# CHAPTER-2

# 2. Review of literature

## 2.1 Introduction

The disease, which has been identified as a significant barrier to the sustainable production of cumin, can be treated with fungicides, biological agents, botanicals, and combinations of these. The effectiveness of these management practices is, however, influenced by several variables, including pathogenic variability. The early pesticides were largely composed of plant extracts, and several plants were more commonly employed as sources of commercial insecticides. However, synthetic agrochemicals largely took over as the main commercial insecticides starting around 1940. The study of plant-derived natural products for use in agriculture initially declined, but this trend has since reversed itself as it has become clear that plant-derived natural products continue to hold great promise for advancing and influencing agrochemical research in the present. There are believed to be at least 250,000 different plant species in existence worldwide. However, it was estimated that just 10% of plant species had been chemically analyzed up until 1993 (Benner, 1993), thus there is a tone of room for more research. The most efficient fungicides discovered in vitro were tested against *Alternaria burnsii* as a spray application for control of cumin blight in pot both alone and in combination with *Azadirachtin*.

74 percent of the substances found in plants and 14 to 28 % of the more powerful species of plants that are utilized medicinally and are pharmacologically active have been further researched, according to estimations by (Ncube et al.2008). Researchers are currently investigating the antibacterial capabilities of botanical medicines because of the acceptance of traditional medicine as an alternative form of treatment and the growth of microbe that are intolerant of the commonly available antibiotic (Neeman and Maoz, 1998; Lis Balchin and Deans,1996: Hammer et.al.1999). Suppressive soil shows comparatively minimal growth of disease despite the presence of a strong fungus and a weak recipient. It has been demonstrated that biological factors dominate in the vast majority of studied systems, even though both biotic and abiotic elements of the ecosystem of soil contribute to the soil's capacity to ward off infection. Many soils have

qualities in common with each other when it comes to the microorganisms that fight disease, yet some only exist in certain pathogen-suppressive soil systems. The strategies used by the microbes that inhibit infections include resistant host induction, it is antibiotics, especially nutrient antagonism (Mark, 2002). Silva and Wagner (2005) claim that the bulk of biocontrol agents have been found in soils that naturally prevent Fusarium wilt.

In Egypt, Cumin (*Fusarium oxysporum*) is a significant rop in spieces. It takes up 3% of Egypt's available planting acreage and is cultivated in the winter, summer, and fall (Glala et al. 2005). It is diverse and has great nutritional value, especially primarily as a vitamin supplement. The significant bioactive components that it includes that have antioxidant properties also have a beneficial effect (Ferraz Da Silva,2009). The application of fungicides to combat the Fusarium wilt disease of tomatoes is impractical and costly because the disease is soil-borne in origin. These substances poison the environment, negatively impact human health, and destroy a variety of useful microbes (Hayes and Laws, 1991).

Some of those typical soil-inhibiting plant pathogenic fungi that cause the wilt of pigeons, peas, tomatoes gram, guava, and many other crops is *Fusarium oxysporum.* According to Mehrotra and Aneja (2003), several different species of fungus are to blame for the respective hosts suffering significant losses. In addition to causing wilt diseases, this fungus species has also been linked to damping off and seedling blight (Agrios, 2005). According to reports, certain plant species have natural compounds that are harmful to several fungi responsible for plant diseases (Mishra and Dixit,1977). Several fungal infections of crop plants have been reported to be treated using some of the poisonous compounds derived from various plant species (Chary et al. 1984).

### 2.1.1 Wilt causing Pathogen-*Fusarium oxysporum.*

Most fungi are saprophytes, which are a varied collection of organisms that subsist on decomposing organic waste. The fungal organism found in soils systemic wilting which harms several economically significant products around the globe occurs by the fungal infection *F. oxysporum*, which lives in the soil (Ortoneda et al.2004). The plant-based disease *F. oxysporum* contains various specific types called *formae* organisms which

attack a range of organisms and cause a variety of diseases, just like different plant diseases. Trees & agricultural land can both harbor the soil-based pathogen *Fusarium oxysporum,* which lives in soil (Orr and Nelson 2018; Snell and Hansen;2019). This pathogen may linger on plant debris for a long period and affect both vegetative and regenerative development stages (Roy & Meena,2020; Postic et al.,2012). According to Velarde- Feox et al., 2018 & Altinok et al. 2018, these species are harmful pathogens for plants growing in greenhouses and open fields. The objective of this inquiry is to determine the pathogenic species and pathogenicity of wilt disease in advanced *A. mangium* seedlings.

One of the common soil-inhibiting plants' harmful fungi is *F.oxysporum* which wilts pigeon peas, tomatoes, guavas, and many other crops. Mehrotra and Aneja (2003) claim that a number of different fungus species are to blame for the large losses suffered by their respective hosts. This fungus species has also been connected to damping off and seedling blight in addition to wilt disorders (Agrios,2005). Several fungi responsible for plant diseases are said to be negatively impacted by natural chemicals found in a few plant species (Dixit and Mishra, 1977). Many dangerous substances produced by various species of plants have purportedly been utilized to treat a variety of fungal diseases in agricultural plants (Chary et al.,1984).

