

# Plant extracts for the management of Black Shank and Fusarium Wilt of Tobacco: A Comprehensive review

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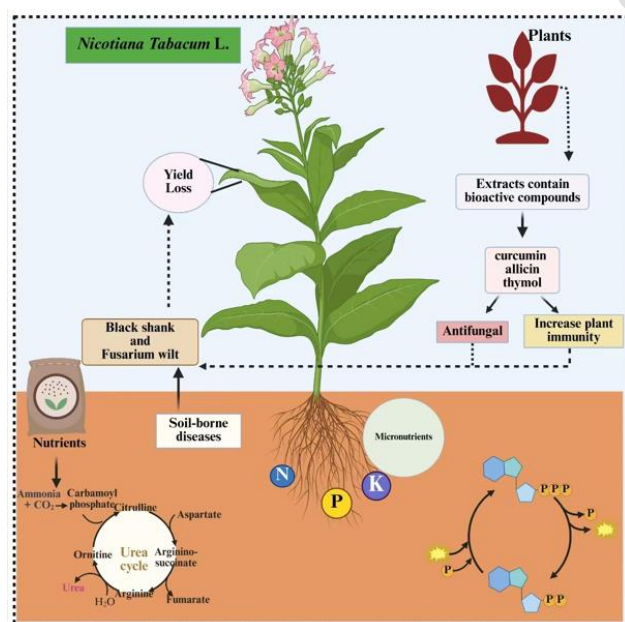
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## Graphical abstract



## Abstract

Tobacco (*Nicotiana tabacum* L.) is severely affected by soil-borne pathogens such as *Phytophthora nicotianae* and *Fusarium oxysporum* f. sp. *nicotianae*, causing major yield losses. Fungicide resistance and restrictions on synthetic pesticides necessitate eco-friendly alternatives. Plant extracts rich in compounds such as curcumin, allicin, and thymol exhibit antifungal and plant defense-inducing activity but face challenges of instability and variable field

performance. Recent advances in nanotechnology, including chitosan- and lipid-based carriers, improve stability, bioavailability, and targeted delivery, while synergistic use with biocontrol agents (*Trichoderma*, *Pseudomonas*) enhances disease suppression. Despite these developments, regulatory hurdles, cost, and low farmer adoption limit large-scale application. This review highlights the potential of nano-formulated plant extracts and integrated biocontrol strategies for sustainable management of tobacco diseases and emphasizes the need for standardized protocols, farmer education, and supportive policies to enable commercialization. Integrating biotechnology and nanotechnology offers a promising path for long-term crop protection.

**Keywords:** *Phytophthora nicotianae*; *Fusarium oxysporum*; botanical fungicides; nano-encapsulation; sustainable agriculture

## 1. Introduction

Tobacco (*Nicotiana tabacum* L.) is a commercially significant crop cultivated widely across many regions of the world, and is valued mostly for its financial returns (Santos *et al.* 2025). However, the production of *N. tabacum* meet major threats from soil-borne diseases, including Black Shank and Fusarium Wilt (Ping sun *et al.* 2025). According to (Gai & Wang 2024), these soil borne diseases not only affect the leaf quality but also reduce the overall yields leading to considerable economic losses for growers (Gai & Wang 2024). In the past, chemical insecticides and soil fumigation have been key

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components of the control of these diseases, but these methods. There has been some degree of control thanks to these techniques, the limitations of traditional methods have been brought to light by increased environmental concerns, the advent of pesticide-resistant disease strains, and customer demand for tobacco products without residue (Abdullah & Zahoor 2023). In light of these challenges, there has been a growing interest in sustainable, environmentally friendly disease management strategies. Among these, the use of plant extracts has emerged as a promising alternative due to their bioactive properties, low environmental persistence, and biodegradability.

Black shank is still one of the most harmful tobacco diseases, and it is brought on by the oomycete pathogen *Phytophthora nicotianae* Breda de Haan (Cochran *et al.* 2024). Black lesions on the stems, drooping of the leaves, and eventually the collapse of the entire plant are the usual symptoms of the diseases (Han *et al.* 2024). The disease is particularly challenging to eradicate because *P. nicotianae* prefers warm, wet soil conditions and develops hardy oospores that can remain for long periods of time in the soil. Despite the use of cultural measures like crop rotation and the adoption of resistant cultivars, they have only partially succeeded, particularly in environments that are conducive to the development of disease (Pandey *et al.* 2025). Likewise, tobacco growers face a serious threat from Fusarium Wilt, which is brought on by *Fusarium oxysporum* f. sp. *nicotianae* (Xie *et al.* 2024). The symptoms of this disease including, vascular discoloration, leaf yellowing, wilting, and ultimately plant death (Vasić *et al.* 2025; Lucas 1975). The Fusarium pathogen, like Black Shank, is showed remarkably resilient to environmental changes. It may live for years in plant debris and soil, and its ability to infect a huge variety of plant hosts makes management attempts even more challenging (Naqvi *et al.* 2025). The efficacy of chemical control methods against Fusarium Wilt has only been average, and the development of strains resistant to fungicides makes controlling the disease more difficult (Fei *et al.* 2025).

Traditional chemical control methods have a number of drawbacks, even with their track record of efficacy (Akbar *et al.* 2024a; Akbar & Khan 2021). Overuse of chemicals like metalaxyl, mefenoxam, and chloroneb has resulted in the selection of resistant disease strains, making treatments less and less effective over time (Akbar *et al.* 2024b) (Lucas *et al.* 2015). Furthermore, the pollution of the environment brought on by the application of pesticides has emerged as a major worldwide issue. Pesticide residues frequently find their way into water and soil systems, where they harmfully impact organisms that are not their intended targets and contribute to long-term ecological imbalances (Ali *et al.* 2021). Many countries are enforcing stricter laws for the use of pesticides in agricultural products, especially those intended for export markets, which restricts the variety of chemicals that growers can choose from (Sandeep *et al.* 2024). Last but not least, smallholder and resource-constrained farmers frequently cannot afford the high costs involved in the

development, registration, and use of novel chemical fungicides (Khoza *et al.* 2025). These complex drawbacks highlight the pressing need for sustainable, alternative approaches to disease management that are safe for the environment.

Plant extracts are becoming more and more popular as a potential remedy for the issues posed by chemical pesticides (Akbar *et al.* 2022). Several secondary metabolites, including phenolics, alkaloids, flavonoids, terpenoids, and essential oils, are produced by plants and many of them have strong antibacterial and antifungal qualities (Manzoor *et al.* 2025). Plant extracts, in contrast to synthetic fungicides, frequently contain several modes of action, which greatly minimize the possibility that diseases would become resistant (lawal *et al.* 2025). The antifungal effectiveness of plant extracts against a variety of phytopathogens has been shown in several reserch. For example, extracts from turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), garlic (*Allium sativum*), and neem (*Azadirachta indica*) have exhibited potent inhibitory effects on soil-borne fungus (Ali *et al.* 2025; Kim *et al.* 2024; Cao *et al.* 2024; Kapooria 2024). Usually, these extracts cause spore germination inhibition, membrane integrity degradation, and disruption of fungal cell wall formation, which results in pathogen death (Zhang *et al.* 2024). Compared to traditional chemical fungicides, plant extracts have a various numbers of advantages. First of all, they decompose quickly in the environment without leaving behind harmful residues because they are biodegradable (Akbar *et al.*, 2024). Secondly, it is in general accepted that plant-based pesticides are safer for non-target microorganism, humans, and other animals. This safety profile is in line with customer desires for agricultural goods that are residue-free and organic. Thirdly, integrated pest management (IPM) techniques are very compatible with plant extracts. Their application can be integrated with biological, mechanical, and cultural control techniques to produce comprehensive and long-lasting disease management programs. Given these qualities, there is increasing hope regarding the potential of plant extracts to manage tobacco disease in the future, especially in the fight against soil-borne diseases like *F. oxysporum* and *P. nicotianae*.

