinfection of greenhouse structures between crops.<br>Workers should adopt hygienic practices and make use of clean and sterilized plant equipment and tools | i) Enhances growing conditions of crops<br>ii) Improves light penetration in greenhouse<br>iii) Helps to control insects, pests and diseases | Cleaning each area of the greenhouse can be challenging especially when greenhouses bear cracks or small corners |
| 6) | Fungicides and bactericides | Application of chemical products to control pathologies | Effective and show rapid antimicrobial action. They can help in preventing further plant damage caused by microbial diseases | i) Products are health hazards and are not environment-friendly<br>ii) Repeated use can progressively induce development of resistance in crop pathogens |


Figure 21. Products of EM in the form of liquid inoculum and 'Bokashi' mix manufactured under the brand named Orgasmic Garden in Mauritius Island.

In another study Chen *et al.* (2013) reported that, tomato seeds treated with *B. subtilis* resulted in reduced severity of wilting in tomato plants and improved the growth of the plants in terms of plant height, flower, roots and branches. These research findings highlight the promising use of formulations of these antagonistic bacteria against various diseases of greenhouse-grown tomatoes.

Biofertilizers are also an essential means of control using beneficial microorganisms such as *Azotobacter* spp., *Azospirillum* spp. and *Bacillus polymyxa*. These organisms, upon application, have successfully increased the yield and quality of many plants **(Zaghloul *et al.*, 2007)**. In addition to biofertilizers, application of compost is also effective in controlling different types of diseases caused by a wide array of pathogens, including fungi, bacteria and nematode species (Litterick *et al.*, 2004; Noble and Coventry, 2005; Bonanomi *et al.*, 2010).

Implementation of bacteriophage as a biocontrol agent is another interesting area of research. Bacteriophages can lyse pathogenic bacteria from the infected soil or tissues of the host plant (Jones *et al.*, 2012). But more thorough research must be done on how to optimize a better formulation and the frequency of its spraying in order to achieve the best control.

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**Natural antimicrobials**

Biopolymers such as chitosan, exhibit antimicrobial activities that can help to control plant diseases **(Hassan and Chang , 2017)**. This polymer is a biodegradable compound derived from shrimps and crab shells (Romanazzi, 2018). Growth of soil and leaf pathogens (bacteria, fungi and virus) can be inhibited by the application of chitosan **(Hassan and Chang, 2017)**. Chitosan has a direct adverse effect on the growth of these pathogens or can initiate a sequence of defense mechanisms (Hassan and Chang, 2017). Chitosan can also increase the shelf life of treated vegetables by decreasing the rate of water loss and reducing the respiration rate (Hassan and Chang, 2017). Table 2 shows the efficiency of Chitosan acting as a biocontrol agent in tomato disease management.

Spraying of small antimicrobial peptides released by some bacteria, oomycetes and fungi on infected plants can hamper the synthesis of protein or nucleic acid in the pathogen, thus inhibiting their growth (Sundin *et al.*, 2016).

**Nanoparticles**

Nanotechnology is the process of creating nanoparticles which have unique chemical and physical properties owing to their size at the nanometre scale. The use of nanotechnology in crop disease management is attributed to the diverse roles of nanomaterials to act as antimicrobial agents, to strengthen the plant's immunity or act as carriers of pesticides or micronutrients (Mittal *et al.*, 2020). These nanomaterials can be biosynthesized by plants, bacteria, fungi, actinomycetes and agro-waste (Kaushik *et al.*, 2020; Gan *et al.*, 2012). Fungi are more efficient producers of nanoparticles as these are released extracellularly which means that no downstream processing is required (Mishra *et al.*, 2014). The shape and size of nanoparticles synthesized can also be modulated by the control of certain parameters such as temperature, pH, reaction time and the concentration of the substrate (Sathishkumar *et al.*, 2010). In a study carried out by Mishra *et al.* (2014), gold nanoparticles were successfully produced by *Trichoderma viridae* within 10 minutes and were an efficient control against pathogenic bacteria. Figure 22 is a simplified pathway showing the synthesis of gold nanoparticles from red algae (Senthilkumar *et al.*, 2019). Extracts of marine algae are known to possess bioactive components such as phenols, flavonoids, alkaloids and terpenes that act as reducing agents (Santhoshkumar *et al.*, 2017).

