

Cite this: *Sustainable Food Technol.*,  
2025, 3, 612

# Recent strategies for controlling the white mould fungal pathogen (*Sclerotinia sclerotiorum*) using gene silencing, botanical fungicides and nanomaterials

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*Sclerotinia sclerotiorum* is a fungal pathogen that causes white mould diseases in several plants of economic importance. In the form of sclerotia, the pathogen is soilborne and can survive for a long period of time. White mould is considered yield burdening to soybean and poses a threat to over 600 host plant species; hence, there is an increasing effort to manage it by using various strategies. One of the common methods of controlling the pathogen is the use of chemical fungicides; however, concerns remain regarding environmental and human safety. Additionally, the pathogen has demonstrated potential to develop resistance to chemical fungicides and some of these chemical fungicides are being removed from the market due to threat to the environment, and the use of environmentally friendly biofungicides and gene silencing are examined. Another recent strategy that has been adopted for controlling the pathogen is the use of nanomaterials. Hence, the use of metallic nanoparticles, metal oxide nanoparticles, carbon-based materials, and their composites for combating the *Sclerotinia sclerotiorum* fungal pathogen are comprehensively reviewed. Finally, future perspectives on the control of the fungal pathogen are suggested.

Received 19th November 2024  
Accepted 12th February 2025

DOI: 10.1039/d4fb00348a

rsc.li/susfoodtech

## Sustainability spotlight

White mould is considered yield burdening to soybean and poses a threat to over 600 host plant species; hence, there is an increasing effort to manage it by using various strategies. One of the common methods of controlling the pathogen is the use of chemical fungicides; however, concerns remain regarding environmental and human safety. Additionally, the pathogen has demonstrated potential to develop resistance to chemical fungicides and some of these chemical fungicides are being removed from the market due to threat to the environment. Without controlling this pathogen, the sustainable development goal of the United Nations (Goal 2) would not be achieved. Therefore, the current research is important for scientists to know the way forward on the control of this destructive pathogen.

## 1 Introduction

*Sclerotinia sclerotiorum* (Lib.) de Bary is a fungal pathogen responsible for white mould that affects plants such as canola, brassica oilseed<sup>1,2</sup> soybean,<sup>3</sup> sunflower,<sup>4</sup> mustard,<sup>5</sup> tomato<sup>6</sup> and other plants (over 600 plant species in total).<sup>2</sup> It is a devastating disease of plants, and its pathogen is not only ubiquitous but also has potential to feed as a hemi-biotroph on these plants.<sup>2,7</sup> In lettuce, it impacts the anthocyanins, flavonoids and phenolic contents leading to reduction in the yield of the plant.<sup>8</sup> Similarly, a significant decrease in the biochemical activities, yield of oil, growth, dry weight and fresh weight of *Mentha arvensis* infected by this fungal pathogen has been reported.<sup>9</sup> The reduction in these parameters was found to increase when the initial inoculum levels of *S. sclerotiorum* were increased. This

showed that the pathogen is a threat to food security and must be controlled.

Apart from white mould, different names are associated with diseases caused by this pathogen, *i.e.*, blossom blight, crown rot, watery soft rot, cottony rot and *Sclerotinia* stem rot.<sup>10</sup> The management of *Sclerotinia sclerotiorum* is challenging due to the fact that it affects such a wide range of host plants coupled with long-term survivability and genetic variability.<sup>11</sup> Additionally, the pathogen exhibits two infection mechanisms. It can damage the basal stem through soil-borne mycelia *via* a myceliogenic germination mechanism. It can also infect the upper part of the plant, *i.e.*, stems, leaves and reproductive organs through ascospore dispersal, resulting from carpogenic germination (Fig. 1).<sup>2</sup> Taxonomically, it belongs to the kingdom, phylum, class, order, family and genus of Fungi, Ascomycota, Discomycetes, Helotiales, Sclerotiniaceae, and *Sclerotinia* respectively.<sup>13</sup> Its presence in the plant cultivars is often detected *via* the use of techniques such as polymerase chain reaction