The results of previous research showed that the application of plant extracts against Fusarium Wilt and Black Shank infections have been promising. Both in laboratory and greenhouse tests, for example, extracts from *Azadirachta indica*, *Ocimum sanctum*, *M. micrantha*, *Senna alata*, *Datura metel*, and *Allium sativum* have been found to considerably lesser disease incidence (Haile 2025; Rifnas 2025). Plant extracts may be a useful part of integrated disease management, according to these studies. However, several knowledge gaps remain in spite of the encouraging results. The majority of research to date has been carried out in carefully regulated lab settings, and field applications are still difficult to move to. The efficacy of plant extracts in field conditions can be strongly impacted by variables like pathogen variety, environmental variability, and host plant reactions.

Moreover, there is a pressing need to better understand the specific modes of action of plant-derived compounds against *P. nicotianae* and *F. oxysporum*. Although broad mechanisms like membrane disruption have been suggested, there aren't enough in-depth molecular research. Standardizing extraction techniques is another important challenge. Variability in efficacy is frequently caused by variations in the plant components utilized, solvent systems, extraction methods, and quantities of active ingredients (Sharma *et al.* 2025). The commercialization of bio pesticides based on plant extracts is hampered by this lack of standardization. Furthermore, not enough research has been done on the possible phytotoxic effects of certain plant extracts on tobacco plants. For plant extracts to be successfully embraced by farmers, it is imperative that they do not adversely affect plant growth or production. Lastly, to optimize the effectiveness of plant extracts in actual agricultural contexts, practical elements of field application, such as the creation of stable formulations, efficient delivery systems, and suitable treatment schedules, must be methodically addressed.

A thorough analysis of the body of research on the application of plant extracts to the control of Fusarium Wilt and Black Shank in tobacco is urgently needed in light of these factors. A review of this kind can synthesize existing knowledge, pinpoint important research gaps, and offer tactical guidance for upcoming studies. Hence, the goals of this review are to: (1) summarize the major findings about the use of plant extracts against Fusarium Wilt and Black Shank of tobacco, (2) examine the mechanisms of action behind the antifungal activities of plant extracts, (3) discuss about the difficulties and restrictions related to their practical application, and (4) determine the future research directions required for the creation of efficient plant extract-based disease management strategies. Through this comprehensive synthesis, we aim to contribute valuable insights into the promising but underutilized domain of plant-based disease management strategies for tobacco. By highlighting the potential and limitations of plant extracts, this review aspires to support the broader goal of promoting sustainable agricultural practices that are both environmentally sound and economically viable.

## **2. Major Diseases of Tobacco: Black Shank and Fusarium Wilt**

### **2.1. Black Shank (Caused by *Phytophthora nicotianae*)**

#### **2.1.1. Black Shank of Tobacco**

One of the most significant and dangerous diseases affecting tobacco (*Nicotiana tabacum* L.) plants is black shank (Pandey 2023). It is brought on by the fungus *Phytophthora parasitica* var. *Nicotianae*, which is found in most areas where tobacco is grown (Zhang *et al.* 2024; Zhang *et al.* 2003). *P. nicotianae* belongs to the Kingdom Chromista, phylum Oomycota, class Oomycetes, and order Peronosporales. Ten different clades were found within the genus *Phytophthora* in recent studies that used genetic markers like beta-tubulin, elongation factor 1

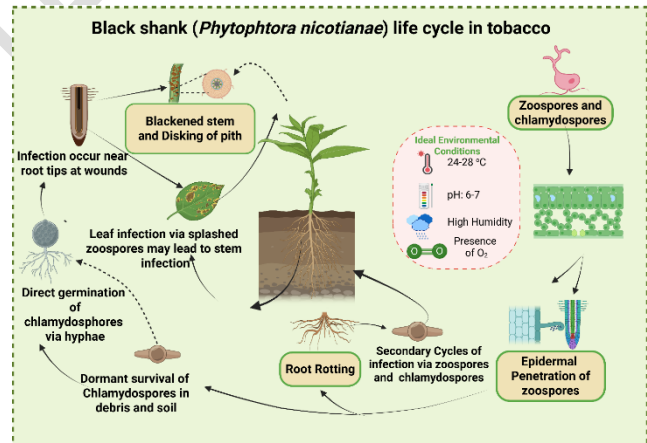
alpha, enolase, heat shock protein 90, 60S ribosomal protein L10, 28S ribosomal DNA, and tgaA (Yan *et al.* 2024). The white hyphae (filaments) of *P. nicotianae* are branched and range in diameter from 3 to 11 micrometers (Pandey 2023). The hyphae may appear fluffier and turn pale yellow as they mature. Although the hyphae lack septa, or partitions, older cultures may produce pseudosepta, or seeming partitions. As they age, the hyphae develop oil globules and turn granular (Gupta & Chugh 2022). The sporangia (structures that produce spores) of this fungus are oval, lemon-shaped, pear-shaped, sympodial (occurring in pairs), and span between 18 and 61 by 14 and 39 micrometers. Sporangia are pale yellow to transparent and develop from the hyphae on short pedicels (Mondal *et al.* 2020). With an apical papilla, these sporangia can generate five to thirty zoospores, which are tiny, mobile spores that range in size from seven to eleven micrometers (Delmas *et al.* 2014). There are several varieties of spores seen in *P. nicotianae*. The concave side of biflagellate zoospores has flagella attached to it (Kasteel *et al.* 2023). Typically measuring 30  $\mu\text{m}$  in diameter and ranging from 14 to 43  $\mu\text{m}$  in length, chlamydospores are spherical or ovoid in shape and non-papillate (Scanu *et al.* 2021). At first, they have thin walls and are hyaline, but as they become older, they thicken and change from yellow to brown. Perpendicular to the vegetative hyphae, chlamydospores develop on short lateral hyphae. Oospores, which are spherical to pyriform and hyaline to pale yellow in appearance, have been observed in laboratory settings but do not have a strong environmental record (Nam *et al.* 2022). The structures that surround the oosphere are called oogonia, and they are hyaline to pale yellow in color. The developing oospore, which is normally 23–30  $\mu\text{m}$  in diameter, is the result of fertilization (Tsai & Thines 2025).

One type of plant pathogen that is frequently seen in tropical and subtropical areas with high humidity and warm temperatures is *P. nicotianae* (Bahadur & Dutta 2023). Temperatures between 28 and 32°C and pH values between 5.7 and 7.0 are usually ideal for their growth. For optimal infection, they also need a temperature of at least 20°C (Pandey 2023). When there is enough oxygen and water in the environment, sporangia can grow and form; the ideal temperature range is between 24 and 28°C. Within 48 hours of the mycelium developing, sporangia may emerge (Benigno *et al.* 2025). When sporangia germinate, they will create secondary sporangia at the same temperature. A tiny projection known as a papilla will allow kidney-shaped zoospores with two flagella to emerge from the sporangia (Crouch *et al.* 2022). Within 72 hours of landing on a host's tissue, these zoospores will swim in circles and germinate to generate new sporangia, which will then produce another generation of zoospores (Moreira *et al.* 2023). Plant pathogen *P. nicotianae* is a polycyclic disease-causing agent that mostly targets the roots of its host plant but can also infect leaves and flowers (Volynchikova & Kim 2022). Its classification as a hemibiotrophic pathogen indicates that, throughout its disease cycle, it undergoes both biotrophic (living off a host) and necrotrophic (producing death in the host)

stages (Rajarammohan 2021). The pathogen *P. nicotianae* first establishes a mutually advantageous association with its host. But after a while, it kills the host cells and goes into a phase when it feeds on the dead host tissue. The necrotrophic phase is the term for this stage (Singh *et al.* 2024). The amount of zoospores in soil is correlated with the severity of the infection, which occurs when asexually generated, multi-nucleated sporangia release zoospores, which are mobile and lack a cell wall (Del Castillo-González *et al.* 2024). The zoospores attract to certain compounds in the soil and go to the root tissue, where they develop into a cyst on the plant. The development of a germ tube, which ultimately becomes an appressorium, is crucial to the *P. nicotianae* disease cycle (Legrifi *et al.* 2023). The pathogen may penetrate and infect host cells thanks to these structures, which causes the host cells to die. Additionally, *P. nicotianae* can persist in the soil for long periods of time as chlamydospores, which can infect subsequent growth seasons (Bag *et al.* 2023). To try to stop this infection from spreading, management techniques frequently focus on the development of germ tubes (Delai *et al.* 2024).