Table 2. Chitosan and its inhibitory effect on specific pathogens (Hassan and Chang, 2017).

| Pathogen | Mechanisms |
|---|---|
| *Alternaria alternata* | Inhibits fungal growth and the production of toxin |
| *Fusarium oxysporum* f. sp. *Lycopersici* | Hinders conidia germination, build-up of phenolic compounds, aggregation of the fungal cytoplasm, swelling of the hyphae and causes constriction of its plasma membrane |
| *Botrytis cinerea* | Reduces rate of respiration, decreases production of internal oxygen and ethylene, increases internal carbon dioxide level and favours formation of harder fruits. |
| Potato spindle tuber viroid | Decreases viral activity |

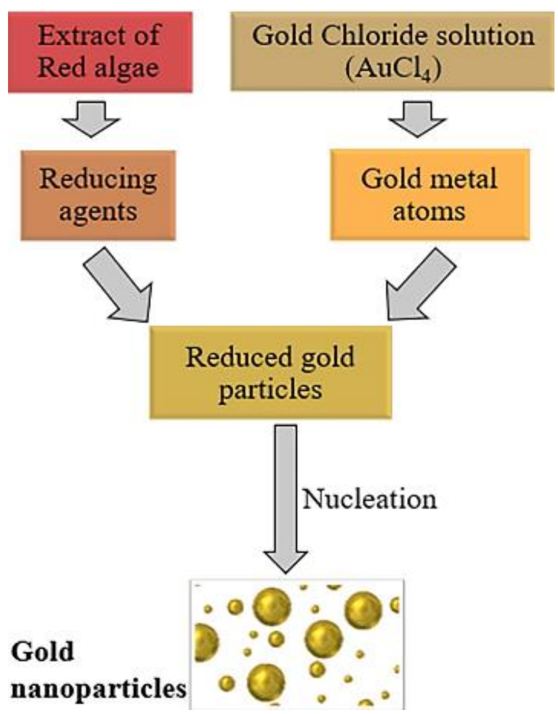


Figure 22. The pathway demonstrated for the synthesis of gold nanoparticles from red algae.

Moreover, it has been reported that some marine algae having good biological constituents can be used for the production of nanoparticles (Ajdari *et al.*, 2016). In this study, extract obtained from *Gelidiella acerosa*, a red marine alga, was mixed with a solution of gold chloride, allowing the synthesis of gold nanoparticles. The reducing agents present in the algal extract gave electrons to the gold ions from the gold chloride salt to form atoms. The atoms were then further combined together to form particles. The mixture was left at 37 °C and then stirred for 1 hour at room temperature under the presence of direct sunlight. They were then washed with distilled water followed by centrifugation and finally dried in an oven at 50 °C. The powdered nanoparticles were stored at 4 °C until further use.

In addition to diseases, agricultural pests also negatively affect crop production. While synthetic pesticides are still largely used for control (Nicolopoulou-Stamati *et al.*, 2016), only about 1% or less of the pesticides actually reach the target organisms (Özkara *et al.*, 2015). In fact, most of the applied pesticides end up in the environment through leaching, runoff and degradation caused by light and microbes (Özkara *et al.*, 2015). As a consequence, farmers tend to apply these products more frequently than needed in order to compensate for the loss, leading to a surge in the cost of production along with adverse environmental impacts (Özkara *et al.*, 2015). Use of biopesticides encapsulated within nanoparticles has attracted significant scientific interest recently and is an emerging research thrust in sustainable agriculture. Nanobiopesticides facilitate the controlled delivery of the active compounds to the infected plants thereby attaining greater desired effects at a much lower dosage. Moreover, controlled delivery of pesticides results in less harmful effects on the environment and on humans, protection of the active compounds from degradation and enhanced biological activity of the compounds on the pests (Sahayaraj, 2014; Campos *et al.*, 2019; Lade and Gogle, 2019). Nanopesticides can include encapsulated plant compounds, microorganisms or metal ions.