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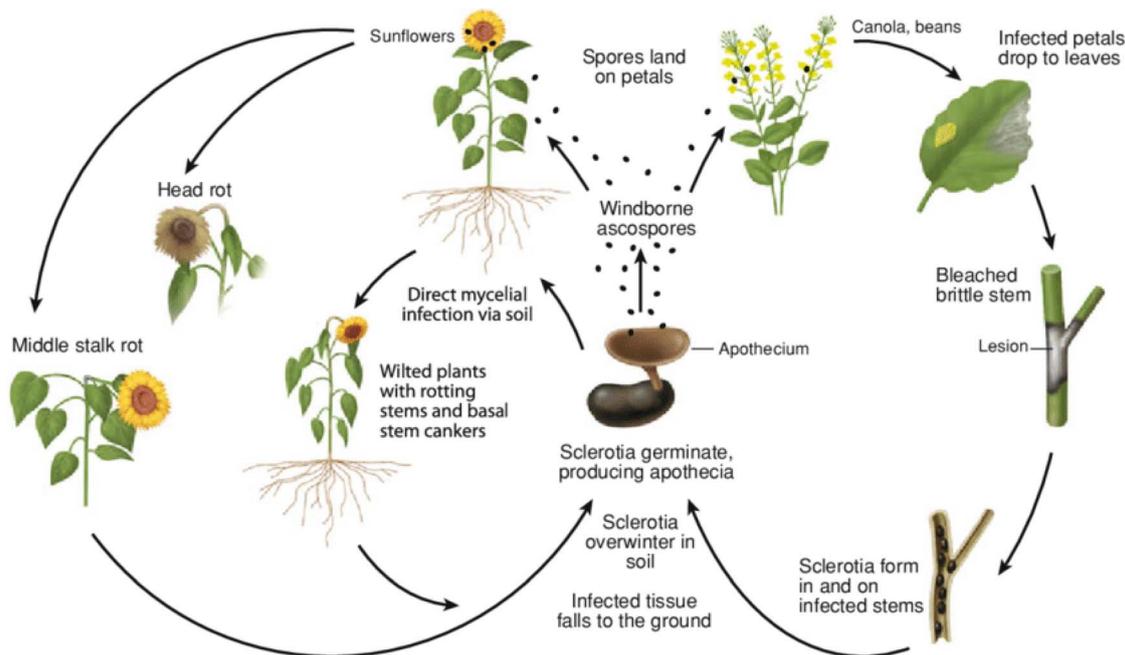


Fig. 1 The disease life cycle of the white mould pathogen (*Sclerotinia sclerotiorum*) on canola and sunflower. Reproduced with permission from ref. 12. Copyright (2014), Springer Nature, License number: 5964070527457.

(PCR)-based techniques,<sup>14</sup> immunochemical detection methods<sup>15</sup> and leaf-reflectance spectroscopy.<sup>16</sup>

The use of fungicides to manage *Sclerotinia* not only aims to protect current yields by mitigating disease establishment and severity but also seeks to safeguard future yields by reducing the accumulation of sclerotia in the soil, thereby preventing the initiation of new infection cycles. However, heavy reliance on expensive commercial fungicides, along with repeated, poorly timed, and sometimes indiscriminate use, has resulted in the pathogen developing an insensitivity against the active ingredient/s, leading to a decreased efficacy of the fungicide in controlling the pathogen. The risk of *Sclerotinia sclerotiorum* continuing to develop resistance to common fungicides, for example benomyl, carbendazim,<sup>17–19</sup> procymidone, fluazinam, and pyraclostrobin, has prompted researchers to explore alternative management strategies against white mould. Besides, most of these fungicides are being removed from the market as they have been found to be highly carcinogenic by inhibiting thyroid peroxidase in animals and some of them release the ethylene thiourea metabolite.<sup>20,21</sup> Another strategy that has been used is the use of cultural practices such as crop rotation, tillage, canopy management, controlled seedling rate, widening row spacing during planting, weed control, changing planting dates, reducing nitrogen-rich fertilizers, planting small grains along with cover crops, avoiding excessive irrigation and the use of resistance varieties. However, all these have been found to reduce the overall yield of the plants.<sup>22</sup> The limitations of some of the other strategies that have been adopted are shown in Table 1.