*P. nicotianae* reproduces mostly asexually, while it can occasionally reproduce sexually by fusing male and female gametangia (Berbeć 2024). Both A1 and A2 mating types must be present for sexual reproduction to produce thick-walled oospores (Babarinde *et al.* 2024). But the uneven distribution of these mating types in the environment raises the possibility that oospore production may not play a major role in the pathogen's life cycle and that worries about virulence and pathogenicity changes brought on by sexual reproduction may be exaggerated (Meng *et al.* 2014). At any stage of growth, the virus can infect the tobacco plant's roots, stems, and leaves, among other areas. This may drastically lower the tobacco crop's output and quality (Sun *et al.* 2024). Tobacco plants may become stunted and fall before their leaves are developed enough to be harvested if they contract Black Shank disease early in the growing season (Bahadur & Dutta 2023). *P. nicotianae* can infect tobacco plants at any stage of growth, although it usually affects plants between 6 and 8 weeks of age (He *et al.* 2022). According to Tong *et al.* (2024), Within 48 hours of the pathogen being introduced, black shank signs can be seen, and the plant may die within a week of infection. Its main symptoms include root and crown rot, consistent wilting, and chlorosis of the leaves, which result in water-soaked lesions on stem tissue that are 15 to 20 centimeters above the soil line (Sapkota *et al.* 2022). Root necrosis may also be seen as the condition progresses (Zhou *et al.* 2023). The defining signs of black shank are brown to black vascular disking and pith necrosis (Cochran *et al.* 2024). On older foliage, a large, concentric, round, dark-brown lesion that is 7 to 8 cm in diameter may also develop as a result of the inoculum being dispersed by rain splash (Cochran *et al.* 2024). Since other tobacco diseases like Fusarium Wilt and Granville Wilt might exhibit similar symptoms, the macroscopic symptoms mentioned above should be utilized in concert with microscopic inspection and molecular characterisation to accurately detect *P.*

*nicotianae* infection. Plant tissue exhibiting signs of illness, water, or soil can all harbor *P. nicotiana* as shown in **Figure 1**. *Ageratina adenophora*, *Ageratum houstonianum*, *Parthenium hysterophorus*, and *Xanthium strumarium*, these invasive species demonstrated antifungal activity against *Phytophthora capsici* (Han *et al.* 2024). While not specifically tested against *Phytophthora nicotianae*, their potential to inhibit *Phytophthora* species suggests they could be further investigated for black shank management. The methanolic extracts of these plants generally exhibit stronger inhibitory effects compared to aqueous extracts (Han *et al.* 2024), indicating that the active compounds are more soluble in organic solvents. *Ipomoea carnea*: Although tested against different fungal pathogens (*Fusarium solani*, *Alternaria solani*, and *Colletotrichum circinans*) (Tao *et al.* 2021), the antifungal properties of *Ipomoea carnea* suggest potential for broader applications, warranting further research against *P. nicotianae*. *Parthenium hysterophorus*: Whole plant is used for organic and inorganic pollutant removal and Cr(VI) remediation (Zhu *et al.* 2024). *Prosopis juliflora* and *Leucaena leucocephala*: These invasive plants, commonly found in Egypt, have shown antifungal activity against *Fusarium solani*, *Alternaria solani*, and *Colletotrichum circinans* (Tao *et al.* 2021). Their water-based extracts exhibited antifungal properties, suggesting a readily accessible method for potential application. Further research is often needed to optimize their use in integrated disease management strategies (Naqvi *et al.* 2024) as shown in **Table 1**.



**Figure 1.** Life cycle and pathogenesis of *P. nicotianae* causing Black Shank disease in tobacco.

## 2.2. Fusarium Wilt (Caused by *Fusarium oxysporum* f. sp. *nicotianae*)

In many countries throughout the world, Fusarium wilt, a disease brought on by the *F. oxysporum* species complex, causes significant damages (Engalycheva *et al.* 2024). In 1916, Maryland had the first documented incidence of the disease in the United States. In the states of Connecticut and Massachusetts, it had developed into the most serious and destructive disease affecting broadleaf cigar wrapper tobacco by the 1980s and early 1990s (Pandey 2023). About 20% of the tobacco producing areas were affected by the disease, which caused significant damage and led to the removal of badly infected fields from

tobacco production (Liang *et al.* 2024). The three species of *F. oxysporum* f. sp. *nicotianae*, f. sp. *batatas*, and f. sp. *vasinfectum* are the fungus that causes wilt in tobacco plants (Berruezo *et al.* 2021). These species were distinguished by their effects on cotton, sweet potatoes, and tobacco, among other hosts. It was discovered that four races of the fungus *F. oxysporum* were harmful to tobacco (Berruezo *et al.* 2018). According to recent studies using cluster analysis, the pathogen consists of at least three different isolate groups, which may show distinct lineages from tobacco and sweet potatoes (Paul *et al.* 2020). All isolates that were initially discovered in tobacco are included in one of these clusters, *F. oxysporum* f. sp. *Nicotianae* (Rahman *et al.* 2021). Sweet potato isolates from North Carolina and Louisiana (Race 0) or California (Race 1) make up the second and third clusters of *F. oxysporum* f. sp. *batatas*. No disease was caused by Race 0 of *F. oxysporum* f. sp. *batatas* in flue-cured tobacco or resistant sweet potatoes, however Race 1 caused wilt in resistant sweet potatoes but had no effect on tobacco. This shows the variability of the tobacco wilt pathogen. The symptoms of Fusarium wilt in tobacco plants include leaf yellowing, drying, and death. These symptoms can occur in a vertical pattern, usually on one side of the plant or the mid vein of the leaf (Wang *et al.* 2022). The vascular tissue of the plant becomes characteristically chocolate-brown discolored due to the disease, and this discoloration may extend to the top of the plant (Dell'Olmo *et al.* 2023). The outside of the green stem gradually shows similar darkening. The leaves will cure on the stalk rather than rot, which will cause the stalk to bend over at the bud and give it a characteristic "crookneck" look (Pandey 2023). The plant will eventually become dry and necrotic as a whole. Sandy loam soils and warm weather increase the disease's severity (Abbas *et al.* 2022). Plant resistance, cleanliness, crop rotation, nutrition, nematode control, and fumigation or bio fumigation are some of the strategies used to prevent Fusarium wilt in tobacco. But not all approaches work the same way (Haruna *et al.* 2024). According to El-Aswad *et al.* (2023), recently there are no chemical management for *F. oxysporum* f. sp. *nicotianae* in soil that are completely effective. The quality and look of wrapper leaves are negatively impacted by the phase-out of the most effective fumigants, methyl bromide and chloropicrin (Villarino *et al.* 2021), but fumigation of soil may result in a little decrease in the severity of disease. Moreover, fumigation with metam sodium does not reduce cotton wilt induced by soil borne Fusarium or *F. oxysporum* f. sp. *Vasinfectum* (McDonald *et al.* 2021). In the crop residue Chlamydospores may also resistant to fumigation. In Connecticut, fumigation has produced mixed outcomes, especially when tobacco is planted in the same area over time. However, it may indirectly limit disease manifestation by affecting plant parasitic nematodes. The recent study by (Lal *et al.* 2024), The development and broad use of tobacco cultivars resistant to Fusarium wilt has shown to be the most effective strategy for managing the disease on a global scale. The broadleaf cigar wrapper varieties including 'B2' and 'C9' resistant to Fusarium wilt