In the encapsulation process, active compounds derived from plant extracts are enclosed and used to provide photoprotection to crops and ensure the stability of these bioactive compounds. Biogenic nanoparticles produced from various sources bear the benefits of being biocompatible. Nanoparticles released by microorganisms have in turn helped in preventing compound degradation and provide an enhanced biological activity (de Oliveira, 2021).

Nanobiopesticides can be synthesized from a variety of nanoparticles to encapsulate different compounds extracted from plants with potential pesticidal activity combined into an appropriate polymer (Lade and Gogle, 2019) (Figure 23).

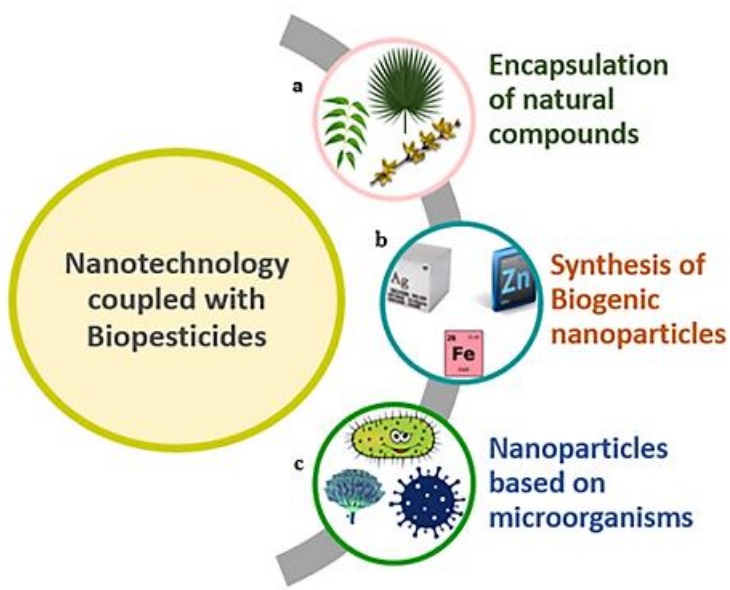


Figure 23. Schematic illustration of using nanotechnology with biopesticides in pest and disease control.

This technique is known as nanoencapsulation and that consists of capturing one substance within another substance that will result in the production of particles having very small diameters. Campolo *et al.* (2017) investigated the use of Polyethylene glycol (PEG) nanoparticles as carriers for essential oils, such as limonene, and monoterpene hydrocarbons obtained from citrus peels. The combination was mostly efficient against *Tuta absoluta* affecting tomatoes and the encapsulation protected the plants against any cytotoxicity effects exhibited by the essential oils.

Several prospecting studies have been performed to investigate the potential of plants and microorganisms as sources of nanoparticles (Figure 23). In this type of synthesis pathway, biomolecules such as polysaccharides, amino acids, proteins and vitamins produced by plants or microbes, also act as reducing agents thereby affecting the shape and size characteristics of the nanomaterials (Akther and Hemalatha, 2019). Silver nanoparticles are known for their antimicrobial properties against plant pathogens in fields and greenhouses. A study revealed that spraying tomato plants, infected with early blight, with 5 μg/ml of silver nanoparticles encapsulating cell extract of biocontrol agent *Trichoderma viride*, led to a decrease in the count of fungal spores (48.57%) as well as an increase in the chlorophyll content (23.52%) and fresh weight of the tomato (32.58%) compared to the untreated infected ones (Kumari *et al.*, 2017). Thus, nanoparticles play a crucial role in the field of agriculture by providing protection to crops, improvement in terms of soil nutrients and fertility and weed control.

