SCREENING AND SELECTION OF TOMATO RESISTANCE TO *Oidium neolycopersici.*

BY

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SR/1589/RPR/20/025

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCES

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc HONS) IN PLANT BIOLOGY AND BIOTECHNOLOGY.

MAY, 2021.

CERTIFICATION

This is to certify that this project work was carried out by Elect Osemwonyenmwen ALENKHE (Miss) in the Department of Plant Biology and Biotechnology, University of Benin, Benin City under the supervision of

_____ _____

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_____ _____

PROF (Mrs.) F. I. OKUNGBOWA DATE
(HEAD OF DEPARTMENT)

_____ _____

EXTERNAL EXAMINER DATE

DEDICATION

I dedicate this research project to God Almighty who supplied all the help I needed to scale through. I also dedicate it to my little sister, Lydia Alenkhe.

ACKNOWLEDGEMENT

I give thanks to God for His grace and mercy that has seen me through, during the course of my studies here in the University of Benin. I will also not fail to thank my parents for loving me unconditionally and providing all the resources I needed to bag my B.Sc degree. I am also immensely grateful to my supervisor, Dr. L. Eboigbe for his fatherly guidance, painstaking patience and kind supervision. I give special thanks to my course advisor, Dr. B. O. Edegbai for his support and counsel to me as one of his students. I will not forget to thank the entire department of Plant Biology and Biotechnology and her H. O. D., Prof. F. I. Okungbowa for providing the platform to carry out this research project.

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ABSTRACT

Nine tomato genotypes collected from Nigerian Institute of Horticulture and National Centre for Genetic Resources and Biotechnology, Ibadan, were cultivated and screened for resistance to *Oidium neolycopersici.* The pathogen was cultured in the laboratory in potato dextrose agar as culture medium and re-inoculated to the leaves and root area of the screenhouse tomatoes. During the course of the experiment, there were expectations to find a resistant variety which will serve as a potential tool for cultivating resistant varieties of tomato. However, all accessions of tomatoes inoculated with the pathogen were evaluated and found to be susceptible to the pathogen. The pathogen totally colonised the plants, caused necrosis of the leaves, prevented flowering and fruiting, and ultimately caused the death of all tomato plants observed in this study. The study showed that powdery mildew (*Oidium neolycopersici*) poses significant threat to greenhouse tomatoes, as well as tomatoes grown in the field.

CHAPTER 1

INTRODUCTION

1.1.0 Tomato

The tomato plant (*Lycopersicon esculentum* Mill. Con. Syn. *Solanum lycopersicon* Lin) is one of the world's most important crops due to the high value of its fruits both for fresh market consumption and in numerous types of processed products. They are also important for the well-being and also contain flavonoids, chlorophyll, organic acids and phenolic compounds (Giovanelli *et al*, 2002). It is cultivated and used in various cuisines all over the world. It can be eaten fresh or cooked as salad sauce, soups or processed into ketchup, purees and paste. One of the main constraints to tomato cultivation is damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which cause severe losses in production. Nigeria is ranked the second largest producer of tomato in Africa and 13th in the world (Eboigbe and Eshikhogie, 2015). World volume of production has increased approximately 10% since 1985, reflecting a substantial increase in dietary use of tomato. Tomatoes and tomato products are rich sources of folate, vitamin C, and potassium. Relative to phytonutrients, the most abundant in tomatoes are the carotenoids. Lycopene is the most prominent carotenoid followed by beta-carotene, gamma-carotene and phytoene as well as several minor carotenoids. The antioxidant activity of lycopene as well as several other carotenoids and their abundance in tomatoes makes these foods rich sources of antioxidant activity (Beecher, 1998).

The production of tomatoes in Nigeria in 2010 was about 1.8 million metric tonnes, which accounts for about 68.4% of West Africa, 10.8% of Africa's total output and 1.28% of world output (Ugonna *et al*, 2015). With a global production of 177 million tons, Tomato

(*Solanum lycopersicum*) is considered as one of the most widely grown food crops in the world. (FAO, 2018).

1.1.1 Centre of Origin and Distribution

Although it is believed to have originated in western South America and Central America, its domestication and use as a cultivated food source may have originated with the Mexicans, before the Spanish and then other parts of the European-colonised world. It did not attain widespread popularity in the United States until the early 20th century. It is cultivated extensively for its edible fruits. The tomato plant was introduced into various parts of Africa by Europeans that established colonies around them. It was brought into Nigeria by the Portuguese who were the first European visitors to Nigeria.

1.1.2 Botany

It is a vascular plant(tracheophyte) as well as an angiosperm. Tomato plants typically grow to 1–3 meters (3–10 ft) in height. They are generally much branched, spreading 60–180 cm (24–72 inches) and somewhat trailing when fruiting, but a few forms are compact and upright (Britannica Encyclopaedia). It has a tap root system that grows to a depth of 50cm or more. Leaves are alternate about 15-50 cm in length and 10-30cm in width. It varies from imparipinnate to bipinnate with 2-6 opposite or subopposite pairs of petiole or subsessile leaflets. Leaflets are ovate to oblong with glandular hairs, petiole is 3-6cm. The five-petaled flowers are yellow, 2 cm (0.8 inch) across, pendant, clustered, bisexual and regular. Calyx is short and hairy. It is divided into five lobes growing about the fruit. Anthers are yellow. They have elongated sterile tip at the apex. Fruits vary in diameter from 1.5 to 7.5 cm (0.6 to 3 inches) or more. They are usually red, scarlet, or yellow, though green and purple varieties do exist, and they vary in

shape from almost spherical to oval and elongate to pear-shaped. Indeterminate tomato plants are perennials in their native habitat but are cultivated as annuals. Although its fruits are botanically classified as berries, they are labeled vegetables for nutritional purposes and are a good source of Vitamin C and the phytochemical, lycopene. The fruit is a fleshy berry, variable in size, shape, colour and pubescence. The immature fruit is green. Ripe fruits range from yellow to orange to red. Seeds are pear or kidney-shaped and numerous (Naika *et al*., 2005).