### 2.1.2 Blight causing pathogen-*Alternaria burnsii*

The fungus *Alternaria* belongs to the dictyosporic genus, family Dematiaceae, and order Hyphomycetes. The Alternaria genus was initially described by Neem in 1816. According to Neergaard (1945), *A. Alternata* belongs to the family *Longicatenatae*. *A. burnsii* developed an Alternaria spot on the leaf on *Asalio* foliage (Padule and Utikar; 1980; Melkania,1980). According to him, *A. burnsii* conidiophores ranged in lengths between 16 to 60.66 µm, were easy to understand brown color, and generally contained 1-3 septa, but occasionally had 4-5 septa. Conidia are cells that were found to be consistent in size and shape, often oval with a crude beak, and to range in color from pale to darker brown. They also measured between 10.26 and 77.52 x 4.56 and 14.82 µm. Additionally, the conidia possessed 0-3 horizontal and 1-6 transversal septum. At a magnification of 50, Simmons and Roberts (1993) used an electron microscope to investigate the three-dimensional sporulation patterns of *A. burnsii*. According to

Melkania (1980), *Alternaria burnsii* leaf spot on cumin leaves was spread by the *A. burnsii*. Small, brown lesions with an uneven form and a discolored tint that originally appeared as symptoms subsequently grew larger and darker. When lower plant leaves come into contact with irrigation channels, the infection process begins. Later, the seed coat and stem also exhibit similar characteristics. The disease is actually spread by infected seeds, soil, and leaves but diseased plant debris also serves as an inoculum for future occurrences. Up to mid-January, the disease's severity worsens. At this stage, the plant's stem and flower components also get a disease. The entire plant finally displays classic blight signs. Fungal colonization in the host plant's xylem causes obstruction and xylem breakdown, which causes signs of the wilt disease including leaf withering and yellowing of the leaves as well as the final death of the plant. It has been demonstrated that many plant species have chemical compounds that are harmful to many fungi that cause infection in crops (Amadioha, 2000; Kagale et al., 2004). The pathogen was later identified as the cause of the Alternaria leaf spot of cumin, which was first noticed in 1967 in the Ethiopian region of Kaffa (Stewart and Dagnalechew, 1967). The first case of the genera Alternaria spores on cress leaves was reportedly produced by *Alternaria burnsii*, according to Melkania (1980).

## 2.2 Isolation, Identification, and Characterization of *Fusarium oxysporum* and *Alternaria burnsii*

According to Chatterjee and Rai (1974), the infectious agent *F.oxysporum* is a pathogen that was discovered from the roots of plants that had wilt symptoms and was kept alive on a PDA medium (Potato Dextrose Agar medium). *Fusarium spp*. was isolated from the soil and roots of cumin plants gathered in the field. Because potato dextrose agar offers a nutrient-rich substrate for growing a variety of fungi, it is used.$10^{-4}$ at a dilution using the pour plating method of Ofunne (1999). Numerous diseases affect the cumin plant (Fusarium wilt), which causes significant crop losses. The diseases include blight, tiny leaves, wilt, and others. One of the major diseases among them that has a significant impact on the number of eggplants in the field is wilting. Wilt is a caused by fungus called *Fusarium oxysporum*. The disease's primary symptoms cause adult and seedling plants to wilt. Several fungi responsible for plant diseases are said to be negatively impacted by natural chemicals found in a few plant species (Dixit & Mishra,1977).

Several fungal infections of agricultural plants were purportedly been treated using several specific poisonous compounds obtained using different types of plants (Chary et al.,1984). The latter causes tomato foot rot (Romberg & Davis 2007; Vawdrey & Peterson 1988); moreover, it can be pathogenic to blossoms, fruits, and plant stems (Nakayama et al. 2010). However, *F. oxysporum* is unique in that it has more than 82 recognized formae specials that are categorized based on host specificity (Smith 2007). In fact, only two different formal specialties exist in the *F. oxysporum* important to cumin (Blancard 2012). Jarvis & Shoemaker, 1978 W.C. Snyder and H.N. Hansen's *Fusarium oxysporum f. sp. lycopersici* 1940. According to Blanchard (2012) and Ignatov et al. (2012), the latter has also been identified as one of the most prevalent diseases of fresh-stored cumin. The great range of species and races makes it extremely challenging to classify this entire species (Sayed 1976; Fisher 1982).

### 2.2.1 Morphology of *Fusarium oxysporum* & *Alternaria burnsii*

In order to practically distinguish between formae specialist of the same species or even races of the same forma specialis, studies of the pathogen colony's morphological, microscopic, and molecular characteristics, as well as the application of pathogenicity tests, are needed (Arie & Hirano 2006, Summerell & Leslie 2006, Capparelli & Iannelli 1982). A virulent *Fusarium* is distinguished by its host plant since it can perfectly manifest itself on both immature and adult plants (Boland & Kuykendall 1998).

Based on cluster morphology, the larger and micro-conidial structure, and conidial measuring, a total of 60 types of *Fusarium* were detected. Following the method of booth,1971 these *Fusarium* isolates have since been shown to be related to the *F. oxysporum* type. Examination of conidia was performed at 40X magnification using the Motic image plus 2.0 program. *Fusarium* has been the subject of several morphological, microscopic, and molecular research, and has been reported on PDA. When *F. oxysporum* first appears, it has an abundant white mycelium that gradually becomes pale pink with a trend toward violet. Microconidia are unicellular oval or ellipsoid in their microscopic form. Macroconidia include a pedicellate basal cell and an attenuated apical cell, and they are branched (or unbranched) in short mono phialides. Chlamydospores are frequently solitary, have a smooth wall, and form in intercalated

or terminal places, according to Smith (2007), Leslie & Summerell (2006), Dillard 1989, Summerell et al. (2003), and Campbell et al. (2013).

