# CHAPTER-9

# Discussion

## 9.1 Introduction

The biodegradation and decaying of cereals, vegetables, fruits, and agricultural goods before and after harvest caused by insect and microbial infestation can result in losses of up to 100%. Different kinds of fungus have been linked to severe losses in grain nutritional quality and seed quality (Koirala et al., 2005). Due to their spectrum of toxicity against non-target organisms, including people, which are known for causing pollution problems, the World Health Organization (WHO) prohibited numerous agriculturally significant pesticides (Barnard et al., 1997). Despite their negative impacts, several underdeveloped nations continue to use these insecticides. In order to solve the pre-harvest and post-harvest issues, pesticides were used excessively in agriculture. Even though they are used to enhance seed quality, harmful synthetic fungicides are typically not used to stop the biodeterioration of grains during storage (Harris et al., 2001). The major reason for this is a lack of knowledge on the screening and evaluation of various plants of their antifungal activity. Finding an alternate approach to avoid grain biological degradation during storage without harming consumers is therefore urgently needed. Numerous higher plants generate insecticides, medicines, and organic substances that are significant to the economy. According to Hamburger and Hostettmann (1991), there may be more than 400,000 different plant compounds. Out of these all, more than 10,000 are secondary metabolites, which play a vital defense function in plants. In order to manage storage pests, plant-based secondary metabolites with defensive functions may be used. The majority of higher plant species, however, have never been surveyed or described. It is yet unknown whether their chemical or biologically active components may serve as new sources of economically viable insecticides (Balandrin et al., 1985). It is expected that biologically active insecticides produced from plants would become more important in crop protection methods. The production of forthcoming commercialized agricultural herbicides strategies with particular reference to the management of plant disease, will be heavily dependent on the utilization of naturally occurring chemicals from plants that inhibit the reproduction of undesirable microorganisms. This approach to plant protection would be more feasible and environmentally sound (Varma and Dubey, 1999; Gottlieb et al., 2002). The adoption of pathogen-resistant varieties, crop rotation, and the application of chemical fungicides are the primary management strategies for the Fusarium wilt disease of cumin. A common practice for controlling diseases brought on by fungi was the application of chemical fungicides. According to Mahal F and MS 1999, these applications have, on occasion, been linked to some very substantial health concerns in the field. However, the use of fungicides has allowed residual harm to collect up in crops and soil, enhanced the contamination of the environment, eliminated unexpected beneficial bacteria in the soil, and disrupted the ecological equilibrium (Rahmah Al, Mostafa et al.; 2013). The harmful effects of chemical fungicides on the environment and human health render it urgently necessary to identify new fungicides that are more environmentally friendly in the natural world. **The following headings have been utilized to convey the findings of the present investigation along with relevant ones.**

Among the most widely cultivated plants worldwide is cumin, because of its excellent nutritional value, abundance in flavonoids, and anti-inflammatory and antioxidant properties. Many diseases have been managed by it using medicinal plants (Lee et al.,2018). Several soil fungi target cumin plants and infect them at various phases of growth, causing diseases like blight, powdery mildew, and wilt. This study emphasized the wilt and blight disease, which reduces the yield of tomato plants in all nations where they are grown. It is known that the fungus *Fusarium oxysporium* is the source of this disease. The extent to which each fungal isolate could cause wilt disease in cumin plants was different. These findings are consistent with those made by Abo-Elyousr and Mohamed, (2009) who found that the disease-causing potential of fungal isolates varies. The most common form of disease control is the application of fungicides (Carvalho, F.P. et al. 2007). However, control of the disease by various agrochemicals is hazardous to both humans and the environment, and the advantages for management decline with time due to pathogenic resistance to medicines, because many research investigations in the past have advised the use of botanical extracts to treat helminthic, fungal and bacterial disease, it was essential to look for natural alternatives to pesticides as an alternative (Enikuomehin, 2010 and Abo-Elyousr, K.A.M, 2009).

Among the many common diseases, affected by several Alternaria species, is known as spot on the leaves infection. widespread and dangerous infections of cumin and other cruciferous plants like cauliflower (Boiteux and Reis 2010 and Cucuzza et al. 1994). Despite the fact that several Alternaria species have been identified as the disease's causal agents (Simmons 2007, Ghosta & Rahimloo 2015 and Siciliano et al 2017).