There are over 2567 research articles on the general investigations involving *Sclerotinia sclerotiorum* between the years

2013–2023. An upward trend in the research was observed from 2017 to 2022 with 325 papers published in the year 2022 alone, indicating increasing interest in the research on *Sclerotinia sclerotiorum*. Most of the control strategies within these periods are the use of antagonistic fungi (such as *Clonostachys rosea*,<sup>30</sup> *Aspergillus flavus* strain NJC04,<sup>31</sup> *Aspergillus pseudoelegans*, *Aspergillus niger*, and *Aspergillus japonicus*<sup>32</sup>), the use of *Trichoderma* spp. (such as *T. afroharzianum* and *T. asperelloides*<sup>33</sup>), the use of mycoparasites such as *Coniothyrium minitans*,<sup>34</sup> and the use of antagonistic bacteria (such as *P. fluorescens*, *P. commune* and *T. asperellum*<sup>35</sup>). Other strategies that have been adopted are the use of fungicides, gene silencing and nanomaterials. While the use of antagonistic organisms for controlling this pathogen has been reviewed,<sup>36–38</sup> the use of fungicides, gene silencing and nanomaterials has not been reviewed. To understand the work that has been done in this regard and how these strategies disrupt the lifecycle of the pathogen, a comprehensive report is required. Hence, the present review examines the recent developments in the alternative control of *S. sclerotiorum* to create a greater array of tools for producers to utilise for integrated pest management.

### 1.1 Life cycle, mechanism of action and morphology of *S. sclerotiorum*

*S. sclerotiorum* spends most of its lifetime in the soil where it exists in a sclerotium form (Fig. 1). Although sclerotia are the main structures indicating the end of the disease cycle, they are also the nutritional and structural bases for myceliogenic and apothecial germination for the start of a new disease cycle.<sup>12</sup> The size of sclerotia can range from a few millimetres to several centimetres, depending on the host plant, where larger sclerotia



Table 1 Advantages and disadvantages of some fungal pathogen control methods

Control method	Descriptions	Advantages	Disadvantages	Ref.
Biofumigation	Adding fresh plant biomass to the soil that releases chemicals which destroy soil-borne pathogens	The properties of the soil are improved; the activities of beneficial microbes are enhanced	It is costly; the efficacy is time dependent since there is ascospore dispersal when applied to flowering plants at a particular stage of development; unreliable	1, 23 and 24
Organic amendment	The use of manure, municipal solid waste compost and biosolids to replace or supplement mineral fertilizers	Enhancement of soil fertility and yields; reduces the cost of the fertilizers	Safety concerns such as accumulation of metals in the soil and groundwater contamination with organic compounds; health impacts from viruses and other pathogens	21 and 25
Crop monitoring <i>via</i> unmanned aerial systems	The use of remote sensing technology such as unmanned aerial vehicles, manned aircraft and satellites to study diseases and pests	More efficient than ground-based surveys; high mobility with near real-time application	Low endurance time (less than 1 h); air safety concern; involves the use of professional software for complex data analysis	26 and 27
Biological fungicides	Spraying bio-fungicides on the growing plants, addition to the soil or treatment of plant materials	Minimal toxicity; ecologically pure	Reapplication is required to obtain high efficiency; they are not tolerant to temperature regimes; their response to phytopathogenic organisms is not rapid	28
Use of ultraviolet/gamma radiation	UV-B, gamma and UV-C are used to kill pathogens in plants	Uniformity of the dose allowing products with different shapes and sizes to be treated; high penetrability	Cannot treat radiation microbial population; mutation of the microbes to a more/less pathogenicity from parent organisms	29