1.1.3 Taxonomy

The cultivated tomato is a member of the genus *Solanum* within the family Solanaceae. The Solanaceae, commonly known as the nightshade family, also includes other notable cultivated plants such as tobacco, chilli pepper, potato and eggplant. Tomato classification has been the subject of much discussion and the diversity of the genus has led to reassessment of earlier taxonomic treatments. Tomato was originally named *Solanum lycopersicum* by Linnaeus in 1753; *Lycopersicon lycopersicum* L. Karsten has also been used (Valdes and Gray, 1998). Miller (1768) in The Gardener's Dictionary used *Lycopersicon esculentum*. For a long time, tomatoes were known as *L. esculentum*, but recent research has shown that they are part of the genus *Solanum* and are now again broadly referred to as *Solanum lycopersicum* (OECD, 2017). The genus *Solanum* consists of approximately 1,500 species. The tomato clade (formerly recognised as the genus *Lycopersicon*) includes the cultivated tomato (*Solanum lycopersicum*) and 12 wild relatives, all natives to western South America. Tomato (*Solanum lycopersicum*) is derived from two wild ancestor species, *Solanum pimpinellifolium* and *Solanum cerasiforme*. Other wild species are useful for breeding disease resistance, colour improvement and desirable quality traits (Ranc *et al*., 2008). The 12 wild members of the *Lycopersicum* clade demonstrate a

high level of phenotypic and genetic variation, including a great diversity in mating systems and reproductive biology. Peralta, Spooner and Knapp (2008) recognised 12 species of wild tomato. Following the approach of Thorne (2000), tomato botanical hierarchy is presented below:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus: Solanum

Species: *Solanum lycopersicum*

1.1.4 Nutritional and Health Benefits

Tomatoes are loaded with all kinds of health benefits for the body. One of the most well-known tomato eating benefit is its Lycopene content. Lycopene is a vital anti-oxidant that helps in the fight against cancerous cell formation as well as other kinds of health complications and diseases. Free radicals in the body can be flushed out with high levels of Lycopene, and the tomato is so amply loaded with this vital anti-oxidant that it actually derives its rich redness from the nutrient. Lycopene is not a naturally produced element within the body and the human body requires sources of Lycopene in order to make use of this powerful anti-oxidant. While other

fruits and vegetables do contain this necessary health ingredient, no other fruit or vegetable has the high concentration of Lycopene that the tomato takes pride in.

Cancers such as prostate cancer, cervical cancer, colon cancer, rectal cancer, and cancers of the stomach, mouth, pharynx, and esophagus have all been proven to be staved off by high levels of Lycopene. Researchers introduced Lycopene into pre-existing cancer cell cultures and Lycopene prevented the continued growth of these cultures. This is powerful evidence that the health benefits of eating a tomato are quite phenomenal. It takes as little as 540 milliliters of liquid tomato product to get the full benefits of Lycopene. This means that a daily glass of tomato juice has the potential to keep a person healthy for life. Tomatoes are by far the healthiest of the fruits and vegetables with the power to ward off some of the worst known diseases to man (Kumar *et al*, 2012).

The provitamin A activity of beta- and gamma-carotene, their modest levels in tomato products, and the high consumption of these foods results in a rich supply of vitamin A activity from tomato-based foods. Tomatoes also contain several other components that are beneficial to health, including vitamin E, trace elements, flavonoids, phytosterols, and several water-soluble vitamins (Beecher, 1998).

Adejumo (2012) reported that a medium sized tomato contributes 40% of ascorbic acid (vitamin C) required in humans, which is important in forming collagen, a protein that gives structure to the bones, cartilage, muscle, and blood vessels and aids in the absorption of iron. Ascorbic acid is necessary for healthy teeth, gums and is essential for proper functioning of adrenal and thyroid glands. It is also an antioxidant and as such acts as a general de-toxicant. Tomato fruits contain vitamin A (5%), vitamin C (17%), and vitamin E (4%), potassium (5%).

Vitamin A is needed for maintenance of skin, mucous membranes, bones, teeth, hair, vision and reproduction processes. The chemical composition of the tomato fruit depends on factors such as cultivar, maturity and environmental conditions, in which they are grown (Davies and Hobson, 1981).

1.1.5 Cultivation and Yield of Tomato

Tomato requires a relatively cool, dry climate for high yield and premium quality. However, it is adapted to a wide range of climatic conditions from temperate to hot and humid tropical. The optimum temperature for most varieties lies between 21 and 24 °C. At these temperatures good quality seeds will take about seven days to emerge. Temperature affects flowering and pollination. The hot and dry weather leads to drying of the flowers and stops pollination. If temperatures are below 15 °C or above 29 °C, pollen release is restricted, resulting in incomplete fertilization of ovules. This causes collapsed fruit walls and formation of deep indentation in the fruit, a phenomenon called catface (Peirce, 1987, Bok *et al*., 2006).

The plants can survive a range of temperatures, but the plant tissues are damaged below 10 °C and above 38 °C. Tomato plants react to temperature variation during the growth cycle for seed germination, seedling growth, flower and fruit set and fruit quality. If cool or hot weather spells persist during flowering, pollen production will be low. This will influence fruit formation. It is possible to sow indoors earlier (in pots or trays). Light intensity affects the colour of the leaves, fruit set and fruit colour. A simple rule of thumb can be used to determine whether local water supplies are sufficient for growing tomato. If there are herbaceous plants (plants with many thin leaves) growing in the natural environment, it will be possible to grow tomato. You should be able to count on at least three months of rain. Water stress and long dry periods will cause buds and flowers to drop off, and the fruits to split. However, if rains are too heavy and

humidity is too high, the growth of mould will increase and the fruit will rot. Cloudy skies will slow down the ripening of tomatoes. However, adapted cultivars are available(Naika *et al*, 2005). Tomatoes grows best in light, free draining, fertile loam soil with pH of 5 – 7. However, tomatoes can be grown in a variety of soils (Purseglove, 1988, Naika *et al*., 2005).

1.1.6 Pests and Diseases of Tomato

Tomato cultivars vary widely in their resistance to disease and various forms of mildew and blight are common tomato afflictions. Some common tomato pests are stink bugs, cutworms, tomato hornworms and tobacco hornworms, aphids, cabbage loopers, whiteflies, tomato fruitworms, flea beetles, red spider mite, slugs and Colorado potato beetles. The tomato russet mite, *Aculops lycopersic*i, feeds on foliage and young fruit of tomato plants, causing shrivelling and necrosis of leaves, flowers, and fruit, possibly killing the plant. The plants are susceptible to a number of diseases, including bacterial spot, bacterial wilt, early blight, mosaic virus, Fusarium wilt and powdery mildew.

Bacterial spot can be caused by several species of *Xanthomonas* but the most prevailing is *Xanthomonas vesicatoria*, a gram negative rod (Jones *et al*., 2004). It affects the stem, fruits and other parts that are above the ground. Bacterial wilt is caused by *Ralstonia solanocearum*, a gram negative rod, motile with one or more polar flagella. Symptoms include dropping of leaves, wilt, adventitious root and leaf epinasty (Lin *et al*., 2010).

Earlier studies indicate as major economically important tomato diseases early blight (*Alternaria solani*), late blight (*Phytopthora infestans*), Septoria leaf spot (*Septoria lycopersici*) and viruses. In course of time, some diseases, which have been considered as minor importance, have become the most important ones. These diseases include Powdery mildew (*Leveilula*

taurica), root knot (*Meloidogyne spp*.) and bacterial wilt (*Ralstonia solanacerum*) (Worku et al, 2018)

1.2.0 *Oidium neilycopersici* L. Kiss (Powdery Mildew)

Tomato powdery mildew caused by *Oidium neolycopersicum* has started to cause severe epidemics on tomato during the last 15 years (Jones et al., 2001). Heavy infections were first recorded in Western Europe and during the 1980s, it was reported for the first time in North America (Mieslerová and Lebeda, 1999). It is a dangerous pathogen, which spread through temperate areas of the world. The host range of the pathogen is broad, and it is reported to attack over 60 species in 13 plant families, particularly members of Solanaceae and Curcubitaceae (Jones *et al*., 2001). Symptoms include powdery white lesions on the adaxial leaves surface and on all other aerial plant parts except on the fruits. The fruits are not directly affected but impaired photosynthesis and premature senescence reduces fruit size and nutritional quality leading to diminished yields (Mieslerová & Lebeda, 1999). In severe outbreaks, the lesions coalesce and the disease is debilitating resulting in fast death of leaves. It is extremely common in greenhouse tomatoes worldwide but increasing in importance on field-grown tomato crops.