## 9.2 Isolation, Identification, and Characterization of Pathogens *Alternaria burnsii* and *Fusarium oxysporum*.

A review of the literature revealed that *Alternaria burnsii* morpho-species have been associated with the disease *Alternaria blight*, which affects cumin and other plants internationally (Rahimloo & Ghosta 2015; Aneja et al.,2015; Peruch; 2006; Al-Lami et al. 2019). Initially defined as small-spored *A. mavae*, *Alternaria*, *A. perangusta* A. *tenuissima*, *A. destruens*, and *A. turkisafriaall* produce Along with small to slightly lengthy foremost and supplementary conidiophores, strands of conidia can be simple, lengthy, or extensively branched.

The investigation on cultural characteristics of *Alternaria burnsii* and *Fusarium oxysporum* was carried out on PDA media and Rose Bengal media and is described in detail in Chapter-3. Three isolates displayed significant differences in culture attributes, including colony margin, colony color, topography, colony width, and sporulation, on solid medium (Tables 3.3 to 3.5). The conclusions that were given are consistent with earlier research. The findings support previous studies by Panday et al. (2005), Pipaliya and Jadeja (2008), and Ramegowda and Naik (2008). The *A. alternata* isolates varied in their cultural characteristics, such as colony color, geographic range, and topography. Researchers (Varma et al.,2008; Tetawal et al.,2002; Naik et al., 2010) also found that both diseases displayed variations in cultural traits such as growth rate, kind of growth, colony, and sporulation. Similar outcomes were reported by Tetarwal et al. (2008) for *A. alternata* isolates and Shahnaz et al. (2013) for *A. burnsii*. As a result, the current findings agreed with those of prior researchers' investigations.

The results published by Mehta and Kumar et al.;2003, identified variations in the size, depth, and quantity of conidial septations among the isolates of *Alternaria burnsii*, which are consistent with the conidial morphology of *A. burnsii.* (Patel R.J. 1968) These algae had three to five cells, were branching, and were septate. Characteristically, the indicated isolate was determined to be *Alternaria burnsii*. The *Alternaria burnsii's* morphology is consistent with earlier findings from (Joshi, 1955; Patel, 1968 Uppal et al., 1938).

*F. oxysporum* has been the subject of numerous morphological, microscopic investigations that have described it in PDA medium. Unicellular oval or ellipsoid microconidia are seen. Macroconidia are likewise widely distributed and have attenuated apical cells. The majority of chlamydospores are solitary, have a smooth wall, and develop in intercalated or terminal positions (Leslie & Summerell 2006; Dillard, Smith 2007; Summerell et al. 2003; Campbell et al., 2013). The results of the morphological and microscopic investigation of the fungal strain demonstrated that *Fusarium oxysporum* was definitely the causative agent of Fusarium wilt isolated from cumin-affected plants. The soil-born pathogen (fungus) *Fusarium oxysporum* was first identified by Patel et al. (1957) as the culprit behind the wilt disease in cumin plants. Macroconidia have an apical cell that has inhibited growth. In intercalated or terminal locations, chlamydospores are often isolated, have a smooth wall, and are formed (Smith 2007; Leslie & Summerell 2006; Dillard 1998; Summerell et al. 2003; Campbell et al. 2013). Conidiophores contained three to five cells, were branching, and were septate (Patel RJ 1968). This type of culture was periodically subcultured and preserved on the same medium. The described isolate was recognized as *Fusaium oxysporum* based on its character pattern. The morphology of the pathogen is consistent with earlier findings made by Joshi (1955); Uppal et al. (1938); and Patel (1968).

Our results correlate with Previous, *Fusarium oxysporum f.sp. ciceris*, the pathogen that causes cowpea wilt, was evaluated against 31 plants in the *Asteraceae* family (Rai and Acharya, 1999). The antifungal activity of Allium cepa "Akugun 12" against two Fusarium isolates, was assessed by Ozer et al. in 2003. *A. sativum* also has anti-fungal properties, according to Sahavaraj et al. (2006). Both Misra and Dixit (1976) and Bowers and Locke (2000) saw comparable outcomes when employing *Allium sativum* against a variety of fungi, including *Fusarium spp*. In their studies on the antifungal efficacy of plant extracts against *Fusarium pallidoroseum*, researchers Jacob and Siva Prakasan (1994) and Arya et al. (1995) discovered that extracts of piper betle and Neem leaves had an inhibitory effect on the mycelial development of the fungus.