have been associated with greater numbers of apothecia.<sup>39–41</sup> Ascospores are forcibly ejected into the air from apothecia and are the primary inoculum which initiates disease. The secretion of oxalic acid by *S. sclerotiorum* at infection sites, *i.e.*, canola petals or heads of sunflower, initiates the breakdown of plant tissue, reducing the pH and triggering the production of polygalacturonase, an enzyme responsible for the depolymerization of pectates in plant cell walls.<sup>42,43</sup> This biochemical reaction leads to the disease symptoms of water-soaked lesions and ultimately the formation of mycelia and sclerotia. Without the secretion of oxalic acid, programmed cell death by oxalate oxidase cannot occur, preventing the formation of sclerotia.<sup>44,45</sup> The entire disease life cycle of the white mould fungus on canola and sunflower is shown in Fig. 1. There is a positive correlation between flowering and susceptibility to the pathogen, as petals are infection courts for ascospores, and fungicide applications need to target this tissue prior to ascospore deposition.

Infection of the stems results in greater risk of yield loss as lesions impair the plant's ability to transport water and nutrients through the xylem and phloem, and in cases of severe infection stems may break and result in no to little yield.<sup>46</sup> The sign of the disease is the formation of white fluffy mycelia which melanise and form sclerotia as the disease progresses. The sclerotium is an irregular to round-shaped structure (Fig. 2a) that becomes encased in hyphae within a week. Subsequently,

the apex of the inner hyphae swells and extends towards the surface to form a melanised rind<sup>47</sup> (Fig. 2b–f). Other fungi such as basidiomycetous *Athelia rolfsii* have spherical sclerotia. However, the most common shape for the sclerotia of white mold is an irregular shape.

In the form of sclerotia *S. sclerotiorum* can remain dormant in the soil for 10 years until conducive weather events permit either carpogenic or myceliogenic germination.<sup>48</sup> The ability of sclerotia to withstand adverse environmental conditions in the soil and in the absence of a host plant is associated with the melanised rind (outermost layer of the cortex) unlike the inner region of the cortex which is hyalinized.<sup>49</sup> The melanin produced by *S. sclerotiorum* has been confirmed to be dihydroxynaphthalene which is an arene stabilized by resonance.<sup>49</sup> The presence of melanin in sclerotia enhances their survival in soil by providing protection against UV radiation and microbial degradation. This resilience allows sclerotia to successfully germinate and produce ascospores, making timely fungicide applications essential for effective disease management as these ascospores are released from the apothecia.

## 2 Alternative strategies to control *Sclerotinia sclerotiorum*

Current methods of managing *S. sclerotiorum* focus on limiting infections through (1) reducing sclerotial viability and