Oidium neolycopersici is a pathogen affecting tomatoes as its primary host. However, unlike other pathogens responsible for powdery mildews (e.g., *Leveillula taurica*), *Oidium neolycopersici* is an external powdery mildew. It immediately produces white powdery spots that cover the upper side of leaflets rather than the underside. In time, dense, white mycelial network colonise the upper surface of the leaves.

1.2.1 Identification of *Oidium neolycopersici*

A new powdery mildew disease on tomato plants was reported in the UK in 1986 (Fletcher *et al*., 1988) but it has now spread world-wide. However, its true identity was uncertain due to the lack of a sexual stage and varying reports of its structure, particularly whether conidia were formed singly or in chains. Consequently, the new mildew pathogen on tomato plants was variously termed *O. lycopersicum*, *Erysiphe orontii* or *E. cichoracearum* (Bélanger and Jarvis, 1994; Boiteux, 1994; Koike and Saenz, 1999) or was simply described as *Erysiphe sp*. (Arredondo *et al*., 1996; Karasevicz *et al*, 1996; Kiss, 1996; Neshev, 1993; Olalla *et al*, 1998; Pernezny *et al*, 1998; Smith *et al*., 1997; Vakalounakis *et al*, 1992). The first appropriate description of the fungus, *Oidium lycopersicum*, appeared to come from Australia (Cooke and Massee, 1888), and the name was re-designated, in 1999, as *Oidium lycopersici*, in accordance with the International Code of Botanical Literature (Mieslerova and Lebeda, 1999). However, confusion remained over classification based on morphological characteristics. Consequently, Jones *et al* (2000) analysed the internal transcribed spacer regions of the nuclear rRNA genes from the new tomato powdery mildew pathogen and were able to differentiate *Oidium* (*neo*)*lycopersici* from *E. orontii* and *E. cichoracearum*. Moreover, Jones *et al* (2000) found *O.* (*neo*)*lycopersici* to be a sister taxon of *Erysiphe aquilegia* var. *ranunculi*. This was further confirmed by Kiss *et al* (2001) from a study of tomato powdery mildew fungi from Europe, North and South America, and Asia. Importantly, Kiss *et al*. (2001) recognized that all recent outbreaks of tomato powdery mildew reported outside Australia were caused by a species that formed conidia singly, or, in high relative humidity, in pseudo-chains of 2–6 conidia, and so created a new species, *O. neolycopersici*, for this pathogen. The Australian isolates, which always formed conidia in chains, retained the name *Oidium lycopersici* (Jones *et al*, 2001).

Characteristics of *Oidium neolycopersici*:

Mycelium white, thin, covering the upper and occasionally the lower faces of the leaves, and also the stems. Hyphae hyaline, septate, 4-8 µm wide, appressoria distinct, lobed to multi-lobed, rarely nipple-shaped, opposite or dispersed. Conidiophores erect, 58-115 µm (mean: 91.5 µm) long, foot-cells cylindrical or sometimes inflated in the middle and constricted at the base, 27-74 µm (mean: 42.4 µm) long, followed by 1-2 shorter cells or a single cell of about the same length. Conidia produced singly, or, in high relative humidity, in pseudo-chains of 2-6 conidia, 22-46 (mean: 33.5) × 10-20 (mean: 13.7) µm, ellipsoid-ovoid or doliform, without fibrosin bodies, germ tubes arising from an end or side of the conidium, substraight or curved, apex enlarged, lobed, rarely simple, germ tubes increasing in width from base to top (Kiss *et al*., 2001)

Habitat: On living leaves and stems of *Solanum lycopersicum* in greenhouses, also in the field.

Distribution: Nearly circumglobal, wherever tomatoes are grown.

1.3 AIMS:

1. To test for resistance and susceptibility of various genotypes of tomato to the fungal pathogen, *Oidium neolycopersici*.
2. To determine the level of damage *Oidium neolycopersici* can potentially cause to greenhouse tomatoes.

1.4 OBJECTIVES

1. Different genotypes of tomato will be observed and their resistance and susceptibility to *Oidium neolycopersici* will be determined with measurable data.

2. The pathogen, *Oidium neolycopersic*i, will be genetically and morphologically evaluated for easy identification.

CHAPTER 2

MATERIALS AND METHODS

2.1. Study Area:

The screening of the tomato genotypes was conducted at the Screen House, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin city [6.20N, 5.73E] located within the tropical rain forest zone. The annual rainfall is between 2000 to 3000 mm with peaks in July and September. It has minimum temperature of 20 °C and 40 °C. Atmospheric humidity is high throughout the year (Onwueme and Sinha, 1991).

2.2. Collection of plant materials: Nine genotypes of tomato seeds were collected from the Plant Genetic Resource unit of National Horticultural Research Institute (NIHORT) and National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan. The nine tomato accessions were labeled namely 1, NHTO(24), NHT0(26), NHT0(8), NHTO(28), NGB00737, NHTO(24), PICCO, 8 and 9. The genotypes labeled 1, 8 and 9 were without identifiers.

2.3. Isolation of Pathogen: The pathogen was previously isolated from infected tomato leaves and cultured in the laboratory. It was used for preliminary evaluation. It was also used to prepare the inoculum for inoculation of the screen-house tomatoes with the pathogen.

2.4. Transplanting of Seedlings/Plant Husbandry: After 2-3 weeks, nursery for transplant was prepared by filling 30 perforated plastic pots (labelled 1-10) with about 0.75kg of loamy soil. The soil was well watered. The seedlings were transplanted from the ten pots to the 30 new pots(seedlings from every one genotype spread in 3 pots) and transferred to the screenhouse. At day 5, wood ash was added to the soil in the 30 pots as liming material. Wood ash is rich in

calcium carbonate and helps to achieve a soil PH close to the neutral pH range, which is preferable for many plants including *Lycopersicon sp*. It also adds potassium to soil.

Plate 1: Arrangement of the tomato genotypes in nursery pots in the screenhouse.

Pla

te 2: The tomato plants 8 weeks after transplant.

2.5. Culturing the Pathogen: The media used to culture the pathogen, *Oidium neolycopersici*, were potato dextrose broth and agar. At day 6, potato dextrose agar was prepared as culture medium and poured into petri dishes. The organism was then inoculated from the infected leaf specimen and cultured in the petri dishes for subsequent study and comparative analysis.