### 2.2.2 Purification of pathogens

Various plant elements, including roots, stems, leaves, flowers, and fruits, can become infected by fungi, which then cause distinctive visual symptoms like spots, blights, anthracnose, wilts, and rots. Cut into little pieces, the contaminated tomato, chili, and maize pieces were collected (Manual of Soil Fungi, Gilman CJ. A, 2019). After thoroughly rinsing tissue in sterile water, the fungus responsible for the symptoms is found in plant tissues exhibiting them.The affected materials and surrounding unharmed parts are subsequently put into sterilized Petri plates carrying 0.1% mercuric chloride, a liquid to perform surface sterilization of tissues from plants using flame-sterilized tools. The tissues are subsequently separated into tiny (2–5 mm square) segments. The plant portions were placed on Plates of PDA for five to seven days of incubation to allow the fungus to fully develop. The resulting fungi were purified on Rose Bengal medium using the hyphal tips procedure, and each isolated fungus was then subcultured on slant media for further research. The fungus was recognized (Nelson PE, Toussoun TA) according to the cultural traits stated by Nelson et al. (1982).

To isolate fungus, soil that had pathogens in it and the plant's roots that were wilting in an area were employed. Following a two-minute immersion in a solution containing 1% sodium hypochlorite, a total of three washings in filtered water, then drying in an atmosphere with laminar airflow, the surfaces of this root sample were sterilized (Suvandi et al.2012). The plant specimens were then cleaned under running water. They were laid in a plate filled with two percent (W/V) agar and one percent tetracycline sulphate, then incubated for 48 hours (Leslie & Summerell 2006; Gardner 1980 et al. 2012).

## 2.3 Koch postulate

There are countless microorganisms that animals and plants come into touch with daily, but only a few of them may harm living things (American Society for Microbiology). Plants and fungi interact in a variety of ways, from mutualism to parasitism (Wikipedia. Koch's postulates;2014). Organisms play a very important role in releasing essential

nutrients into the soil that are required for the plant (Jayawardena, R.S.; Hyde, K.D, 2019, Enke;1955)

They can significantly harm forestry and agriculture as plant pathogens (Hyde, K.D Jayawardena R.S 2019:) Since pathogens with infectious properties have specific characteristics that increase their ability to pass on disease, it was once thought that they had characteristics that were wholly distinct from other types of bacteria. (Casadevall, A.; Pirofski, 2002). As a result, microorganisms are now divided into groups based on how likely they are to cause disease. Some organisms cannot be viewed from the perspective of the pathogen since their properties of the infectious agent, rather than the qualities of the microbe (Amponsah, N.T., 2011). For instance, Staphylococcus epidermis and Candida albicans can only infect persons who have weakened host defenses or altered microbiota, but other organisms can infect both healthy individuals and those with weakened immune systems (Méthot, P.O.; Alizon, 1998). As a result, a pathogen cannot be classified only by its capacity to harm hosts with compromised defense (Phillips, A.J.L.2001; Hyde, 2019). Relations between hosts and pathogens can be divided into categories according to the situation that impairs the host's normal tissue structure or function (Ross, L.N. and Woodward,1998). The concept of a pathogen and its relationship to its host are linked. Thus, a creature that may harm the host might be referred to as a pathogen (Cambau, E.; Drancourt, M 1994). One of biology's most intricate processes is the relationship between hosts and pathogens (Schneider, D.J.; Collmer,1995). Based on how they live, three types of pathogens are Hemi biotrophs, biotrophs, and necrotrophs. (Glazebrook, J. et al 2005). Rusts, powdery mildew, and Peronospora are examples of biotrophs that rely on live plant cells for nutrition (Harrach, B.D.; B. Barna; 1922). Through the use of cells wall-degrading enzymatic agents' Necrotrophic pathogens like the bacteria *Botrytis Cinerea and Chiofolos, heterostrophus* rapidly devastates its host cells (K.D. & Jayawardena 2017). The ability of plants recognizes pathogens and react to them results in the rapid activation of defense systems. Numerous fungal species that affect various hosts and have distinct tissue specificities can cause plant diseases, which manifest in a wide range of symptoms (Guarnaccia, V.; Groenewald, J.Z, 2018). The anthracnose infection turns brown, post-bloom berries, seedlings blight & foliage disease. The loss brought on by Colletotrichum species can affect a variety of hosts (Crous, P.W.; Hawksworth,

2015). A variety of plants are susceptible to infection by Diapor organisms, which can cause seed degradation, dieback, shoot cankers, foliage pigmentation, and roots and produce rots. (Bhunjun, C.S.; Dong, 2020). Post-harvest infections, canker lesions, plant dieback, green spots, pin blight, top disease, grey destruction, severe chlorosis, fruit turning brown, and additional pestalotiopsis organisms can affect several economically important plants. (Phukhamsakda, C.; McKenzie). The agriculture industry, ornamental crops, and fungal infections can all result in catastrophic diseases with significant economic effects (Vohník, M.; Borovec, O.; Kolaříková, 2019). Some diseases, such as a species that was accountable for the Irish potato-based bipolarism and Famine, between oryzae, that was accountable for the 1943 Bengal famine in India (Huhnorf; 2012, Schoch C.L.; 2017) have played a role in humanity's lack of food. Fungus is thought to wipe out thirty percent of the world's agricultural produce each year (Aime, M.C.; Lucking;2020). In addition to surface infections, pathogenic fungi can also result in invasive infections in humans that can be fatal (Brooks, T.M.; Cuttelod, A et al. 2015). More than 1.6 million people die because of fungal infection each year, according to estimates (Lindahl, J.F and Graminimize et al.1965). As a result, a rise in the knowledge of the infection, recipients, and means of propagation of fungi is critical for assuring worldwide biosecurity (S. Kasuga, D.J.19945; Kroken and Taylor et al.1995). This is essential that one remembers that pathogens can be beneficial to the environment. According to TDS (Tree Disease Concepts), rainforests require a Healthy amount of disease because infections operate as government agencies, terminators, and recovery of resources processes, helping woodlands to survive throughout the period. (Andersen;2002, K.F. Nielsen et al. 2009). This idea makes clear that the pathogen effect only has an influence on forest health when it rises over what is necessary for the system to remain sustainable.