## 9.3 Koch's postulates

A large variety of crops and fruits are affected by the wilting, decay, leaf blight, and leaf spot caused by the fungal genus. Cumin wilt is affected by *Fusarium oxysporum* and cumin blight was affected by *Alternaria burnsii.* According to Pragya et al.2017, *A. tomato Phila* and *A. burnsii* are capable of inflicting the tomato disease known as early blight, it appears on the leaf surface as small, black, necrotic, clumping together, and circular sores. *A. burnsii* caused dark brown leaf spot lesions in Tomatoes that resembled the symptoms that developed in cumin (Kwon, et al. 2021). Additionally, it has been noted that some *Alternaria* species may infect several hosts (Wiltshire, S.P. Danish 2018). *A. burnsii* has consistently been identified as a pathogenic fungus that affects cumin and manifests as light to dark brown patches and finally withers and drops its leaves. According to Parkunan, V.; Li, S.; Fonsah 2013), an *A. alternate* infection can result in spots on the leaves of seven banana cultivars, such as Novaria, Cavendish, and others.

In this study, we have described a cumin disease in some villages of Rajkot City situated in Gujarat. It was identified as a cumin leaf blight disease in accordance with the symptoms in affected leaves. The disease was brought on by a species of *Alternaria,* as determined by pathogen isolation and morphological observations. Because of their similar morphologies, *Alternaria species* are difficult to differentiate (Dagno and Crovadore et al. 2021). The possible pathogens we identified from infected cumin plants, according to the findings of our morphological identification. The possible pathogens we isolated from infected cumin plants were more likely to be *F.oxysporum* and *Alternaria burnsii*, according to the results of our morphological identification.

## 9.4 In vitro evaluation of plant extract against *A. burnsii* and *F. oxysporum*.

Botanical preparations for antimicrobial properties might be due to the existence of antimicrobial chemicals. Higher-growing plants have been found to have antimicrobial chemicals, which are useful for preventing plant infections. (R.K. and Singh et al. 1987). Treatment of synthetic fungicides, using pathogen-resistant varieties, as well as alternate cultivation are the main approaches to control for fungus *Fusarium oxysporum* in cumin. The application of chemical fungicides has been the traditional method of controlling wilt disease caused by *Fusarium oxysporum.* Cumin disease is a major health hazard for which new eco-friendly fungicides need to be found (Alam S. et al., 1999). The result of this study showed that the plant extracts of Neem (*Azadirachta indica*), Custard Apple (*Annona reticulata*), Bullet wood (*Mimuspos elengi*), and Betel leaf (*Piper betel*) were most effective against *Fusarium oxysporum* causes cumin wilt.

In this current study, 16 plants with different solvents were in vitro evaluated for antifungal efficacy against significant *Alternaria burnsii* and *Fusarium oxysporum*. These herbs were chosen based on a knowledge of traditional therapies and at-chance selection from the local flora. Only eight plants were found to be capable of preventing test fungus from growing mycelium at a 25% concentration, according to the screening. In our investigation, different concentrations of plant extracted (5%,10%, and 15%) prepared in water, acetone, and cow urine inhibited the development of *F. oxysporum* in vitro to varying degrees. In comparison to the other levels, 15% had the greatest impact in reducing the radial development of pathogens. The research on *F. oxysporum* by Mousa et al.,2011 is in the same context as this. A similar finding was made by Kareem et al. (2008), who discovered that C. procera leaf extracts have antibacterial action against *Fusarium oxysporum*. Furthermore, Nikam et al. (2007) used preparations from the root, foliage, and plants of C. procera for evaluating biological investigations regarding antimicrobial effects towards *F. oxysporum*. Olufolaji et al. (2015) demonstrated that phytochemicals, such as alkaline substances steroids, saponin, flavonoids or steroids, flavonoids, and phenols present in various plants and our plant, may be harmful to the pathogen and restrict its growth as an alternative.

These findings agreed with the results reported by Gangopadhyay et al. (2010) who investigated the results from six botanical extracts on the development and spore germination of *A. burnsii*, including *Aloe vera*, *Eucalyptus globulus*, *Azardiratcha Indica* leaves and *A. Indica* seed. According to the study, the inhibition was both in vitro and in vivo and most effective at 10% concentration.

The results of this study represent a significant advancement in crop protection measures for antifungal activity against significant *Alternaria* species. Custard apple, Neem, Betel leaf, Bullet wood, Vinca rosea, and Adulsa have been shown to be significant alternative plants for preventing the biological degradation of grains during storage. *Ocimumbasilicum, Eucalyptus amygdalina, Ailanthus Excels und Lantana camera* extracts of plant species belonging to different families were found to have significant levels of antifungal activity against *Fusarium oxysporum* (Bansal; 2012).