2.5.1 How to Prepare Potato Dextrose Agar.

Apparatus/Materials for preparation of the culture medium include measuring cylinder, weighing scale, 200g Irish potatoes, agar powder, glucose and conical flask. Peel 200g Irish potatoes and add 1 litre of water. Boil for about 15-30 minutes. Measure 20g glucose for 1 litre of water in the measuring cylinder (10g for 500ml and 5g for 250ml). Measure 15g agar powder on the scale for 1 litre of water, 7.5g for 500ml and 3.75g for 250ml. Filter the water from the potatoes, and measure 50ml potato water. Mix the glucose, agar and potato water together in the measuring cylinder. Add distilled water till the mixture is at 250ml. Mix properly. Pour mixture into the conical flask, cork and autoclave. Apparatus required to autoclave with a pressure cooker include a conical flask, pressure cooker and heat source (eg. gas cooker). To begin, wash the conical flask thoroughly. Then pour the medium (i.e. potato dextrose broth) into the conical flask. Cork and seal the conical flask with foil paper. Pour water into the pressure pot. Place empty bottles inside the pressure pot to provide balance and support for the conical flask. Afterwards, place the already sealed conical flask in the pot. Cover the lid of the pot securely and place the pot on the source of heat e. g. Gas cooker. When it starts to steam at 10-15 minutes, cover the with steam lid. Time for another 15 minutes after it starts spinning. Drop from source and allow to cool before opening the pot.

2.5.2 How to Inoculate to Petri Dish: Apparatus/Materials required include inoculating loop, absolute ethanol burner, bleaching agent, petri dishes, a piece of cloth. To inoculate, first sterilise the inoculation chamber with a bleaching agent and absolute ethanol burner. Spread a clean piece of cloth over the flat surface. Carefully place the infected leaf on the piece of cloth. Using the inoculating loop, inoculate the powdery patches onto the petri dishes.

2.6. Koch's Postulate: Re-inoculation of Pathogen to Screenhouse Tomato Plants.

At nine weeks from time of transplant, 3ml of the pathogen cultured in the laboratory was inoculated to the leaves and root area of the screenhouse tomato plants. Inoculation was done by means of a syringe.

2.7. Data Collection:

After inoculation, the various genotypes were observed to determine their individual reactions to the fungal pathogen. Over a period of approximately 7 weeks, observable data was collected from the plants and recorded. Leaves of each specimen was observed for traces of powdery patches and the number of infected leaves per specimen was counted and recorded.

2.8. Measurement of Disease Incidence

The disease progression measured against time was calculated using Area Under Disease Progress Curve. The trapezoid method which is the most common way to calculate AUDPC was employed. It is performed by using a formula devised by Campbell and Madden (1990) or by plotting a graph of percentage of infection against time and summing the trapezoids between time intervals. Area Under Disease Progress Curve is a quantitative measure of disease intensity with time. It is used in plant pathology to indicate and compare levels of resistance to diseases among varieties of plants.

2.9. Morphological and Genetic Identification of Pathogen

Infected leaves were collected from the screenhouse tomato plants and powdery patches were inoculated onto petri dishes containing potato dextrose agar as culture medium. The powdery patches were also inoculated into agar slant bottles. The isolate was observed under a microscope.

CHAPTER 3

RESULTS

The results from this experiment show the serious damage this fungal pathogen causes in tomato genotypes. All the genotypes evaluated in this experiment were affected. The various ways the pathogen presented itself is shown in plates 3, 4 and 5.

Plate 3 shows a sample of the inoculated plants and degree of infection at 5 weeks from inoculation date and 12 weeks after transplant date. The pathogen exhibits itself on the leaves in the form of white powdery substance (powdery mildew) and black sclerotia-like substances, which when inoculated to Potato Dextrose Agar plate produced fungal growth.

Plate 3: Pathogen-inoculated tomato cultivar exhibiting symptoms of powdery mildew. No fruits produced after inoculation.

Plate 4 shows the control plants with fruits in clusters 12 weeks from transplant date. The control plants, grown in the field and not inoculated with the pathogen, show healthy growth.

Plate 4: The control tomato plants fruiting 12 weeks after transplant.

Plate 5 shows the severe damage the disease caused the pathogen-inoculated tomato cultivars at 12 weeks after inoculation.

Plate 5: The pathogen-inoculated cultivars at 12 weeks after inoculation.

Plate 6 shows a microscopic image of the pathogen cultured in the laboratory.