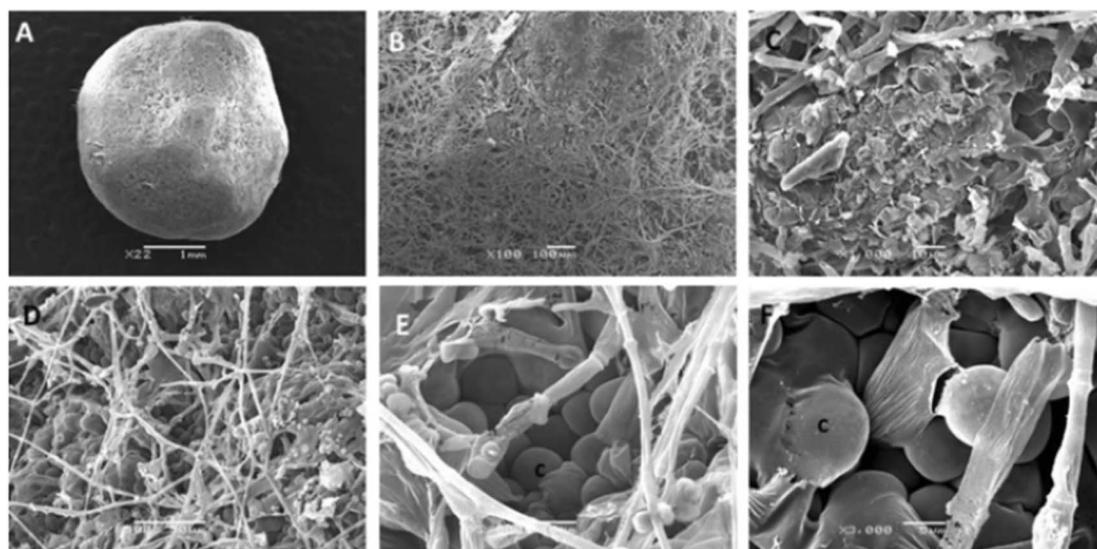


Fig. 2 Scanning electron images of the sclerotium after 8 days in the maturation stage. (a) The round structure of the mycelium; (b) sclerotium covered by the mycelium; (c) layer of membrane developing on the surface of the sclerotium. (d) Hyphae projecting into the surface (e and f) swelling cells on the tips (c) of hyphae. Reproduced with permission from ref. 47. Copyright (2014), Springer Nature–License number: 5964071117957.

germination ability, (2) inhibiting ascospore germination and (3) enhancing the plant's ability to withstand penetration mitigating or restricting disease establishment. However, the need to combat *S. sclerotiorum* requires novel approaches which disrupt the disease cycle unconventionally, *i.e.*, targeting sclerotial formation.

### 2.1 Use of a gene silencing target to control *Sclerotinia sclerotiorum*

Host-induced gene silencing has been adopted for controlling the fungal pathogen. This approach depends on the potential of eukaryotes to silence the transcription of genes through a transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS) process.<sup>50</sup> TGS involves promoter methylation leading to a decrease in the synthesis of RNA while PTGS involves degradation of RNA for a specific sequence.<sup>51</sup> Irrespective of the process involved, the response of the plants to the external stimuli and their nature are altered by knocking down the gene expression. To use this strategy in controlling pathogens, it requires identifying and silencing the genes in the pathogens that are responsible for pathogenicity, development and growth.<sup>52</sup> By following this, Andrade *et al.*<sup>53</sup> silenced the gene that was responsible for the synthesis of chitin in the white mould pathogen. The specific selection of the gene was carried out in such a way that specific phytopathogens were efficiently targeted without any impact on other organisms in multiple cultures. Generally, some of the effectors that have been identified in *S. sclerotiorum* for silencing are *Ss-caF1* which is a putative Ca<sup>+</sup> binding protein; *SsiTL* which is an integrin; *SspG1d* which is an endopolygalacturonase that targets a protein involved in calcium signalling known as iPG-1 and *SsSSVP1*, that targets QCR8 in the cytochrome of the mitochondria.<sup>50</sup>

In an investigation, a forward genetic approach was used to identify a protein that plays a critical role in the formation of sclerotia. The protein is *SsGAP1* and it is responsible for activating RAS-GTPase. Considering the role played by the RAS signalling component in the formation of sclerotia, it has been used for host-induced gene silencing for reducing the virulence of white mould diseases in *Arabidopsis thaliana* and *Nicotiana benthamiana*.<sup>54,55</sup> Knocking down *SsOah1* (SS1G\_08218) and *SsCyp51* (SS1G\_04805) genes of *S. sclerotiorum* using spray-induced gene silencing led to slowing down of the disease progression within two hosts (*B. juncea* and *N. benthamiana*). Lesion development reduction and disease initiation delay were also observed. Depending on the host, the growth and morphology of the host have been altered when this gene was deleted.<sup>56</sup> Gene deletion can also be used to identify the exact gene that contributes to the parasitic nature of a particular strain against the white mold pathogen. For instance, Lv *et al.* identified S8 serine protease-encoding gene *CrKP43* which has been important for the parasitic nature of *Clonostachys chloroleuca* against *Sclerotinia sclerotiorum*. With the deletion of this gene, inhibition of conidiation and deformation of the fungal morphology and cell wall were observed. Their findings further revealed that deletion of *CrKP43* weakens the antagonistic mycoparasite ability of *Fusarium oxysporum* against *Sclerotinia sclerotiorum*. Other genes that have been deleted and found to be suitable for the control of *Sclerotinia sclerotiorum* through host-induced gene silencing are shown in Table 2. Despite the prospect of this technique, Nunes and Deans<sup>62</sup> reported some limitations for its feasibility and real-world application. One of the limitations is the challenge of identifying fungal targets. Apart from the points raised by these scientists, the technique is expensive and requires a deep understanding of sequencing data and protein–protein interactions.