**9.5 Botanical extracts are evaluated for their antimicrobial properties by applying the poison food method.**

Several workers agree with the findings. According to Jadeja and Pipliya (2008), 5% and 10% extracts of ginger rhizomes and garlic cloves were most efficient in inhibiting *A. burnsii* by 78.5% and 73%, respectively. According to Vihol et al. (2009), in vitro mycelial growth of *A. burnsii* was 67% reduced by a 15% extract of garlic (*Allium sativum*).

According to the results of the food poison technique, the botanicals used can inhibit the growth of the *Fusarium oxysporum to* a greater extent with higher botanical concentrations, which suggests that higher botanical concentrations are preferable due to the presence of more antifungal compounds. Riaz et al. (2008) found 54.0 to 79.0% growth suppression of *F.oxysporum* by utilizing the extract of the plant in acetone at 2.0 to 8.0% doses.

### 9.6 In vivo (Pot trial) evaluation of plant extract against pathogens

According to the result of the pot trail the wilt and blight caused by fungi were most susceptible to Neem, Adulsa, and Bullet wood which also showed the greatest degree of inhibition. The biocontrol ability of different plant extracts against Foc varied. Different plant extracts exhibited a range of 50.0 to 66.0 percent growth inhibition. The findings of Oriole and Adejumo (2009) were similar. Based on the outcomes of potted research, the soil treatment with phytochemicals greatly reduced the potential of Foc for infecting the crops. The reduction in the wilt disease may be caused by a decline in the pathogen (Foc) population in the soil brought by botanical extracts. The most effective antifungal and most promising solvent for reducing *F. oxysporum* was acetone extract.

Our findings are supported by Ghorbany et al.2010 study that Mentha sp. extract inhibits the development of *F. oxysporum*. According to Sharma &Trivedi (2002), the extract of D. stramonium inhibited the development of *Fusarium oxysporum* by 72.0%. Six phytoextracts were tested against *A. burnsii* by Polra and Jadeja in 2011. For inhibiting pathogen radial development and spore germination, ginger rhizome extract and garlic clove extract turned shown to be the best sources of plant origin.

The antifungal effectiveness of Custard apple towards *Fusarium oxysporum* and *Alternaria alternate* was also investigated by Govindachari et al. in 1998. According to Subramanian and Srinivasa Pai (1953), Azadiractin is responsible for Azadirachta indica fungicidal spectrum. D Joshi et al. (2011) also examined plant extract extracts from buds, flowers, stems, and mature leaves. They discovered various physiologically active substances, including various phytochemicals which are similar to our findings and may have harmful effects on a variety of pathogens. Similarly, some writers claimed that plant extract inhibited the development of photogenic fungi and lessened the severity of sickness, which may be attributed to secondary metabolites such as phytochemicals like alkaloids, phenolic compounds, etc. (Mohamed, N.H, 2008; Ashour, M.L. Wink, M; Wink, M El- Asaf, S.; Numan, M,2021, Readi,2012, Jan, R.;).

Our findings demonstrated that Neem aqueous extract greatly decreased wilt infection in all tested doses. The maximum seed germination and seedling were produced by plant extract of piper betel, Neem, Adulsa, and Bullet wood at 15%, followed by the other levels. Numerous studies have found that neem, garlic, and ginger seeds germinated more readily and had higher vigor indices when treated with plant extracts (Khan, M.I,1992, Ahmed, M.; Mehbub, H, 2013 et al., 2022). Our findings showed that after 5, 10, and 15 days of treatment, extracts boosted antifungal activity in cumin plants that had been inoculated compared to untreated inoculated plants. Increased POD activity has been linked to systemic-induced resistance in plants to fungus, according to research by several authors (Naz, R.; Bano, A.; Nosheen, et al.2021 and Yu, Y.; Gui, Y, et al 2022). Plant extracts have been utilized and proven to promote plant growth and output in many plants by inducing systemic resistance, lowering the incidence of disease, and so on (Hussein Mohamed, 2018 Ramamurthy, V.; Samiyappan,2001). The total phenol and flavonoid content of plants treated with Neem at all doses was found to be considerably higher than that of untreated control. Phenolic compounds are hazardous to pathogens and their buildup at infection sites was linked to a limitation on pathogen growth, our findings are similar to those of several researchers (K.A.M. and Abo-Elyousr, et al., 2012). Additionally, modifications in the pH of cytoplasm in plant cells brought on by a rise in phenolics may also encourage resistance, preventing the growth of pathogens. Many plants contain phytochemicals that are useful as medicine in control of various plant diseases caused by bacteria, fungi, and viruses. For an evaluation of a plant's potential therapeutic value as well as to identify the active ingredients that regulate the known natural behaviors expressed by plant life, a physicochemical examination of an extract of the plant is required. Additionally, it offers a foundation for additional studies and the specific identification of certain molecules. The specific kind of solvent applied is a key factor in the method of extraction of a phytochemical from a material.