Moreover, a decrease in the severity of plants infected with Tomato mosaic virus and Potato virus Y was observed after 7 days of spraying with silver nanoparticles (Shafie *et al.*, 2018). Examination under Transmission Electron Microscopy (TEM) revealed the binding of the nanoparticles to the coat protein of both viruses, thus inhibiting their ability to cause disease (Shafie *et al.*, 2018). Figure 24 demonstrates some other examples of synthesized silver nanoparticles, having three different diameter sizes of 20nm, 60nm and 100nm (from left to right) captured by TEM.

In addition, certain microbes have inherent biopesticidal activity against many agricultural pests in the world (Lade and Gogle, 2019) (Figure 23C). Nevertheless, these microorganisms, i.e., bacteria, fungi, viruses and nematodes are quite sensitive to parameters such as temperature, relative humidity and UV light, which lead to their reduced efficacy (Vimala Devi *et al.*, 2019). Nanotechnology can be used to optimize the formulations of these beneficial microorganisms for enhanced efficacy (Grasso *et al.*, 2019). In a study performed by Murthy *et al.* (2014), a powder of isolate *Bacillus thuringiensis* var. *kurstaki* (DOR Bt-1) was exposed to a process called high pressure homogenization to produce nanoparticles of size ranging from 32 to 1139nm. These nanoparticles have effectively killed most *Helicoverpa armigera* larvae at very low dosages (Murthy *et al.*, 2014).

Nanoemulsion is another technology that has been frequently used together with the administration of biopesticides. Oil/water emulsions consist of scattered droplets of oil in water. Nanoemulsions follow the same concept, but they range from sizes 10 nm to 1 μm, unlike the typical emulsions that are greater than 1 μm **(Jaiswal *et al.*, 2015)**. In a study carried out by Ali *et al.* (2017), citronella nanoemulsion together with 5% neem oil and neem nanoemulsion together with 5% of citronella oil were among the best combination created. The ED50 against *Sclerotium rolfsii* and *Rhizoctonia solani* for citronella nanoemulsion was 20.88 mg/L and 25.64 mg/L respectively and for neem oil nanoemulsion, it was 14.71 mg/L and 13.67 mg/L respectively. It has been concluded that the nanoemulsion exhibited a higher antifungal effect in contrast to the crude citronella and neem oil.

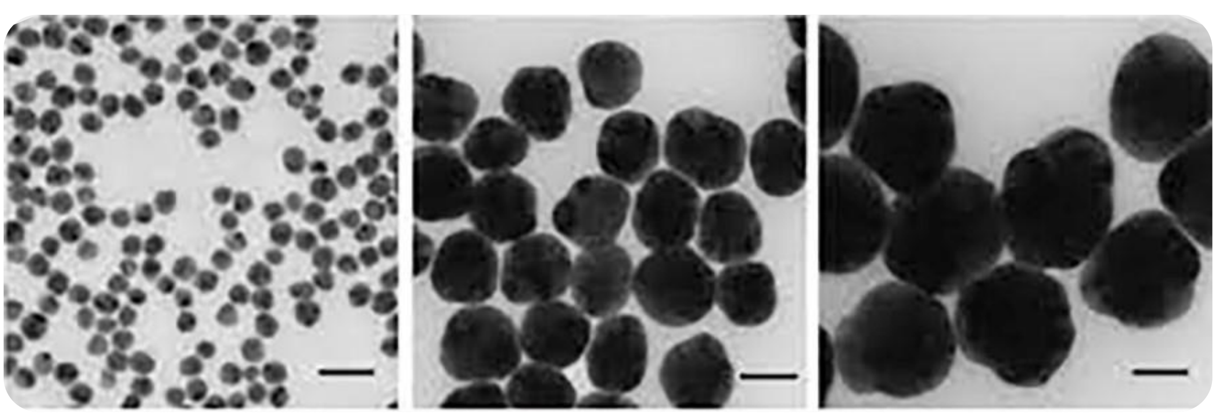

Figure 24. Images of silver nanoparticles observed under the TEM **(Merck, 2021).**