We emphasize the significance of correctly classifying fungal pathogenic species using a polyphasic method in this study. It must be demonstrated whether the fungus is likely a pathogen and connected with plants. We go over the background, significance, and difficulties of using Koch's postulates. According to the American Society for Microbiology, Robert Koch makes a number of hypotheses, or evidence criteria, regarding mycobacterium bacteria that will later become his most well-known work. The evolution of Koch's postulates, an ensemble of norms that define how

microorganisms interact., is a well-known event that took place in 1884, and it is remembered on the American Society for Microbiology's homepage's timeline of the growth of medical microbiology.

However, what is it that everyone seems to understand about these hypotheses that, for instance, has led to a 1.5-word entry in (Cunningham A. Transforming Plague, 1992)? The German doctor Robert Koch was a pioneer in the field of healthcare bacterial science during the year 1870 and the year 1880. Today, many people still rely on Koch's postulates, a set of rules for demonstrating infectious causation in an experimental process. According to reports, Koch created these postulates at the beginning of the 1880s. Separation of a probable infection in materials, cultivating of related microorganisms within purest cultures), and pandiculation (in testing on animals meant to mimic its initial form pathology) are each of the processes most commonly mentioned in health care words. The hypotheses, according to researchers such as Roy Porter and Erwan, represent a transformation of bacteria in late-nineteenth-century medicine (Burckhardt J. U at el. 1881). Medical philosophy regards them as a vital step in building an investigation program centered on explanations of causes in medicine more broadly. KC Carter and colleagues (2023). Thousands of times are referenced in the postulates of experimental medicine. It is commonly contested in this field as to whether a specific bacterium qualifies as a pathogen of a specific disease. The virus's successful discovery was announced in terms of its ability to fulfill these specific requirements when the search for the pathogen responsible for SARS first got underway a few years ago: "The etiology of SARS: Koch's postulates fulfilled" (Halvor Tv. A bacteriological, 1993). Numerous of these references, as noted by Robert Merton, are merely instances of name-dropping or eponymy and reflect the well-known propensity of physicians to provide historical context for events (Thagard P, 1999). Therefore, publications have names such as "Koch's postulates apply with/adapted with/fulfilled modified about" (Kuiken T et al., 2004) have been published and similar ones are published in contemporary research. Throughout these situations, the hypotheses may be referred to as being "an unchangeable requirement for establishing causation in medicines." (Fredericks D, Relman D, et al. 1918). Something remains to be altered during a century to meet Koch's postulates. It's possible that the issue was put in a paper with a very lenient definition of time. Although there does not seem to be a core set of

the postulates, writers list anything from two and 10 of them, therefore meeting the postulates is probably more difficult than it first appears This is also vital to observe such references with the suggestions through the other point of view, especially as devoid of anything, flexible declarations with no duty to comply to any therapeutic microbiological norm. Several Hippocratic oath readings, on the other hand, are likely comparable activities that fail to verify whether the person who speaks was involved in any detectable amount of moral thinking (Aldershot: Ashgate; 2002).

## 2.4 *In vitro* evaluation of *pathogens*

The efficacy of the plant extract against two different pathogenic fungi was tested in vitro using the Poisoned Food Method (Thapliyal and Nene,1993; Carpenter; 1972). *Ocimum sanctum* (Tulsi), *Lantana camara* (Wild sage), *Azadirachta indica* (Neem), *Calotropis gigantea* (Madar), and *Eucalyptus globulus* (Eucalyptus), among the botanicals utilized. After being cleansed with sterile distilled water, plant parts were left to air dry. Measured plant components have been crushed using a 1:1 w/v proportion in a pestling device. The mixture was homogenized for five minutes before being filtered using Whatman's filter paper No. 41 and two layers of muslin cloth extraction liquid (100%) and agar made from PDA media were used to create concentrations of 3, 5, 7, and 9%. *F. oxysporum sp. lycopersici* and *A. brassicas* have been obtained from marinara and coriander foliage, correspondingly. They took 3-mm cultural disks through a seven-day-old colony and placed them in the middle of Petri plates with potato dextrose and the appropriate concentrations of botanical extracts. A control plate was used that contained no extract medium. There were kept three replications. According to Prasad and Barnwal (1999) and Kamlesh and Gurjar (2002). The mycelium's radial growth was noticed during ten days of incubating around 27±2ºC, while a method for determining the percentage of inhibition was applied. The preferred analytical program was Minitab. These include garlic bulb extracts, as well as mentha and datura leaf extracts (Singh et al., 2003; Chattopadhyay et al., 2002; Shivpuri and Gupta, 2001). Some researchers have discovered results that are almost equivalent while investigating the in vitro impact of different extracts from plants on the *solani* strain and other species of *Fusarium* (Arya et al., 1995; Lolpuri, 2002). These inhibitions are also thought to be caused by the presence of cannabinol in cannabis

(*Cannabis sativa*) and essential oils in plants like Tulsi (*O. sanctum*), datura (*D. alba*), and garlic (*A. sativum*) (Anonymous, 1972). According to Vanitha (2010), the EC formulation of wintergreen oil completely inhibited Alternaria chlamydospore's ability to develop mycelial. The same results regarding the effectiveness of some botanical extracts towards *Alternaria alternata* were documented by (Mosallanejad and Zakar;2010 and Raghavendra et al 2009.