pathogen were introduced in Connecticut (LaMondia 2013). This resistance is caused by several genes building up for increased effectiveness (Sadeghpour *et al.* 2024). These resistant cultivars may still exhibit wilt signs in severe environments since they are not totally immune to Fusarium infection (Lal *et al.* 2024). Broadleaf tobacco resistant to wilt has often been found to contain *F. oxysporum*, and this resistance has been found to be associated with the pathogen's slower migration inside the plant's vascular tissues. Moreover, within 24 hours of inoculation, resistant plants exhibited rapid reactions, such as the development of vesicles to block xylem, the deposition of callose, and the production of lipoidal material (Chen *et al.* 2024; LaMondia 2013). Chlamydospores spread through soil on agricultural equipment that moves between fields, and the habit of utilizing tobacco stalks for fertilizer and rubbish disposal are two of the many factors contributing to the pathogen's rapid spread. Studies have revealed that *F. oxysporum* is resistant to composting, in contrast to many other plant diseases (Bouchtaoui *et al.* 2024). These days, tobacco stalks are either buried or dispersed in non-tobacco areas rather than remaining on tobacco fields. By implementing sanitation procedures, such as removing soil clumps from field equipment before moving it to another field, the wilt pathogen and other soil-borne illnesses, like tobacco cyst nematodes, can be prevented throughout their spread as shown in **Figure 2**. Despite these precautions, *F. oxysporum* continues to spread between farms more rapidly and extensively than expected (Ismaila *et al.* 2023); (Wichuk *et al.* 2011). Wounding roots through close cultivation, hoeing, or drip tape irrigation creates holes for infection and increases Fusarium wilt (Bhumarkar *et al.* 2021). Reducing injury lowers the incidence and severity of wilt. Beyond merely generating new infection sites from wounds, tobacco cyst nematodes (*Globodera tabacum*) and root knot worms (*Meloidogyne* spp.) also create Fusarium wilt (Khan & Sharma 2020). Prior to fungal exposure, plants with nematode infestations showed higher rates and severity of wilt than those exposed concurrently. It was discovered that an infestation of *G. tabacum* caused more wilt than an equivalent amount of *Meloidogyne hapla*. In field tests, early season nematode control reduced the frequency and intensity of Fusarium wilt in tobacco (Makunde *et al.* 2023). By modifying the pH and nitrogen levels of the soil, several Fusarium wilt infections have successfully managed (Habte & Dobo 2025). The incidence and severity of the diseases in tomatoes and chrysanthemums have been related to factors such soil pH, lime, calcium, and nitrogen sources (Valenzuela 2024). Calcium was the most successful nutritional component studied for controlling Fusarium wilt in tobacco, according to (Okiro *et al.* 2025). Since calcium controls the synthesis of callose, a plant defense mechanism against vascular wilt pathogens, its deficiency has been connected to the onset of the diseases. Calcium is thought to be the most easily manipulated nutrient in Connecticut broadleaf tobacco cultivation (Daunoras *et al.* 2024) (LaMondia 2015). Although fusarium wilt can develop in soil with varying pH



values, the illness may be affected if the pH is raised to above 6.4. However, soils with a pH of 5.6 to 6.0 might develop severe black root rot (Šišić *et al.* 2025). Although Fusarium wilt in susceptible broadleaf tobacco can be suppressed by applying substantial amounts of gypsum to the soil, this effect was minimal at low disease incidence levels. The 13,400 kg/ha of gypsum needed to minimize wilt is far more than tobacco growers typically use (340–560 kg/ha), and it may have detrimental agronomic impacts like slower crop growth (LaMondia 2015).

**Table1.** List of plant species used against Black Shank of Tobacco

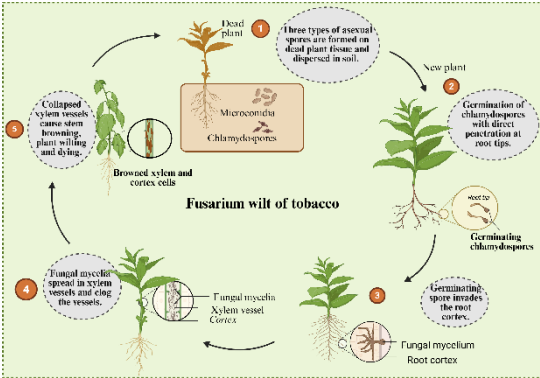
S. No	Plant	Active ingredients	Mechanism	Reference
1	<i>Sophora flavescens</i>	Matrine and oxymatrine	Part of a mixed plant extract that, combined with fungicides, successfully controlled black shank in greenhouse experiments.	Alsarhan <i>et al.</i> (2014)
2	<i>Forsythia suspense</i>	forsythiaside A, arctigenin	Part of a mixed plant extract that, combined with fungicides, successfully controlled black shank in greenhouse experiments.	Alsarhan <i>et al.</i> (2014)
3	<i>Nicotiana plumbaginifolia</i>	Nicotine	Contains theNpPP2-B10gene, which, when transferred to <i>Nicotiana tabacum</i> , promotes resistance to <i>P. nicotianae</i> .	Deaton <i>et al.</i> (1982)
4	<i>Tagetes erecta</i> L.)	lutein, $\beta$ -carotene	Rotation with marigold reduces disease incidence in continuously cropped tobacco fields, influencing soil microbial communities.	Boro <i>et al.</i> (2024)
5	<i>Sophora flavescens</i> , <i>Forsythia suspense</i> ,	Oxymatrine, sophocarpine	Induces PR proteins (PR-1, PR-4, and PR-5) in tobacco plants, enhancing resistance against black shank and tobacco. The extract, alone or combined with fungicides, shows successful disease control in both non-continuously and continuously cropped land.	Wang <i>et al.</i> (2018)
6	<i>Syringa oblata</i>	syringic acid, caffeic acid derivative	Inhibits mycelial growth of <i>P. nicotianae</i> in a dose-dependent manner, disrupting extracellular pH and electrolyte leakage. Minimum inhibitory concentration (MIC) of eugenol is 200 $\mu$ g/mL.	Zhu <i>et al.</i> (2024)
7	<i>Zanthoxylum bungeanum</i> Maxim.	berberine, chelerythrine	Increases soil organic matter, hydrolysable nitrogen, available potassium, and total phosphorus while decreasing pH, promoting plant growth (increased plant height, root length, and dry weight. Acts as a bio-fumigation material against tobacco black shank.	Dhuldhaj <i>et al.</i> (2023)
8	<i>Azadirachta indica</i>	azadirachtin, nimbin, nimbidin	Disrupts fungal membrane integrity; inhibits mycelial growth and spore germination.	Mahmoud <i>et al.</i> (2011)
9	<i>Allium sativum</i>	Allicin	Increases mycelial membrane permeability; causes cell death in <i>P. nicotianae</i>	Wang <i>et al.</i> (2019)
10	<i>Syzygium aromaticum</i>	Eugenol, $\beta$ -Caryophyllene	Damages mycelial membranes, inhibiting growth & spore germination	Jing <i>et al.</i> (2017)

Recent studies as shown in **Table 2**, have demonstrated the efficacy of various plant extracts in controlling *Fusarium* wilt pathogens across different crops. *Datura metel* leaf extract completely inhibited mycelial growth of *F. oxysporum* f. sp. *cubense* in banana at 10% concentration (Hassan *et al.* 2022). For tomato pathogens, *Aloe vera* extracts significantly inhibited growth and sporulation of *F. oxysporum* f. sp. *Lycopersici* (FOL) under both laboratory and greenhouse conditions (Al-Gallas *et al.* 2021), while clove (*Syzygium aromaticum*) essential oils

Without a vulnerable host, the fungus in a field lasts for several years. Based on the chlamydospores' survival, crop rotation's ability to reduce the density of *Fusarium* wilt pathogens in the soil varies widely (Garzón-Nivia *et al.* 2025; Obiazikwor *et al.* 2025) and the pathogen's capacity to infect other plant species that are not impacted as well as the roots of resistant crops (Nowicki *et al.* 2025). It is necessary to assess how resistant plants and rotation crops affect the soil's pathogen populations over time for each unique pathosystem and crop.

reduced fungal growth and spore populations (He *et al.* 2021). Extracts from *Eucalyptus camaldulensis*, *Chromolaena odorata*, *Bidens pilosa*, and *Wedelia trilobata* also showed effective control of *F. oxysporum* in tomatoes (Al-Gallas *et al.* 2021). In chickpea, neem (*Azadirachta indica*) suppressed *F. oxysporum* f. sp. *ciceri* activity *in vitro* (Meena *et al.* 2021), and garlic (*Allium sativum*) induced physiological and biochemical defenses against *Fusarium* wilt in both chickpea and tomato (Selva Amala *et al.* 2024). Other effective botanicals included

resins from *Commiphora swynnertonii* and latex from *Synadenium glaucescens* against tomato wilt (Fenollosa & Munné-Bosch 2020), as well as *Monsonia burkeana* and *Moringa oleifera* extracts (Jamil *et al.* 2021). *Xanthium strumarium* demonstrated efficacy against *F. oxysporum* in pomegranate (Powell *et al.* 2024), while combined neem and willow (*Salix babylonica*) extracts reduced tomato wilt severity by inducing antioxidant enzymes (Farag Hanaa *et al.* 2011). These findings highlight the potential of plant-derived solutions for integrated *Fusarium* wilt management.