The current study confirms previous research (Singh, 2000; Majumdar, 2001; Rao, 2006; Pramod Kumar, 2007) that *Fusarium oxysporum* is inhibited from growing when different kinds of plants have been extracted in water, cow urine, and acetone. As stated earlier, no evidence of the use of plant extracts produced from cow urine to cure the wilt and blight on cumin caused by *A. burnsii* and *F. oxysporum* has been found in the review of the literature. The seeds and oil from the widely recognized and utilized neem tree are commonly utilized to treat infections and ward off insects. According to Mordue and Nisbet (2002), the substance that is extracted contains significant amounts of *Azadiractin*, the drug's active ingredient. In 1998, Govindachari et al. looked into the antimicrobial activity against *Fusarium oxysporum*, *Drechslera oryzae*, and *Alternaria alternata*. Neem oil is a substance that generates a variety of acids, sulfur, etc. Decatylimbin. Azadiractin, and Meliantiol, which also contain quercetin and sitosterol, are all substances found in seeds. Azadiractin, a terpene belonging to the C25 family, is said to be in charge of the fungicide spectrum produced by *Azadirachta indica*, as stated by Shankar and Srinivasa Pai (1953).

## 9.7 Biochemical Parameters

According to the biochemical analysis of plant extract, it was found that all the plant extracts contain more or a smaller number of biomolecules. The extract was created in methanol specifically for the screening of phytochemicals. The presence of phenols, total sugar, protein, and chlorophyll was measured in the entire plant extract. It is possible that the presence of different phytochemicals, including saponin, flavonoids, steroids, tannins, and phenol, gives their therapeutic qualities to medicinal plants. The results of the initial screening tests might eventually influence the creation of new drugs. These tests make it easier for them to quantitatively estimate and find chemical substances that are pharmacologically active. Utilizing methanol and solvent pure ethanol extracts as a basis for comparison, the overall polyphenol compounds in the samples were calculated using the Folin technique. When the phenols in the solution have oxidized into a combination of purple tungsten and molybdenum oxides, phosphotungstic acid and phosphomolybdic acid, respectively, are used to generate the chemical. The amount of phenolic chemicals initially present is related to the amount of blue coloring that is created, whose maximal absorption is at about 750 nm. For the quantitative estimation of proteins in the leaves, extracts were prepared in phosphate buffer. The protein content was determined by the Foline procedure. The results of initial screening tests might eventually influence the creation of new drugs. These tests make it easier for them to quantitatively estimate and find chemical substances that are pharmacologically active (B. N. Ames, M. K. Shigenaga, et al. 1993).

The significance of these biochemical parameters is correlated with the research work of several scientists. Natural antioxidants may be found in plants. In epidemiological studies, fruits and vegetables have been found to be protective against a number of plant diseases. Carotenoids, Vitamins C and E, phenolic compounds, and Thio (SH) molecules are just a few of the substances that have been scientifically linked with these natural antioxidant effects (G. Paganga et al. 1999). Strong antioxidants as well as free radical scavengers, phenol compounds have been studied for their biological effects (M. P. Kahkonen, A et al. 1999, C. A. Rice-Evans et al. 1995, and N. Sugihara, T. Arakawa et al. 1999). These compounds can function as agents for reduction, donors of hydrogen, as well as selective oxygen suppressors because of their electrochemical characteristics, which contribute to their substantial antioxidant capacity (N. Ramarathnam, 1997; C. A. and Evansns; 1996). The functions of phenolic substances derived from plants in nutritional and antifungal properties have become of increasing interest (Tew, G.N. and G. Mathe;2002). The antifungal capacity of various plant components against diseases of plants is important, so this biochemical evaluation was carried out in the current study to correlate the effectiveness of plant extract with phytoextract.