Using an approach of poisoned food, the pathogen toxicity of the crude plant extract was assessed (Mishear and Tiwari,1992). The percent of mycelial development that is suppressed in comparison to the control was determined by using the following equation.

$$L = [(C – T)/C] \times 100$$

The numerous botanicals, including plants of C. *Pluricutis* and *Convolvulus*, *Adhatoda vasica*, *Azadirachta indica*, and *Cinnamomum camphora*, suppressed by growth of *F. oxysporum* (Prasad & Ojha, 1986;), and *Ocimum sanctum*, as well as others, inhibited the mycelial development of many species of *Fusarium* (Furgal wegrazyake, 1984 and El. Sharma et al.; 1986).

## 2.5 Pot trail

The cumin plant (*Solanum melongena L*.) is susceptible to several infections, which severely damage the harvest. Wilt, blight, tiny leaf, and other diseases are among them. One of the major diseases producing a significant decrease in the field is the withering of eggplants. Wilt is brought on by the fungus *Fusarium oxysporum*. Seedlings and adult plants wilt as a result of the disease's primary symptoms.

Older leaves on the fungus-infected plant cause wilt droop and eventually turn yellow. On one side of the plant, leaf yellowing may happen, and over time, the majority of the leaves may turn yellow and wilt. Chemical management techniques were used to protect agricultural plants from infections and to avoid plant diseases. Because of the expensive expense of synthetic pests and associated possibly serious negative consequences, the application of materials that break down, including natural extracts of plants from parts,

has evolved into more significant for the management of plant diseases during the past thirty years (Mitral et al 1983; Fowcett and Spenser, 1970 Ahamed and Grainge,1988).

Three replications of the experiment were carried out in 30 cm pots using the Randomized Block Design (RDC). For the experiment, the pots were filled with a 3:1:1 combination of soil, sand, and FYM. Cumin seeds from the local landrace were surface sterilized (0.1% $HgCl_2$ for 2 minutes) for each treatment. Following germination, trimming was carried out to keep 10 plants in each container. The experiment was carried out in pot culture three times using a Completely Randomized Design (CRD). Spray inoculation with a conidial slurry with $1x10^4$ conidia ml-1 concentration was performed on 45-day-old plants. (1–5). The PDI and Percentage Efficiency of Disease Control (PEDC) were determined using the method provided by Chester (1959). The severity of the condition was recorded using a recognized 0–5 scale developed by Gawande and Patil in 2003. Symptoms of *Fusarium oxysporum* on infected cumin plants have been assessed after 15, 21, and 30 days based on a visual index that is graded according to a severity scale (Rodrigues & Menezes 2006):

0: No infection;

1: A minor disease that affects about 25% of the body.

2: One or two yellowing leaves signify a mild infection.

3: A widespread infection, two to three yellowed leaves, and the drooping of half the leaves. 75% of the plant's leaves began to wilt and droop and all of the leaves became yellow;

4: Total infection. The entire plant's leaves started to wilt and become yellow, and soon the plant died.

Based on this scale, the following formula has been used to determine sickness occurrence (%): However, according to a scale of disease severity created by Batson & Roy (1982), symptoms on tomatoes have been recorded up until the $16^{th}$ day following inoculation (Pandey et al. 2003):

Σ of all ratings maximum rating grade × total number of observations × 100

Lesion sizes are as follows: 0, no infection;

1, 5mm or less;

2, 6mm or more;

3, 11mm or more; and

4, greater than 15mm.

In addition, repeated use of fungicides promotes the growth of unusual disease strains with resistance (Littrell and Smith, 1980). William 1984 observed that the tolerance showed by an assortment of crops in a particular region might not prove beneficial in others due to the presence of pathogens and/or variations among external variables. Furthermore, prolonged use of pesticides encourages the emergence of unique infection forms having resistance.

Now a days need for novel strategies to combat plant diseases is highlighted by these elements (Wilson et al., 1987). Therefore, it is crucial to find fungicide substitutes that are less harmful to both humans as well as atmosphere. Compounds that are naturally found in trees, oily seeds, fruit and vegetables, and herb are known as defenders or nutrients (Kitts et al, 2000). These factors emphasize the need for novel approaches to combat plant diseases (Wilson et al., 1987). Finding safer fungicide substitutes is thus urgently needed for both environmental and human health reasons. Functional foods and antioxidants both contain natural components that can be found in vegetation, vegetables, fruits, oily seeds, and plants, according to Kitts et al. (2000).

According to research by Zhang et al. (2005), environmentally friendly plant extracts offer a lot of potential as a substitute for synthetic fungicides. Plant extracts are thought to be affordable, non-toxic, readily accessible, and easily biodegradable. More and more studies from around the world have been reporting on the antibacterial activities of plant extracts (Cowan, 1999). Secondary metabolites, which are found in plant-derived compounds including alkaline compounds, phenolic compounds, and tannins, for example, saponin, flavonoid, and glycosides might possess anti-fungal activities (Elzaawely, and Maswada 2013; Hassan, 2012).