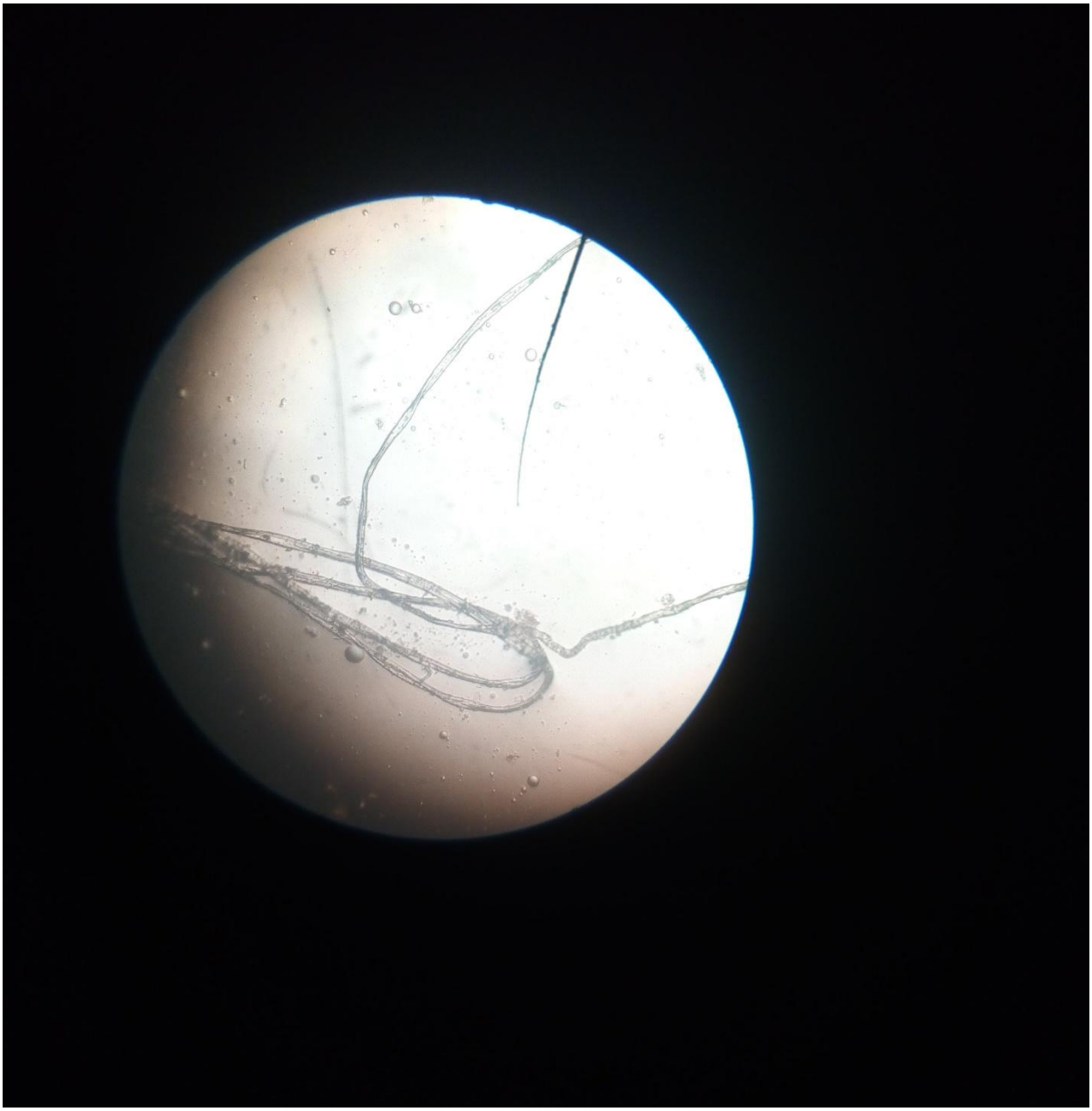

Plate 6: A microscopic image of *O. neolycopersici* culture.

AUDPC PLOT OF PATHOGEN INOCULATED CULTIVARS

Figures 1-9 show the levels of leaf symptoms in the tomato genotypes evaluated. Symptoms were observed in plants after ten days of inoculation. From that point, there was a progressive increase until the death of the plants. All the tomato genotypes screened were susceptible.

CULTIVAR 1:

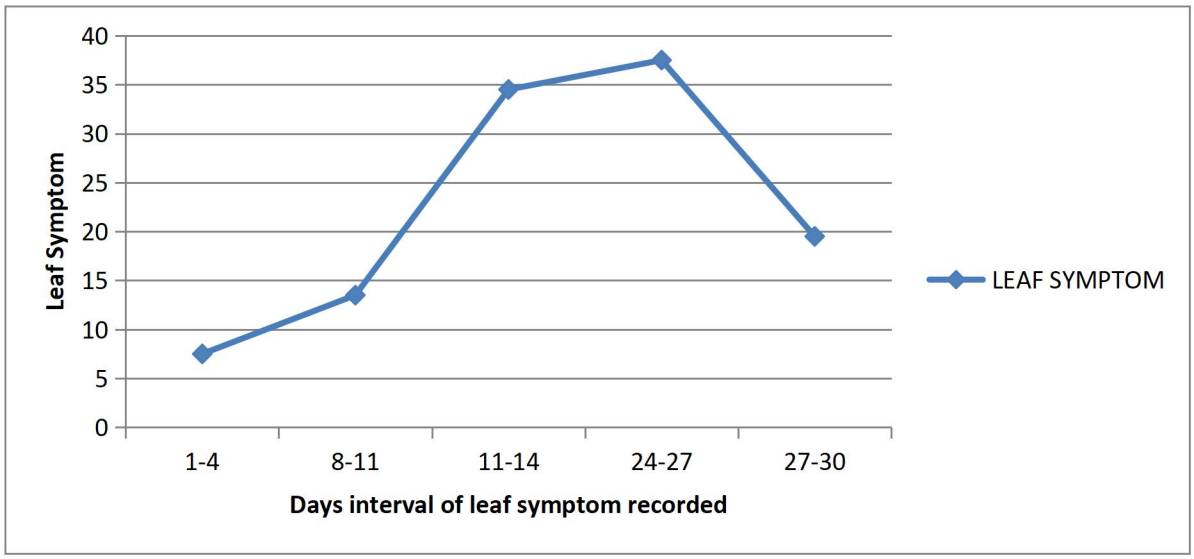

Figure 1: Evaluation of leaf symptom in Cultivar 1

CULTIVAR NHTO(24):

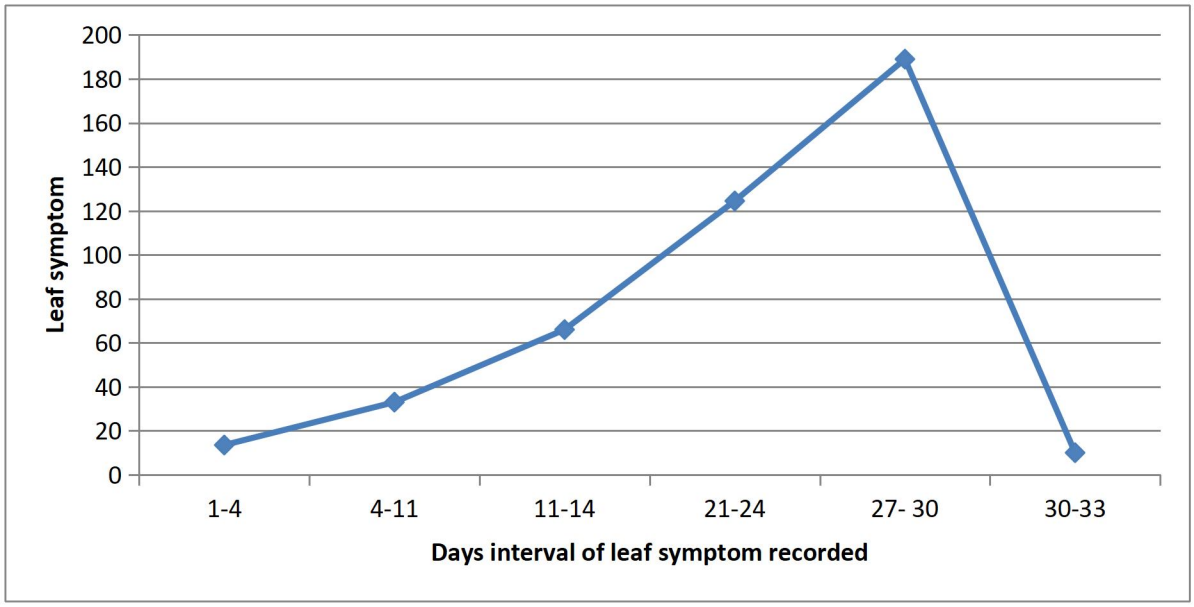

Figure 2: Evaluation of leaf symptom in Cultivar NHTO(24)

CULTIVAR NHTO(26):