**Figure 2.** Disease cycle and pathogenesis of *Fusarium* wilt in tobacco.

**Table 2.** Antifungal effects of plant extracts against *Fusarium oxysporum* pathogens in different crops.

Plant Extract Source	Target Crop	Pathogen	Observed Effect	References
<i>Datura metel</i> (leaf extract)	Banana	<i>F. oxysporum</i> f. sp.cubense	Complete inhibition of mycelial growth at 10% concentration.	Hassan <i>et al.</i> (2022)
<i>Aloe vera</i>	Tomato	<i>F. oxysporum</i> f.sp.lycopersici	Inhibited growth and sporulation of FOL under laboratory and greenhouse conditions.	Al-Gallas <i>et al.</i> (2021)
<i>Syzygium aromaticum</i> )	Tomato	<i>F. oxysporum</i> f.sp.lycopersici	Essential oils from clove also significantly reduced growth and spore population	He <i>et al.</i> (2021)
<i>Eucalyptus camaldulensis</i> , <i>Chromolaena odorata</i> , <i>Bidens pilosa</i> , <i>Wedelia trilobata</i>	Tomato	<i>F. oxysporum</i>	Effective control of <i>F. oxysporum</i> in vitro and in tomatoes.	Al-Gallas <i>et al.</i> (2021)
<i>Azadirachta indica</i> (Neem)	Chickpea	<i>F. oxysporum</i> f. sp.ciceri	Suppressed activity of <i>F. oxysporum</i> f. sp.ciceriunder in-vitro conditions.	(Meena <i>et al.</i> (2021)
<i>Allium sativum</i>	Chickpea, Tomato	<i>F. oxysporum</i> f. sp.ciceri, <i>F. oxysporum</i> f. sp.lycopersici	Used as inducers on physiological and biochemical activities in tomato against <i>Fusarium wilt</i> .	Selva Amala <i>et al.</i> (2024)
<i>Commiphora swynnertonii</i> (resins), <i>Synadenium glaucescens</i> (	Tomato	<i>F. oxysporum</i> f. sp. <i>Lycopersici</i>	Extracts were effective against <i>F. oxysporum</i> f. sp. <i>Lycopersici</i>	Fenollosa & Munné-Bosch (2020)
<i>Monsonia burkeana</i> , <i>Moringa oleifera</i>	Tomato	<i>F. oxysporum</i> f. sp. <i>Lycopersici</i>	Extracts were effective against <i>F. oxysporum</i> f. sp. <i>Lycopersici</i>	Jamil <i>et al.</i> (2021)
<i>Xanthium strumamrium</i>	Pomegranate	<i>F. oxysporum</i>	Effective against pomegranate isolated pathogenic fungi.	Powell <i>et al.</i> (2024)
<i>Neem</i> ( <i>Azadirachta indica</i> ) and <i>willow</i> ( <i>Salix babylonica</i> )	Tomato	<i>F. oxysporum</i>	Reduced <i>Fusarium</i> wilt disease in tomato seedlings, induced antioxidant defensive enzymes.	Farag Hanaa <i>et al.</i> (2011)

### 3. Current Management Strategies

Current tobacco management of *Fusarium* Wilt and Black Shank depends on an integrated strategy that combines biological, cultural, and chemical control techniques. Still, each approach has important drawbacks that jeopardize

sustainability and long-term effectiveness. According to Sapkota *et al.* (2023), fungicides continue to be the main line of defense against these debilitating diseases. Metalaxyl and fosetyl-Al are frequently applied to manage Black Shank, while benzimidazoles (like carbendazim) and triazoles (like tebuconazole) are used to manage *Fusarium*

Wilt. But there are concerning trends in resistance as a result of the overuse of these fungicides. More than 60% of *P. nicotianae* populations in important tobacco-growing regions are resistant to metalaxyl, according to recent surveys, making the fungicide useless in many places (Clement *et al.* 2025; Van Jaarsveld *et al.* 2002). Also, benzimidazole resistance has grown widely in *F. oxysporum* f. sp. *nicotianae*, with resistance frequencies above 80% reported in China and Brazil (El-Nagar *et al.* 2023). In addition to their resistance, chemical fungicides can contaminate soil and water, harm beneficial microorganisms without their intended target, and threaten the health of farmers. The EU's prohibition on methyl bromide and impending limitations on phosphonates are just two examples of how regulatory bodies are regulating synthetic fungicides more and more, which further reduces the range of alternatives (Nader *et al.* 2020). Crop rotation and resistant cultivars are examples of cultural methods that provide some partial solutions but are difficult to apply. Although switching tobacco to non-host crops (such as maize or sorghum) can decrease the pathogen burden, this strategy is compromised by the long-term survival of *F. oxysporum* chlamydospores (decades) and *P. nicotianae* (up to 5 years in soil) (Ristaino *et al.* 2021). Although resistant tobacco cultivars have been developed (such as Black Shank's "K 326"), resistance frequently breaks off in 5–10 years due to pathogen adaptation (McCorkle *et al.* 2018). According to Mavroeidis *et al.* (2024), resistant cultivars may degrade leaf quality, which is essential for commercial viability. *Trichoderma harzianum* and *Pseudomonas fluorescens* are biological agents showed promising results in laboratory condition but they exhibit different results in field, this is due to environmental sensitivity and competition with native microbiota (Ayaz *et al.* 2023). Although *Trichoderma* spp. can lower the incidence of Black Shank by 30 to 50% in greenhouse experiments, their effectiveness is greatly diminished in field condition with varying moisture and temperature (Naorem *et al.* 2023). Similarly, efficacy rates for Bacillus-based bio fungicides vary from 20% to 70% depending on the location, and they frequently fall short of commercial-scale consistency (Barros-Rodríguez *et al.* 2024).

### 3.1. Plant Extracts as Sustainable Alternatives: A Path toward Eco-Friendly Disease Management

Research on plant-derived antimicrobials as sustainable substitutes for traditional disease management techniques has increased due to the rising limitations of these methods for preventing Fusarium Wilt and Black Shank in tobacco (Deresa & Diriba 2023). Plant extracts contain complex combinations of bioactive compounds that attack pathogens through several processes concurrently, greatly minimizing the possibility of resistance development, in contrast to manufactured fungicides that target single metabolic pathways (Ayaz *et al.* 2019). Alkaloids, flavonoids, terpenoids, and phenolic compounds are among the hundreds of secondary metabolites that have been discovered by recent developments in phytochemical research to have strong

antifungal effects against both *Fusarium oxysporum* and *Phytophthora nicotianae* (Deresa & Diriba 2023). For example, *P. nicotianae* zoospores' cell membrane viability is damaged by curcumin from turmeric (*Curcuma longa*), which also prevents mycelial growth by interfering with mitochondrial activity (Wang *et al.* 2019). Plant extracts' mechanisms of action go beyond simply suppressing pathogens; they can also cause tobacco plants to develop systemic resistance (Yang *et al.* 2024). Plants are primed for improved immune responses by compounds like thymol from thyme (*Thymus vulgaris*) and allicin from garlic (*Allium sativum*), which have been demonstrated to activate the salicylic acid defense system and upregulate pathogenesis-related (PR) proteins (Anisimova *et al.* 2021). Because plant extracts have both direct antibacterial activity and host defense potentiation, they are very useful for integrated disease management. Field experiments in China's main tobacco-growing regions showed that neem (*A. indica*) kernel extract and chitosan together decreased the incidence of Black Shank by 68–72%, which is similar to synthetic fungicides but without the hazards of resistance (Ibrahim *et al.* 2023). Plant extract formulations have historically presented difficulties that are being addressed by recent technological advancements. Utilizing biodegradable carriers like as lignin and chitosan, nanoencapsulation strategies have greatly increased the stability and bioavailability of volatile chemicals (Deng *et al.* 2023). Oregano (*Origanum vulgare*) essential oil Nano emulsions, for example, retained 85% antifungal activity after 30 days of field exposure, but unformulated oil only exhibited 35% (Hosny *et al.* 2021). Additionally, developments in extraction methods, including supercritical fluid extraction and ultrasound-assisted extraction, have reduced processing time and energy usage while increasing active compounds yields (Khadhraoui *et al.* 2021).