## 2.5.1 Management of Pathogens

Pathogenic fungi have been eliminated using a variety of disease control techniques. Cultural, physical, chemical, and biological techniques are among them (Kata, 2000). The fact that pathogens are soil-borne makes it extremely difficult to manage the disease. In addition to damaging the environment, synthetic chemicals used to treat plant diseases are also dangerous to human health. Toxins for both humans and the

environment have accumulated as a result of the intensive use of fungicides to control plant diseases. Chemical pesticide usage has come under more and more restrictions. Plant farmers are faced with the difficulty of maintaining plant health with a reduced input of agricultural pesticides in light of the negative consequences of these chemical residues found in eatables. Because they have a less detrimental effect on the environment than synthetic fungicides, using natural materials to treat fungal infections in plants is seen to be a beneficial alternative. It has been demonstrated that many higher plants and the components of them are both non-toxic and safe, as well as effective at controlling plant diseases. Plants are renewable natural resources for many physiologically active substances. The effects of these substances on the environment range from anthelminthic to anticancer to sedative to laxative to cardiac tonic to diuretic.

Economic & environmental researchers studying plants are taking these factors into account. Actively looking for more economical and ecologically friendly biochemicals for crop disease prevention and treatment based on preparations of several plants (John and Naidu,1982; Haagsma and Gerresten, 1951; Kumar et al. 1957). As a result, organic products are being used more and more in control strategies. Compared to their synthetic counterparts, botanical pesticides are less expensive, and even farmers can simply synthesize their basic extracts. Additionally, these pests are less likely to develop resistance or return. *Azadirachta indica* has been demonstrated to have antifungal properties that inhibit *F.oxysporum* (Jnandaik and Sharma,1994; Sivakadadacham,1988). Neem extracts bioactivity has been linked to a number of substances, including *salannin, nimbin*, and *nibbidin. Azadirachtin,* however, is the most potent antifungal substance. In general, *Adulsa* and *piprer betle* have a good level of antifungal effect on pathogens against aqueous and ethanolic extracts. The pathogen proved most resistant to the *Lantana camara* aqueous extract. Althaea rose's ethanolic extract was also the least effective against the infection. According to Leicach and Yaber grass,2011; and Leicach et al 2010, the bioactive chemicals tannins terpenoids, glycosides, alkaloids, steroids, flavonoids, and phenolics may be responsible for the antifungal action of plant extracts. These are naturally occurring secondary compounds generated by plants used for medicinal purposes to give defense versus stressful circumstances or the introduction of pathogens. They additionally participate during

host-plant interactions, allowing the crop to stay alive versus competitors including pathogenic microbes (Matsuki et al.,2011). Neem leaf extract diluted in acetone has proven to be more efficient than water. An earlier study (Gull et al., 2012) verified these results. Garlic inhibits the synthesis of penicillin acid and aflatoxins and has antifungal properties, according to Graham & Graham (1987) and Ismaiel (2009). Allicin is the only part of garlic that has antibacterial effects. Based on investigations on the antifungal characteristics of ginger, it is high in anion-binding complexes that include nitrogen oxides, chlorine, sulfides, and organosulfur molecules, which can readily dissolve the in fluid that play a role behind the ability to kill bacteria (Yadav,2008 and Shobana et al., 2009) demonstrated the antifungal effects of these medicinal herbs by testing 46 extracts of different part of the plants against *Alternaria burnsii*. Similar findings were likewise reached by Ranawane et al. (2010), who asserted that a variety of fungi were negatively impacted by *Datura stramonium* extract. Sharma et al. (2015) also looked into the antimicrobial activity of *D. stramonium* watery extracts from the leaves towards *Alternaria solani* and *Fusarium oxysporum*. Furthermore, to its psychoactive properties, plants produce seeds and flowers that contain biologically active substances like hyoscyamine, a drug called and the drug atropine (Goyal and Kushik; 2008, Sharma and Rajesh;2002). Considering it has a higher concentration of tannins, phenol compounds, Vitamin-C, protein molecules, and sugars compared to the water-based extract, citrus reticulates ethanol extract beat comparable. A significant number of flavonoids, or 92.4 mg CT/gm of catechin equivalent, are present in the ethanolic extract. Tannin concentration was 132 mg TAE/gm and vitamin C content was 115.5 mg AAE/gm, according to Justin et al. (2014). Abdel-Ghani et al. (2013) found that the aqueous extract of *Malva parviflora* was more effective than the ethanolic extract.

Plant extracts' antifungal properties could be attributed to the existence of specific antifungal toxins. According to R.S. Dwivedi and Singh; 1978, upper-growing plants have been shown to have compounds called antifungal compounds that can fight against diseases in crops. The adoption of pathogen-resistant cultivars, crop rotation, and the administration of chemical fungicides are the main management strategies for Fusarium wilt in cumin. The conventional approach to managing the *Fusarium oxysporum*-induced wilt disease has been to apply chemical fungicides. New environmentally friendly fungicides must be developed since cumin poses a serious threat to human

health (Alam, S., Alam, M. S., & Mahal, F. (1999). According to the study's findings, *Fusarium oxysporum*, which causes cumin wilt, is more susceptible to the plant extracts of *Psidium guajava, Azadirachta indica, Triumfetta pilosa*, and *Senna alexandrina.* These findings are consistent with earlier research regarding the pathogen's resistance to different botanical preparations' antimicrobial properties *Fusarium solani* at various concentrations, including *Azardiachta indicia, Artemisia Annua, Ocimum sanctum*, and *Rheum emodi* (Kumar, A., Rahal, A. et al. 2003). *Azardiachta indica* was additionally demonstrated as efficient versus *A. Solani* (Maya, C., &Thippanna, M. (2013). Various amounts of plant extracts of medicinal plants inhibited the mycelial development of several species of *F.oxysporum* (El-Shami and Allium;2017). *Adhatodavasica Indicas* (N.L.Ojha, 1986; Krishna A and Prasad 1987) *Ocimumbasilicum, Eucalyptus amygdalina, Ailanthus* and *Lantana camera* (Bansal, R. K., & Gupta, R. K. (2012) by extracts of all plant species belonging to different families were found to have significant levels of antifungal activity against *Fusarium oxysporum.* After 36 hours of inoculating the most pathogenic (Ab01) isolate, solutions of diaphane - M 45 (0.3%), tebuconazole (0.2%), azoxystrobin (0.2%), and *Azadirachtin (*0.2%) were sprayed. Tebuconazole was also combined with *Azadirachtin.* For comparison, an appropriate inoculation control was kept in place without the use of fungicides or herbal remedies. After 10 days observations of disease severity were collected on a common disease rating scale (1–5 score), and PDI was computed. For comparison, the infected control was kept alive without the use of fungicides or herbal remedies.