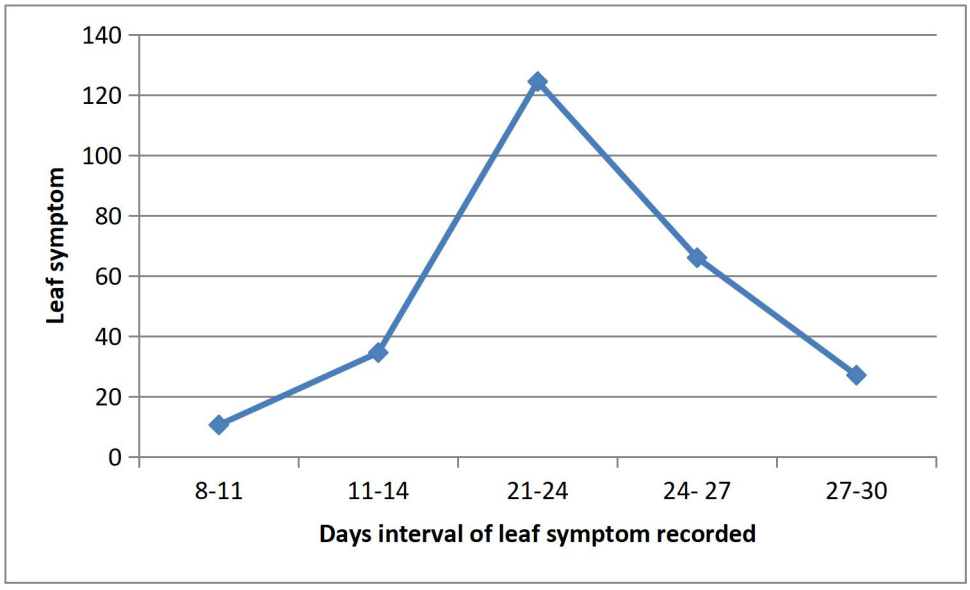

Figure 3: Evaluation of leaf symptom in Cultivar NHTO(26)

CULTIVAR NHTO(8):

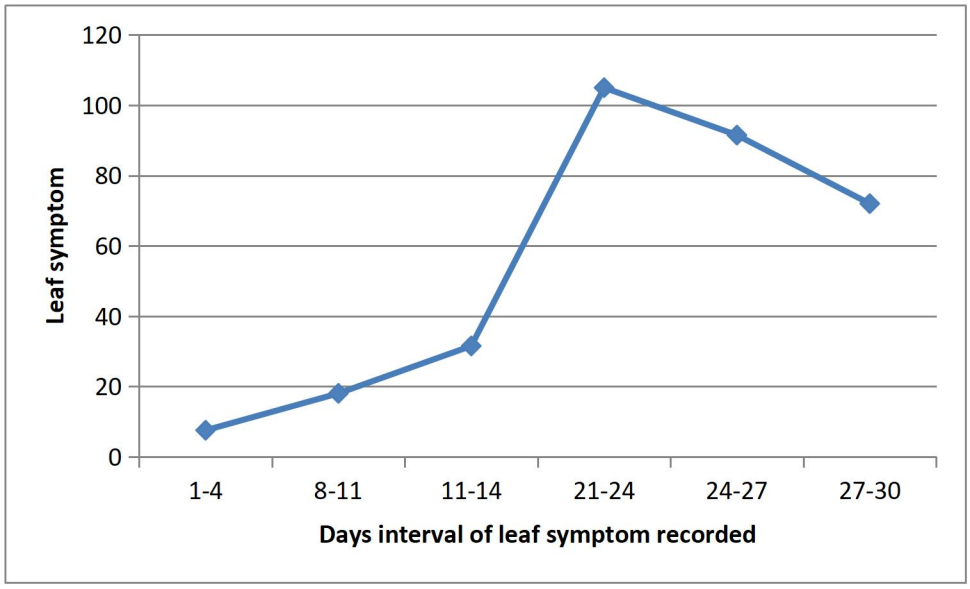

Figure 4: Evaluation of leaf symptom in Cultivar NHTO (8)

CULTIVAR NHTO (28):

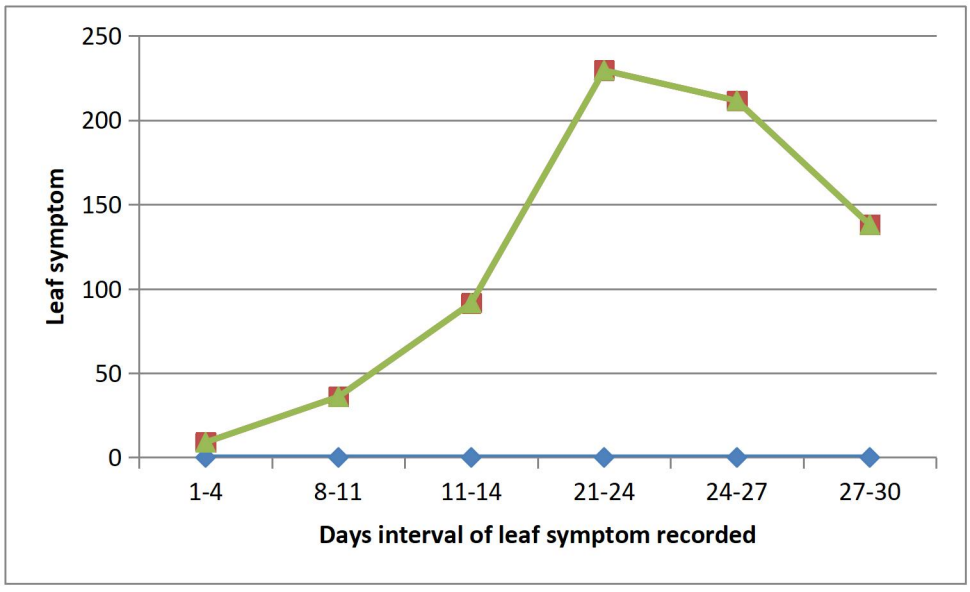

Figure 5: Evaluation of leaf symptom in Cultivar NHTO(28)

CULTIVAR NGB00737:

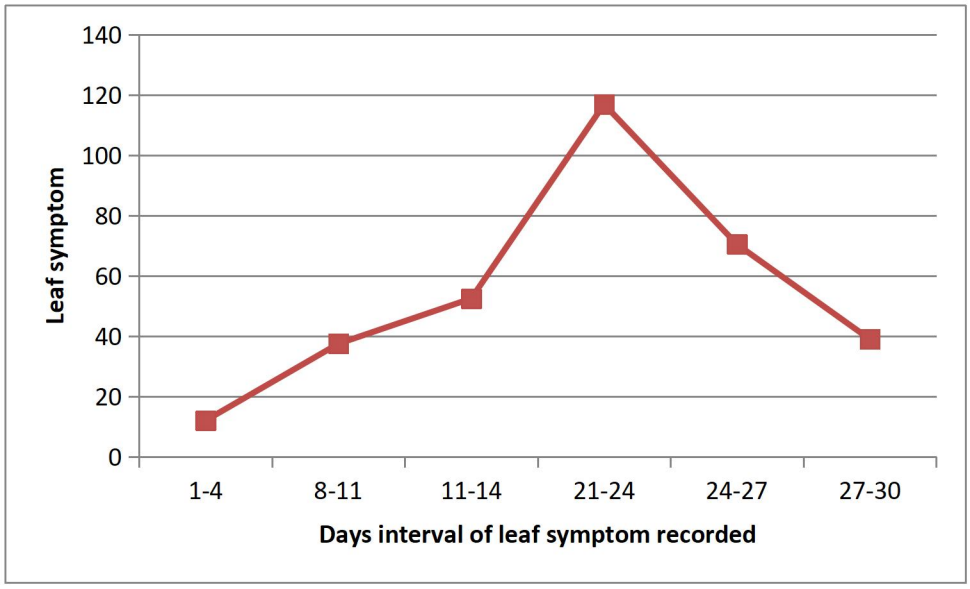

Figure 6: Evaluation of leaf symptom in Cultivar NGBOO737

CULTIVAR PICCO:

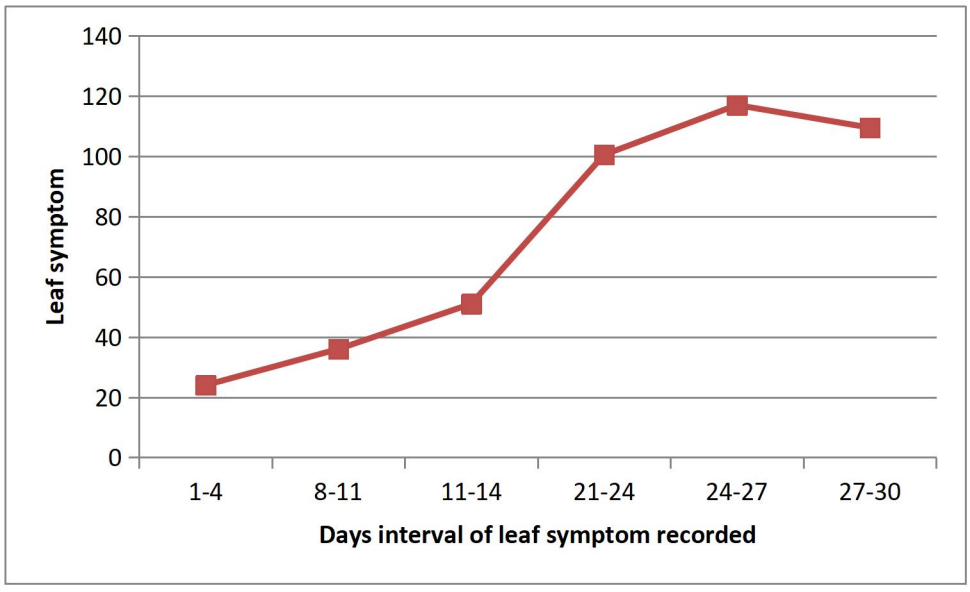

Figure 7: Evaluation of leaf symptom in Cultivar PICCO

CULTIVAR 8:

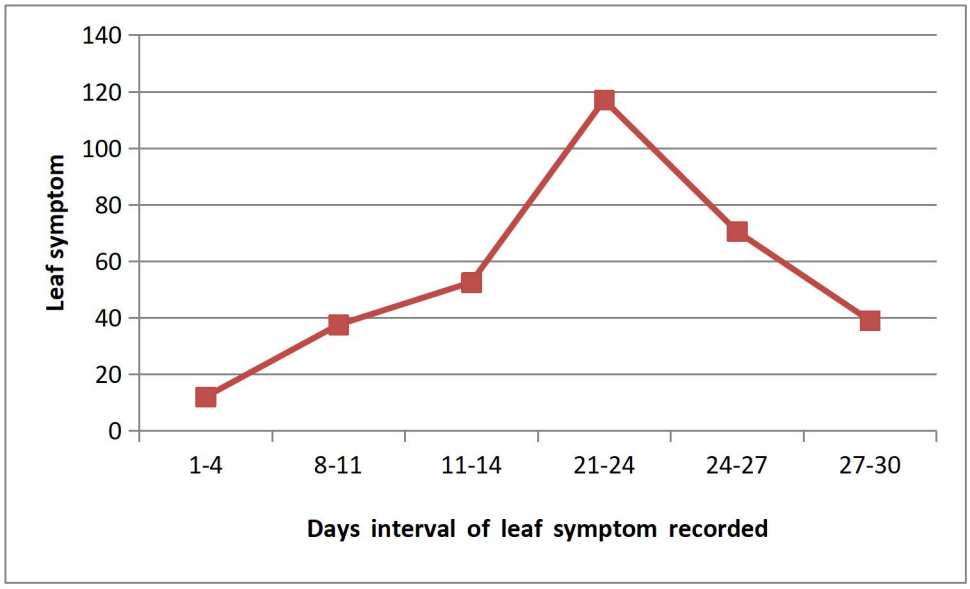

Figure 8: Evaluation of leaf symptom in Cultivar 8

CULTIVAR 9:

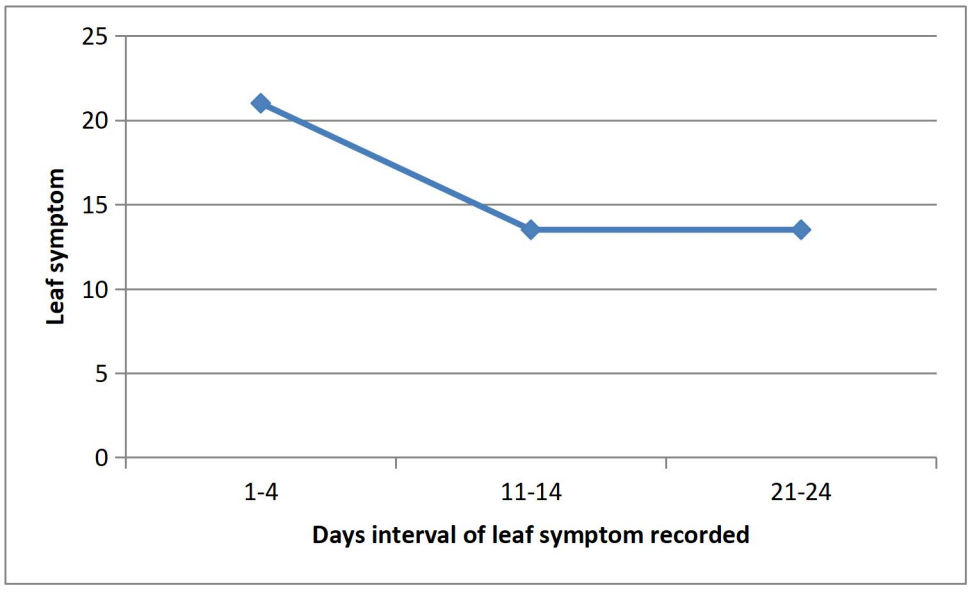

Figure 9: Evaluation of leaf symptom in Cultivar 9

CHAPTER 4

DISCUSSION

In plate 4, white powdery spots indicative of powdery mildew is observed on the leaves of the tomato plant. This disease is caused by many different species of fungi in the order Erysiphales, and the disease is easy to identify from other plant diseases, as its symptoms are quite distinctive. Infected plants display white powdery spots on the leaves and stems. The lower leaves are the most affected, but the mildew can appear on any above-ground part of the plant. As the disease progresses, the spots grow denser as large numbers of asexual spores are formed, and the mildew may spread up and down the length of the plant. *Oidium neolycopersici* is only one amongst other causative organisms for powdery mildew. However, powdery mildew caused by *Oidium neolycopersici* can be distinguished from powdery mildew caused by other fungal pathogens. The white mildew observed in this experiment is expressed as a mycelial network colonizing the leaf surface, topped by numerous conidiophores producing isolated hyaline conidia.