Plant-based disease management has significant positive effects on the environment. According to life cycle assessments, botanical pesticides decompose entirely in soil in 2–4 weeks and have carbon footprints that are three to five times lower than those of synthetic counterparts (Yin *et al.*, 2023; Ristaino *et al.*, 2021). In response to the increased consumer demand for "clean label" products, this quick degradation removes residual problems in cured tobacco leaves. Most significantly, local production of plant extract formulations employing native species in tobacco-growing countries can open up new business prospects for smallholder farmers (Ouma, 2024). Considering these benefits, there are still issues with obtaining regulatory permissions, maximizing application timing, and standardizing extract potency. Next-generation botanical fungicides are being developed more quickly, though, because to the fusion of traditional ethnobotanical knowledge with contemporary analytical methods (such as metabolomics and machine learning). Plant extracts have the potential to evolve from additional treatments to essential elements of sustainable tobacco production systems across the globe as resistance to traditional fungicides keeps growing and regulatory demands increase.



### 3.2. Modes of action of plant extracts

Plant extract or botanicals act against microorganisms are even less known. These mechanisms rely on the composition of botanicals, which is multifactor dependent (Radulovic *et al.*, 2013). A few studies reveal that the major components are mainly responsible for the biological activity of botanicals, but others conclude that several components act in synergy (Amenu, 2014; Chaachouay, 2025). Furthermore, as botanicals contain a mixture of diverse components, their antifungal activity is probably not attributable to a single mechanism. The main mechanisms reported so far are membrane disruption, metal chelation, interaction with DNA, and induction of plant defense reactions (Redondo-Blanco *et al.*, 2020). Several studies report that EO or some of their components are able to disrupt cell wall and membrane integrity and to easily penetrate into the cells (Yap *al.*, 2021). This disruption causes mitochondrial membrane damage, which induces changes in the electron transport chain. Consequently, free radicals are produced, and they oxidize and damage lipids, proteins, and DNA. In contact with reactive oxygen species (ROS), EO phenolic compounds are oxidized and release reactive phenoxyl radicals (Hajam *et al.* 2023). The induction of plant defenses by EO has also been investigated as shown in **Figure 3**. Thyme oil application on tomato roots efficiently triggered peroxidase accumulation in roots, which are well-known to be part of the plant defense mechanisms (Saltos-Rezabala *et al.* 2022). Similarly, Farooq *et al.* (2024) found evidence of the induction of plant defense responses against *F. oxysporum* f. sp. *lycopersici* using different plant extracts. Although the antimicrobial mechanisms of action of botanicals have been carefully studied for their pharmaceutical or food preservative uses, less information is available concerning their use to control plant pathogenic microorganisms (Oulahal *et al.* 2012

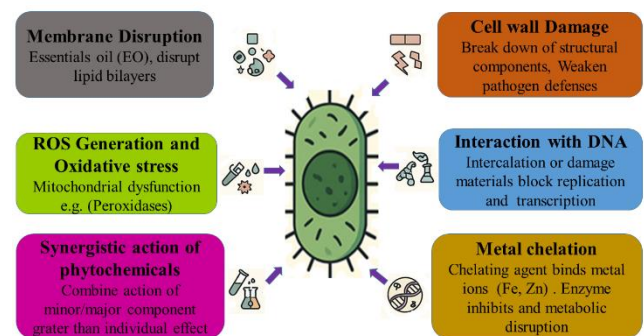
### 3.3. Molecular Modes of Action of Plant Extracts

Plant extracts possess inherent complexity, making it difficult to attribute their bioactivity to a single mechanism; yet, growing evidence highlights both particular molecular targets and synergistic interactions among their phytochemical components. Principal molecules frequently dictate primary function; nevertheless, minor components can substantially enhance effectiveness through synergistic interactions (Amenu 2014; Chaachouay 2025). A fundamental antibacterial process involves the degradation of microbial cell membranes. Terpenoids, such as carvacrol (from oregano) and thymol (from thyme), integrate into lipid bilayers due to their lipophilic properties, causing destabilization of plasma membranes, ion leakage ( $K^+$ ,  $H^+$ ), ATP depletion, and ultimately cell lysis (Bakkali *et al.* 2008; Yap *et al.* 2021).

This membrane disruption frequently impacts organelles, particularly mitochondria, hence disrupting the electrochemical gradient and impairing the electron transport chain (ETC). The resultant surplus of reactive oxygen species (ROS), including superoxide anions ( $O_2^-$ )

and hydrogen peroxide ( $H_2O_2$ ), exceeds microbial antioxidant defenses such as catalase and glutathione, resulting in lipid peroxidation, protein carbonylation, and DNA strand breaks (Camele *et al.* 2019). Phenolics exacerbate this stress by generating phenoxyl radicals by microbial oxidation, hence perpetuating oxidative chain damage (Hajam *et al.* 2023).

Besides these broad harmful effects, plant metabolites significantly influence microbial physiology. Flavonoids and tannins chelate essential metals such as  $Fe^{2+}$  and  $Zn^{2+}$ , thereby inhibiting pathogens from acquiring crucial cofactors for their enzymes (Miklasińska-Majdanik *et al.* 2018). Alkaloids, including berberine, directly intercalate with DNA, obstructing replication and transcription. Conversely, thiol-reactive compounds, such as allicin (derived from garlic), irreversibly inhibit cysteine-dependent enzymes. Plant extracts possess direct antibacterial properties and enhance the body's defenses against infections. For instance, the application of thyme oil to tomato roots induced systemic resistance by enhancing the expression of pathogenesis-related proteins (PRs) and activating critical defense enzymes such as peroxidase (POD) and phenylalanine ammonia-lyase (PAL), which promote phytoalexin biosynthesis (Saltos-Rezabala *et al.* 2022). Farooq *et al.* (2024) similarly demonstrated that plant extracts can prime defense mechanisms to combat *Fusarium oxysporum* f. sp. *lycopersici*. While several processes have been explored in food preservation, their implementation in agricultural disease management remains an insufficiently studied yet highly prospective research domain (Oulahal *et al.* 2012).



**Figure 3.** Mode of action of Plant extracts against pathogenic microorganisms

### 3.4. Recent research on plant extract interactions with pathogen resistance genes

Recent research has improved our understanding of how plant extracts influence disease resistance by interacting with microbial virulence factors and host defense pathways. *Xanthomonas* species employ type III effectors (T3Es), including Xop proteins, to suppress host immunity. Recent studies indicate that phytochemicals can modify these interactions, hence diminishing pathogen pathogenicity (Medina *et al.* 2017). Plant extracts assist plants in managing oxidative stress caused by heavy metals, so safeguarding them from environmental stressors and enhancing their resilience (Mirkov *et al.* 2020). Pathogenic fungi face oxidative stress from plants during infection and must maintain redox balance to

develop disease, highlighting a critical vulnerability that can be targeted by bioactive phytochemicals (Park & Son 2024). Terpenoids, the primary constituents of essential oils, alter the integrity of lipid bilayers, hence compromising fungal and bacterial membranes (Konuk & Ergüden 2020). Moreover, their efficacy can be augmented by synergistic combinations, as demonstrated by the conjunction of oregano oil and blue light, which amplifies bactericidal activity against multidrug-resistant *Pseudomonas aeruginosa* by targeting both planktonic and biofilm cells (Lu *et al.* 2022). The antifungal effectiveness of terpenoids is markedly linked to phenolic –OH groups that induce membrane instability (Konuk & Ergüden 2020).