## 2.6 Biochemical parameter

The plant is protected from many diseases by the natural components of several plants that include phytochemicals (Apparao RK, Kaladhar, et al. 2011). According to their function in plant metabolism, primary and secondary metabolites, or phytochemicals, are generally plant compounds with defensive properties but little nutritional value. Carbohydrates, amino acids, proteins, and chlorophyll make up primary metabolites (Prabha SS, A. Rajaram, et al. 2013). Phytochemicals are the name for the bioactive organic compounds found in medicinal plants that can fight the different diseases in plants. These consist of phenols, tannins, alkaloids, saponins, steroids, flavonoids, and terpenoids, among others (RNS Yadav et. al 2015). However, there is not much

investigation on the phytochemical content of *Costus speciosus*. Plants are a constant

supply of medications, whether they are found in herbal remedies or as pure active ingredients. Consequently, to find plants or plant extracts that might be utilized to make the medications or that could take the place of some pharmaceutical preparations must be bought and imported (Farnsworth NR, Akerele O, 1985). A multidimensional strategy integrating phytochemical, botanical, biological, and molecular approaches is now being used in drug development from medicinal plants.

Alkaloids, also aromatic oils, and phenolic compound nucleoside, according to Abad et al. (2007), maybe the root of Malva's antifungal activity. Juvatkar et al. (2012) claim that the glycosides, saponins, alkaloids, tannins, and flavonoids may act separately or in concert to give *Artemisia herba-alba* its antifungal properties. Findings by Zahra et al. (2011) showed that *Nigella sativa's* aqueous extract has greater antifungal efficacy than its ethanolic extract. The fungus *Aspergillus niger* has been showed to be susceptible to thymoquinone, a component of *Nigella sativa* (Al Jabre et al., 2003). The findings of Zahra et al. (2011) are consistent with findings obtained from the water-based extract of *Nigella sativa*, which demonstrated greater antifungal effectiveness over the extract made with ethanol. These might be a result of the water-soluble extract having more glycosides, or saponins, tannins, flavonoids, and alkaloids than the dry extract. *Nigella sativa*, also known as Nigella contains thymoquinone, which has been shown to have antifungal action against *Aspergillus niger* (Al Jabre et al., 2003).

### 2.6.1Chlorophyll

At two different wavelengths, 645 nm, and 663 nm, the absorbance measurements of chlorophyll extracts were examined. In the samples of fresh, leafy greens that were extracted using acetone, and chlorophyll, the calculation was carried out. The absorbance value was obtained and totaled, and the levels of chlorophyll were calculated by using Arnon’s 1949 equation. Utilizing a portable chlorophyll meter with the proper correction algorithms, José Francisco et al. (2008) calculated the amounts of chlorophyll contents in various tropical tree varieties. An inexpensive and destructive way to determine chlorophyll concentrations is to use a portable chlorophyll meter. Compared to *Uranium regatta L., Codium tomentosum*, *Cladostephus*, and

*Verticillatus*, Shukran Dare 1998 showed that the quantity of pigment in pure water from *Cladophora glomerata* was exceptionally high. In olive plants through the Defuel area, the characteristics of chlorophyll fluorescence, chlorophyll content, and interactions among two types of chlorophyll were studied (khaleghi, et al., 2012). The concentration of chlorophyll in the leaves is proportionate to the result obtained using the technique, even though it expresses relative chlorophyll concentrations in relative amounts in every mass of leaves unit of the region as compared to the total amount or per leaf. Richardson et al.2002; Kousar et al. (2007) utilized a variety of techniques to extract, calculate, and identify the chlorophyll and other pigments in the leaves of black gram plants; the primary color is chlorophyll a, b, and pheophytin.

### 2.6.2 Sugar

For many physiological investigations of plant development and resource allocation, a separate assessment of carbohydrates or glucose in materials is required. The tissue sample is typically depleted of water-soluble carbohydrates before the starch content of the residue is determined. The process costs money and takes time. A heated ethanol solution (Ebell 1969; MacRae et al. 1974; Rose et al. 1991) can be used to extract soluble sugars. There is no comparison of how well the two methods perform in terms of determining the quantities of sugar and subsequent starch, even though it is thought that both methods are successful at removing sugars that dissolve in the tissue of plants.