In an attempt to identify this pathogen under the microscope, I noticed that there was the presence of conidia, branched hypha and fruiting bodies which are typical of the fungus. As seen in this study, powdery mildew (*Oidium neolycopersici*) also halts fruiting, and several affected leaves are partially necrotic, others completely dry. The pathogen inexorably colonises the tomato plants, causing devastating damage and ultimately death of the plants. The symptoms exhibited and the characteristics of the microorganism easily distinguishes it from others such as powdery mildew (*Leveillula taurica*) and powdery mildew (*Oidium lycopersici*). *O*.

neolycopersici produces single, isolated spores. *O*. *lycopersici* bears branched spore during final development stage (Jones et al, 2001).

In this experiment (refer to the AUDPC plot graphs), none of the tomato cultivars is found to be resistant to the pathogen, *Oidium neolycopersici*. This fungus wreaked havoc on the tomato plants. It caused all the leaves to wilt, prevented the plants from fruiting and ultimately led to their death. The plants were inoculated with root dip method, and the disease appeared on the plant after inoculation. However, the control plants (plate 5) fruited and outlived the inoculated plants.

It is also important to note that the screenhouse serves as an ideal environment for the disease to thrive. According to Keinath *et al* (2012), greenhouses provide an ideal moist, temperate environment for the spread of the disease. This causes har, to agricultural and horticultural practices where powdery mildew may thrive in a green-house setting.

The pathogen, like other fungi, can be controlled using chemical methods, bio-organic methods, and genetic resistance. However, the goal of introducing genetic resistance as a method to prevent powdery mildew and other fungal diseases has been challenged by pathogens coming up with new strains and the growth habits of tomato which expose such resistant variety to other pathogens. The implication of this genetic shift is that a previously resistant variety becomes susceptible.

Over the last decade, much research effort has focused on testing wild tomato species for their resistance to *Oidium neolycopersici* infection. Resistance at various levels has been found in *Lycopersicon cheesmanii*, *L. chilense, L. chmielski L. hisutum, L. minutum, L. parviflorum, L.*

pennelli and L. peruvianum (Huang *et al*., 1998; Lindhout *et al*., 1994a; Mieslerova *et al*., 2000). These wild species are grouped into the 'peruvianum' and 'esculentum' complexes, according to how easily they can be crossed with the commercial tomato, *L. esculentum* (Rick, 1995). The 'esculentum' complex comprises *L. esculentum*, *L. cheesmanii*, *L. hirsutum, L. pimpinellifolium, L. parviflorum* and *L. pennelli,* and these species form the main focus of disease resistance screening against *O. neolycopersici*, due to the relative ease with which they can be crossed with commercially grown tomato cultivars (Lebeda *et al*, 1999; Mieslerova *et al*., 2000).

A control method against the spread of O. neolycopersici that is likely to develop in the coming years is the use of biological control for *O. neolycopersici.* A number of mycoparasites with activity against powdery mildews are known, including *Ampelomyces quisqualis*, *Sporothrix flocculosa*, *Stephanoascus rugulosus*, *Tilletiopsis sp.* and *Verticillium lecanii* (Falk *et al*, 1995; Verhaar *et al*., 1997) and there is high hopes of curtailing powdery mildew if these are combined with foliar sprays of phosphate and potassium salts, acting to cause direct effects on the pathogen and infer resistance on the plant.

According to Sajjad *et al* (2011), tomato like other vegetables is more prone to disease mainly due to its tenderness and softness. Based on the stem girth, there is no tomato that can stand when heavily fruited. Breeders are interested in high yielding genotypes. The components of yield traits are in number, size and weight of fruits. These facts made it almost impossible for the tomato not to creep on the soil. Since contact with one another and creeping on the ground are unavoidable, tomato plants are highly vulnerable to a lot of pathogens in general and fungi in particular (Eboigbe and Eshikhogie, 2015). This pathogen, which is relatively new, will pose a great threat to tomato cultivation if not controlled. Therefore, further research should focus on

studying the genetics of the fungus, possibly sequencing the whole genome of the fungus in search of all disease-causing genes present in the organism.

It is also important to note that, according to Rabab *et al* (2017), greenhouse climatic conditions provide an ideal condition for the development of many foliar, stem and soil-borne plant diseases.

CONCLUSION

This study shows that cultivated tomato is highly susceptible to powdery mildew caused by *Oidium neolycopersici*. Therefore, it is important to not only be aware of this pathogen and the resulting disease it causes to plants, but also to pay attention to prevention management practices, as the accompanying damage can significantly reduce important crop yields. Currently, *Oidium neolycopersici* not currently poses a significant threat to greenhouse-grown tomatoes, it is also of increasing importance on field-grown tomato crops. Therefore, further research efforts should be put into finding a lasting solution to this menace.

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APPENDICES

1. Number of infected leaves from observed day 10-39 after inoculation.

VARIETY	DAYS								
	10	13	17	20	23	30	33	36	39
1	0	5	5	4		11	12	13	0
NHTO(24)	3	6	7	15	29	40	43	54	72
NHTO(26)	0	2	3	4	19	57	26	18	0
NHTO(8)	0	5	4	8	13	35	35	26	22
NHTO(28)	0	6	6	18	43	71	82	59	33
NGBOO737	6	14	17	31	39	33	21	22	
PICCO	5	11	3	21	13	29	38	40	33
8	3	5	8	17	18	45	33	14	12
9	10	4	6	8	1	2	4	7	8

2. Trapezoid values of leaf symptoms.

To get these values, the trapezoid method which is the most common way to calculate AUDPC was employed. It is performed by using a formula devised by Campbell and Madden (1990). The average value of two successive values (Appendix 1) were multiplied by the number of days' interval. The procedure was repeated for all consecutive pair of values.

VARIETY	TIME INTERVAL(DAYS)						
	1-4	8-11	11-14	21-24	24-27	27-30	
1	7.5	13.5		34.5	37.5	19.5	
NHTO(24)	13.5	33	66	124.5	145.5	189	0
NHTO(26)	3	10.5	34.5	124.5	66	27	
NHTO(8)	7.5	18	31.5	105	91.5	72	
NHTO(28)	9	36	91.5	229.5	211.5	138	
NGB00737	30	72	105	81	64.5	33	
PICCO	24	36	51	100.5	117	109.5	
8	12	37.5	52.5	117	70.5	39	
9	21		13.5	9			