Besides their antibacterial effects, plant metabolites also influence the host's stress response and the body's ability to combat infections. Thymol enhances the salt tolerance of Chinese cabbage seedlings by augmenting their antioxidant capacity, maintaining redox equilibrium (AsA/DHA and GSH/GSSG ratios), and activating crucial ROS-scavenging enzymes such as SOD, CAT, APX, and POD (Sun *et al.* 2024). This mechanism prevents oxidative damage and sustains seedling growth during salt stress. Extracts of *Borreria verticillata* and silver nanoparticles exhibit nematocidal properties against *Meloidogyne incognita* in tomatoes, presenting a sustainable alternative to chemical nematocides (Fabiya & Olatunji 2024). Hydrocarbons and plant-derived compounds are detrimental to membranes since they accumulate in lipid bilayers, disrupt normal cellular functions, and alter microbial food degradation processes (Sikkema *et al.* 1995). These findings jointly highlight that plant extracts function as both direct antimicrobials and modulators of pathogen resistance genes, host oxidative stress responses, and environmentally viable crop protection strategies. This expanding body of research indicates their potential utility in integrated disease management and sustainable agriculture.

## 4. Challenges and Future Perspectives

### 4.1. Limitations of Plant Extracts

For the management of Black Shank and Fusarium Wilt diseases in tobacco, plant extracts have gained attention as environmentally friendly alternatives (X. ping Sun *et al.* 2025). However, a number of restrictions hindered them from being used in practice. The chemical composition of plant extracts varies greatly depending on the plant species, growth conditions, harvesting time, and extraction techniques, making this one of the main problems (Raudone & Savickiene 2024). Because of this inconsistency, antifungal efficacy is frequently unpredictable, which makes it challenging to standardize therapies for trustworthy disease management (Tang *et al.* 2012). Furthermore, many plant extracts have comparatively weaker direct toxicity against soil-borne infections such as *Fusarium oxysporum* and *Phytophthora nicotianae* than synthetic fungicides, which can limit their efficacy, particularly when disease pressure is high (Mirmajlessi *et al.* 2024). Since variables like pH, moisture,

microbial community dynamics, and organic matter content can change the stability, bioavailability, and activity of bioactive chemicals in plant extracts, the intricate interactions within the soil environment also have an impact on their performance (Adeniji *et al.* 2024). The sustainable sourcing of raw plant materials, preserving the stability and shelf-life of extracts, and creating affordable formulations appropriate for large-scale agricultural use are additional logistical and financial challenges associated with scaling up the production of plant extracts (Lisboa *et al.* 2024). Considering their typically good safety profiles, careful assessment of any non-target and environmental consequences is necessary to guarantee sustainable use (Punniyakotti *et al.* 2024). These problems showed the need for more study to improve formulation stability, refine extraction methods, comprehend soil-plant-pathogen interactions, and successfully incorporate plant extracts into all-encompassing tobacco cultivation disease management programs.

### 4.2. Emerging Technologies to Enhance Efficacy

The effectiveness of plant extracts in controlling Fusarium Wilt and Black Shank infections in tobacco is being quickly improved by emerging technologies, which is viable, eco-friendly substitutes for traditional agrochemicals (Ahmad *et al.* 2024). According to Dewi *et al.* (2022), nanotechnology is essential because it enhances the stability, transport, and bioavailability of plant extracts. Polymeric nanoparticles, particularly those based on chitosan, are useful for the targeted use and regulated release of bioactive substances, which improves systemic plant resistance and antifungal activity (Zhou *et al.* 2024). In addition to directly inhibiting pathogens like *Phytophthora nicotianae* and *Fusarium oxysporum*, metal and metal oxide nanoparticles, like copper oxide (CuO) and silver nanoparticles, have built-in antimicrobial qualities that trigger plant defense mechanisms by producing reactive oxygen species (ROS) and activating antioxidant enzymes (Chen *et al.* 2022). Sensitive bioactive chemicals are kept safe from environmental stresses by nanoencapsulation and microencapsulation procedures, which enhance their functional stability and regulated release in the field (Guía-García *et al.* 2022). By changing plant immune signaling pathways (salicylic acid, ethylene, and hypersensitive response) and reshaping the rhizosphere microbial community to suppress pathogen abundance, synergistic strategies that combine plant extracts, nanocarriers, and beneficial microbes such as the co-application of chitoooligosaccharides with *Bacillus* strains have shown increased control efficacy. By providing antioxidants and strengthening plant structural defenses at the same time, advanced formulations such as silicon-stabilized hybrid lipid nanoparticles functionalized with quercetin act as nanobiostimulants that increase plant resistance (Gutsch *et al.* 2023). Early disease detection and the timing and dosage of plant extract applications are optimized by precision agriculture technologies that use UAV-borne hyperspectral remote sensing and machine learning algorithms, maximizing

their efficacy while minimizing inputs (Padhiary *et al.* 2024). By improving the stability, bioavailability, targeted distribution, and effectiveness of plant extracts, decreasing reliance on chemical fungicides, and advancing environmental health, these integrated technologies work together to enhance sustainable tobacco disease control (Ashraf *et al.* 2021). By improving efficacy through regulated and sustained release mechanisms, emerging technologies such as nano-encapsulation are transforming drug delivery systems (Ayyaril *et al.* 2023). In order to prevent premature drug degradation and guarantee targeted administration, active pharmacological ingredients are encapsulated into nanoscale carriers such as liposomes, polymeric nanoparticles, or dendrimers (Petrovic *et al.* 2024).

A promising strategy to improve sustainable agriculture and disease management is the coupling of nano-encapsulation with biocontrol agents, such as *Trichoderma* fungus and plant extracts

(Saberi-Riseh *et al.* 2021). According to Zhou *et al.* (2024), nano-encapsulation can shield these delicate biological agents from environmental deterioration, guaranteeing their stability and long-term effectiveness. To maintain a steady inhibitory impact against infections, for example, *Trichoderma* spores or plant-derived bioactive chemicals can be encapsulated in polymeric nanoparticles or lipid-based carriers for gradual and regulated release (Zafar *et al.* 2024). In addition to increasing the biocontrol agents' field performance and shelf life, this synergistic strategy lessens the requirement for frequent applications (Teixidó *et al.* 2022). Furthermore, to maximize these compounds' antibacterial and growth-promoting properties, nano-formulations can improve their adherence and targeted

distribution to plant roots or foliar surfaces (Mahmoud *et al.* 2024). Farmers can minimize their reliance on chemical pesticides and achieve more efficient, environmentally friendly crop protection by combining nanotechnology with biocontrol techniques (Jaiswal *et al.* 2022).

A new method for improving the absorption and effectiveness of bioactive extracts used in pharmaceuticals and agriculture is the genetic engineering of tobacco plants (Padhiary *et al.* 2024). Researchers can maximize tobacco's capacity to absorb, produce, and store advantageous substances like antibacterial agents or growth-promoting phytochemicals by altering important genes involved in metabolic pathways (Sun *et al.* 2024). For example, the potency of the plant extract may be increased by overexpressing transporter proteins or enzymes that promote the accumulation of particular secondary metabolites (Shitan 2016). Furthermore, CRISPR-Cas9 gene editing may be used to inhibit opposing pathways, focusing greater resources on the synthesis of the targeted chemical (Jiang *et al.* 2021). These genetically modified tobacco extracts may allow for more effective and prolonged delivery of biocontrol chemicals when paired with nano-encapsulation, further enhancing crop protection and production (Chadha 2020). There is a lot of potential for creating next-generation bio pesticides and plant-based medicines with this creative combination of genetic engineering and nanotechnology. We have compiled examples from contemporary literature about the nano-encapsulation of plant extracts for antifungal applications, highlighting nanocarrier type, particle size, loading/encapsulation efficiency, and asserted efficacy (Table 3).