In the enzymatic method based on NADPH absorption, a distinct enzyme must be produced for each of the sugars (Blunden and Wilson 1985; Hendrix 1993). It is thus a time-consuming and costly process. Certain oligosaccharides, such as sucrose, are not included in processes used to test reducing sugars unless they are first hydrolyzed (Miller 1959; Ashwell;1957). The anthrone technique can be used to detect all types of sugars (Scott and Melvin 1953), however, it is well known to lead to errors when analyzing sugar mixtures due to the considerable variety in coefficients of absorption between all of the sugar types. Phenol-sulfuric acid is used in a less complex colorimetric approach to determine sugar (Duboise et al 1966). Although Buysse and Merckx (1993) changed the quantity of phenol in the phenol-sulfuric acid technique to optimize it for a combination of sugars that included glucose, fructose, and sucrose, this approach has not been optimized for a mixture that included all of the major sugars

found in plant extracts. Plant extracts contain chlorophylls, lipids, and proteins that interact with concentrated sulfuric acid in the glucose test, altering the absorbance reading significantly (Ashwell 1957). Hexane is activated charcoal, and ion-exchange columns are considered to be less feasible and effective at eliminating the aforementioned substances than chloroform. It is used to remove contaminants, Ashwell (1957) indicated that parallel testing for each sample with and without a color developer could offer an adjustment to the absorption values. The possibility of adjusting the absorbance results by repeating parallel tests for all samples with and without a color developer has not yet been tested in phenol-sulfuric acid-based sugar tests. In research studies for sugar based on phenol-sulfuric acid, the method has not yet been evaluated.

### 2.6.3 Protein

The Bradford protein assay technique is employed in this experiment to assess the protein concentration since it is affordable and accessible (Gornall AG and Bardawill CJ, 1949). Another benefit of this approach is the ability to quickly and accurately calculate the absorbance of a specific solution (Lazaro N 2019). Because the absorbance of the BSA and Bradford reagent solution mixture must be determined within sixty minutes, the dye-binding procedure takes just about two minutes to perform (Yuan B.L 2010). Furthermore, the visible light spectrophotometer provides its measurements in a matter of seconds. The Bradford protein assay technique is the colorimetric test employed in this investigation. The fundamental idea behind the techniques, which were created by Dr. Marion Bradford in 1976, (Santos GA et al. 2012), is that Coomassie Blue G-250 binds to the essential amino acids in proteins (Kingsley GR.1997), changing the color of the solution to blue (Saha UK, Sonon L at el.) with a peak absorbance at 595 nm (Rayment GE, Hill R, Greaves A.). Numerous proteins testing methods, including the Nessler technique, Dumas technique, Kjeldahl technique, Berthelot's technique, Folin-Ciocalteu technique, Lowry technique, Biuret technique, direct alkaline distillation, dye binding, and additional techniques, including the Bradford method, the bicinchoninic acid (BCA), and the near-inf reflectance (NIR), approach (Okutucu et al.2007). (Plaza et al., 2013), have been developed over the past 20 years. Folin and Lowry's technique is widely used for biochemical analysis compared to nutrition assessment because of

the high sensitivity required. IR spectroscopy is important for total protein evaluation because of its rapid and non-destructive approaches, yet UV absorption and chromatography methods for separation are frequently used in tandem (Santos et al., 2012; Rukke et al., and Rayment et al., 2012). It can be done to use various protein quantification techniques effectively if you have a greater awareness of the advantages and disadvantages of each method and considers account factors like the type of protein found in the specimen, whether or not the material contains impurities, and the method's velocity, precision, and specificity. The range of sensitivity for the concentration of proteins and its linear response to conventional solutions have to be considered deciding on a dietary supplement evaluation strategy (Janeiro., 2015). Another among the early colorimetric protein detection techniques for determining the amount of protein present in the material was the response of the biuret assay (Gornall et al., 1949). The bonds of peptides react with the reagent that contains biuret to produce a complex that is purple. (Boyer,2000). According to Switzer and Garrity (1999), the coordination compound produced by the element copper and both nitrogen atoms that make up each protein sequence achieves its highest level of toughness and stability within approximately 15 minutes and is constant for a considerable amount of duration. The procedure is reliable, and the chance of accuracy was estimated to be considerably less than five percent. According to Kingsley,1939, it has been found that the biuret approach and the approach proposed by Kjeldahl agreed well on the overall amount of energy. When contrasted with methods that depend on the existence of specific amino acids, the Biuret technique is a favored choice in clinical labs since it is quick, easy to use, and dependable (Gornall et al., 1949). The sensitivity of the biuret test is evaluated using protein samples with information that varies from 0.01 to 5.00 mg/mL (Janeiro et al., 2015). The Lowery method, which can recognize samples of proteins with concentrations between 2 and 100 g, is one of the most sensitive procedures. The reagent known as Folin-Ciocalteu is utilized in the technique to promote the formation of color, with the biuret reaction serving as its basic foundation. After the protein has been dealt with using alkaline copper sulfate in the presence of tartrate, the Folin reagent is applied (Gordon et al., 2013). The shade of blue- color that can be identified within 660 nm to 700nm is produced when the FC reagent reacts with the side chain residues of tyrosine, tryptophan, and cysteine as well as cuprous ions (Stoscheck, 1990). Copper produces a cuprous complex when it combines with the nitrogen atoms in the

peptide.

### 2.6.4 Phenol content

The overall phenol compound, hydroalcoholic, and extracts of ethanol were established using the Folin -Lowry technique with the chemical gallic acid as a reference. The reagent is composed of phosphotungstic and phosphomolybdic acids, which then decrease to a mixture of blue tungsten and molybdenum oxides once the phenols are oxidized. The outcome's blue color corresponds to the total amount of phenolic compounds that initially existed and has a maximum absorbance in the 750 nm region. After five minutes, ten milliliters from a 7% carbonate of sodium solution were added to the solution. Standard solutions for gallic acid were produced at concentrations of 20, 40, 40, 60, 80, and 100 g/ml following the combination had been maintained for a period of 90 minutes beginning at a comfortable temperature, the absorbing capacity of the test and typical solutions at 550 nm was measured using a UV/visible spectrometer in comparison to the reagent blank that was used. mg of GAE/g of extract was employed to calculate the total amount of phenol.