**Use of Ultraviolet (UV) light**

UV light can be more effective than existing fungicides in controlling plant diseases (Thompson, 2019). In an experiment by Onofre *et al.* (2021), UV light was used to kill the mycelium of the fungi responsible for powdery mildew. Moreover, a study revealed that pre-treatment with UV light in the greenhouse can boost the plant defense system against these harmful microorganisms (Alsanius *et al.*, 2019). One major disadvantage of using this method is that sometimes the doses required to kill the pathogen can prove to be injurious to the plant tissues (Singh and Pandey, 2012). In addition, sporulation of some fungi like *Botrytis*, *Alternaria* and *Stemphylium* can occur when they are exposed to light in UV range of less than 360nm (Singh and Pandey, 2012). Thus, to control these diseases, the greenhouse has to be covered or constructed with a UV-retaining vinyl film that does not allow light transmission below the 390nm wavelength (Singh and Pandey, 2012).

**Soil Solarization**

The capture of solar energy is facilitated by the use of a transparent polyethylene sheet that increases the normal temperature by 10 to 15 °C. This method can kill pathogens found in the soil and weeds. Fungal diseases such as fruit rots, blight, wilts and damping off, nematode diseases (e.g., *Meloidogyne* spp.) and bacterial canker affecting tomatoes have been managed using this technique (Singh and Pandey, 2012).

**Steam Sterilization**

This method is mostly used in greenhouses where steam is released through punctured pipes at a depth of 15 cm below the soil level to allow sterilization of the upper soil layer. Hence, bacteria, viruses and any other microbes are killed and this makes the soil suitable for the next batch of cultivation. Important considerations when carrying out steam sterilization of infected soil in greenhouses include ensuring that the soil pH is 6.5 or greater, is dry and dug deep enough so that the steam can penetrate well inside beneath soil layer (Meuldijk, 2021). In addition, steam can be used for decontamination of diseased plants. For instance, Garibaldi *et al.* (2014) and Newell (2003) used steam to sterilize tomato roots infected with nematodes and the fungus *Fusarium*.

## CONCLUSIONS AND FORWARD-LOOKING RECOMMENDATIONS

In the light of the expose, we can conclude that greenhouse-grown tomatoes are widely preferred because they are cultivated under controlled conditions, have a better quality and are more uniform in shape, size and colour compared to field-grown ones. In fact, greenhouse farming is gaining more interest because the greenhouse structure limits certain types of pests and diseases and also allows better control of the interior environment unlike open-field cultivation. Nevertheless, greenhouse-grown tomatoes are still susceptible to certain microbial diseases because the closed environment can be conducive to certain diseases. These pathologies can have detrimental effects on both yield and marketable quality. Therefore, proper control measures remain the sine qua non to disease prevention. As greenhouse-grown tomatoes are increasingly considered as premium produce and with the growing consumer demand for vegetables that are certified “bio”, use of biocontrol methods such as Effective microorganisms or natural antimicrobials coupled with nanotechnology are becoming the subjects of active research and development. With the impacts of climate change, the prevalence of certain diseases in tomato greenhouses may likely increase. In any case, early detection of the disease and an effective treatment regime remain an imperative to contain the spread of diseases.

## AUTHOR CONTRIBUTIONS

Nooreen M. Ally: Methodology, Investigation, Data curation, Writing - Original Draft preparation, Funding acquisition. Hudaa Neetoo: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition. Vijayanti M. Ranghoo-Sanmukhiya: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition. Teresa Ann Coutinho: Writing - Review & Editing, Supervision.

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