**Table 3.** A comparative investigation of nanocarriers utilized for the transport of plant extracts in relation to fungal diseases.

Nano carrier type	Plant extract / compound	Particle size (nm)	Encapsulation / Loading efficiency (%)	Antifungal target / efficacy	Reference
Chitosan nanoparticles	Thyme essential oil	80–150 nm	65–85%	Suppressed growth of <i>F. oxysporum</i> and reduced mycelial biomass.	Salem <i>et al.</i> 2020
Liposomes	Clove oil	100–200 nm	70–90%	Enhanced stability, prolonged release, and robust activity against <i>Candida albicans</i> .	Fathi <i>et al.</i> 2021
Solid lipid nanoparticles (SLNs)	Curcumin extract	120–180 nm	75–92%	Inhibited the germination of <i>Aspergillus flavus</i> spores, hence reducing aflatoxin synthesis.	Ghosh <i>et al.</i> 2021
Polymeric nanoparticles (PLGA)	Garlic extract (allicin)	150–250 nm	60–80%	More efficacious against <i>Botrytis cinerea</i> than the free extract	Li <i>et al.</i> 2022
Nanoemulsions	Oregano essential oil	50–120 nm	80–95%	acts against <i>Alternaria alternata</i> and improves absorption and penetration throughout the body.	Ahmad <i>et al.</i> 2022
Silica nanoparticles	Eucalyptus oil	90–200 nm	70–85%	Reduced <i>Rhizoctonia solani</i> infection in tomato seedlings	Mahmoud <i>et al.</i> 2023
Zeolite-based carriers	Cinnamon oil	100–250 nm	65–78%	Prolonged release improved antifungal effectiveness against <i>Penicillium expansum</i> .	Hassan <i>et al.</i> 2023

#### 4.3. CRISPR-Cas9 applications in enhancing plant extract efficacy

CRISPR-Cas9 is an excellent method for enhancing the efficacy of plant extracts through precise genomic modifications. It can enhance crops, increasing their resilience to stress and illnesses. (Gan & Ling 2022). By targeting critical biosynthetic genes, it can enhance the production of secondary metabolites in medicinal plants. This is typically executed in conjunction with multi-omic approaches to achieve optimal quantity and quality of metabolites (Jeyaraj *et al.* 2024). CRISPR-Cas9 exhibits superior accuracy and efficiency compared to TALENs and ZFNs. This enables the enhancement of beneficial molecules while reducing undesirable metabolites (Angon and Habiba 2022). Moreover, altering stress-response and immune genes improves plant resilience to biotic and abiotic stressors, ensures consistent extract production, and fosters sustainable agriculture (Bhattacharjee *et al.* 2022). Innovative delivery technologies, including virus-based systems and nanoparticles, facilitate the modification of plant genes to enhance their resistance to diseases (Gan & Ling 2022; Zhou *et al.* 2023).

CRISPR-Cas9 possesses significant potential; yet, it is also associated with challenges like as off-target mutations, delivery obstacles, regulatory concerns, and ethical dilemmas (Mohamed *et al.* 2024). Researchers are developing methods to enhance the selectivity and efficiency of guide RNAs, ribonucleoprotein complexes, novel promoters, and transformation protocols [Bortesi & Fischer 2015]. Research indicates that crops can endure higher salinity, modify their lignin and pectin synthesis, and enhance disease resistance (Ly *et al.* 2024). The integration of CRISPR-Cas9 with sustainable methodologies presents a promising approach to enhance the efficacy of plant extracts, elevate crop quality, and bolster food security (Borrelli *et al.* 2018).

#### 4.4. Regulatory and Commercialization Hurdles

Significant regulatory obstacles remain in the way of the commercialization of botanical pesticides, such as genetically modified plant extracts and nano-encapsulated biocontrol agents, especially the requirement for approvals from organizations like the Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (EPA) (Waidyanatha *et al.* 2024). These products must pass extensive safety, efficacy, and environmental impact evaluations because they are made from natural sources but may also contain innovative delivery systems or genetic alterations (Aware *et al.* 2022). Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the EPA controls pesticides and requires comprehensive information on toxicity, impacts on non-target organisms, and residual levels (Dietz-Pfeilstetter *et al.* 2021). In the meanwhile, the FDA may impose further measures to guarantee the safety of humans and animals if the product has pharmaceutical applications (Dietz-Pfeilstetter *et al.* 2021). Small and medium-sized businesses frequently face difficulties due to the drawn-out and expensive approval procedure.

Furthermore, different international legislation and public opinion make market entry much more difficult. Stakeholders must make significant investments in preclinical and clinical research, interact with regulatory agencies early on, and guarantee open information regarding the dangers and advantages of the product in order to get beyond these obstacles. Adoption of creative, sustainable pest management techniques may be accelerated by streamlining these procedures through legislative lobbying and standardized international standards. Farmers' awareness and adoption of nano-encapsulated agrochemicals and biocontrol agents continue to be major obstacles to their widespread commercialization, despite the potential advantages of these products (Vishnu *et al.* 2024). Due to a lack of knowledge about these cutting-edge technology, many farmers especially those in developing nations are skeptical or reluctant to abandon traditional methods (Kuhl 2020). Misconceptions regarding safety, cost-effectiveness, and application techniques may also arise due to the intricacy of nanotechnology and genetically modified solutions (Saleh & Hassan 2023).

Furthermore, small-scale farmers frequently have limited resources, which makes it challenging for them to invest in more expensive nano-formulated products in the absence of convincing proof of long-term advantages (Yadav *et al.* 2023). In order to overcome these obstacles, focused education and extension initiatives are required to highlight the benefits of slow-release nano-encapsulation, including decreased labor costs, increased crop yields, and less chemical usage. Governments, agribusinesses, and research organizations working together can help to further support adoption through pilot programs, farmer training, and subsidies. The full potential of these cutting-edge technologies may go untapped in actual agricultural systems in the absence of efficient outreach and financial incentives.

### 5. Conclusion and Recommendations

In tobacco, plant-derived extracts have shown great promise in the fight against soil-borne diseases such as *Fusarium oxysporum* (Fusarium wilt) and *Phytophthora nicotianae* (Black Shank), providing a sustainable substitute for synthetic fungicides. According to research, bioactive substances found in neem, garlic, turmeric, and other therapeutic plants have immune-stimulating and antifungal qualities that lower pathogen viability and increase plant resilience. Their broad usage is hampered by issues such uneven efficacy, deterioration in field settings, and low farmer uptake.

#### 5.1. Recommendations for Improved Implementation

- To improve stability, gradual release, and targeted distribution, research should concentrate on standardizing plant extract quantities and creating formulations that are nanoencapsulated.
- Integration with Biocontrol Agents: Through synergistic effects, combining plant extracts with

helpful microorganisms (*Trichoderma*, *pseudomonas*) may enhance disease suppression.

- Genetic enhancement of tobacco, CRISPR-based breeding, or transgenic methods may be investigated to create tobacco cultivars that demonstrate increased sensitivity to treatments with plant extracts.
- Support for Farmer Education and Policy, to boost adoption among smallholder farmers, governments and agricultural organizations should raise awareness through field experiments, subsidies, and training initiatives.
- Future research should focus on developing standardized extraction and preparation methods to ensure the consistency, reproducibility, and comparability of studies evaluating the efficacy of plant extracts.
- To guarantee adherence to organic agricultural laws, more research on residual effects and environmental safety is required.

#### Credit authorship contribution statement

**R A and J S:** Writing—original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **BI and A. A. K:** Writing—review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization, Data curation. **S A and LL:** Writing review & editing, funding acquisition, Formal analysis, Conceptualization, Data curation. **S.M.K and IH** Writing—review & editing, Software, Methodology, Data curation, Formal analysis, Validation.

#### Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Conflict of Interest

The authors declare no conflicts of interest.

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