Table 2 Control of *Sclerotinia sclerotiorum* using gene silencing

Gene silenced	Role of the gene	Method of investigation	Effect of the gene silenced	References
SsCak1(a putative protein kinase)	Involved in the growth and pathogenicity of <i>S. sclerotiorum</i>	Next-generation sequencing and forward genetic screen	Complete loss of virulence; defective sclerotia and mycelium development; penetration of the host; formation of appressoria	57
CrGlu6 (glucose-6-phosphate 1-epimerase) in <i>Clonostachys chloroleuca</i>	Generates energy for the growth of <i>Clonostachys chloroleuca</i> (mycoparasite of <i>S. sclerotiorum</i> )	Protein–protein interaction	Significant conidiation reduction; abnormal hyphal morphology; decreased control efficiency, mycoparasitic activity and antifungal activity against white mould disease	58
SsTOR	Target of rapamycin (TOR) regulates stress responses of eukaryotes and controls intracellular metabolism and cell growth	Use of pathogenicity assays; study of cell wall integrity; phosphorylation analysis	Retardation of the hyphal growth; disruption of compound appressoria and sclerotium formation	59
SsPDE2	Regulation of the accumulation of oxalic acid; virulence and infection cushion functionality; sclerotium formation	Next-generation sequencing; forward genetics approach	Sclerotium formation impacted	60
MAPK (mitogen-activated protein kinase cascade)	Controls hyphal fusion, compound appressoria formation, sclerotium development and mycelial growth	Forward genetic screen	Deformed appressoria; impaired apothecia formation; attenuated host penetration	61

## 2.2 The use of botanical fungicides to control *Sclerotinia sclerotiorum*

Safety and environmental friendliness are the two factors that triggered the investigation of natural products for controlling *Sclerotinia sclerotiorum*.<sup>63</sup> Different botanical fungicides (antifungal agents sourced from the extracts of plants) have been tested against *Sclerotinia sclerotiorum*. Some of them are vapours from peppermint oil and sweet basil oil. The two essential oils inhibited the growth of the fungus in a dose-dependent manner. This led to further investigations into the antifungal potential of the major constituents of these essential oils (eugenol and linalool in sweet basil oil; menthone and menthol in peppermint oil). It was however noted that while linalool alone displayed a moderate antifungal activity, eugenol alone did not show any antifungal activity at all. Surprisingly, the mixture of the two major constituents at a ratio equal to their natural existence in sweet basil oil showed a synergistic antifungal property. Similarly, there was synergistic antifungal activity when a menthone and menthol mixture was used whereas menthone did not show any antifungal activity and menthol displayed dose-dependent antifungal activity.<sup>64</sup>

An antimycotic natural ingredient that is commonly found in soil, known as natamycin, has also been investigated for controlling *Sclerotinia sclerotiorum*. Natamycin showed an inhibitory effect on the growth of the mycelia of the pathogen with the half-maximal effective concentration ( $EC_{50}$ ) ranging between 0.53 and 4.04  $\mu\text{g mL}^{-1}$ . The mechanism of action of natamycin was found to involve an increase in

malondialdehyde content and reactive oxygen species (ROS). Also, there was a reduction in the ergosterol and oxalic acid content. In the structural alteration, the integrity of the cell membrane, formation of sclerotia and formation of hyphae were significantly impacted.<sup>65</sup>

Hinokitiol is a natural monoterpene that is present in the wood of the Cupressaceae family and has also been used as an antifungal agent against *Sclerotinia sclerotiorum*. Through its antifungal properties, the shelf-life of carrot was increased. This is due to enhancement of antioxidant activities, prevention of the formation of malondialdehyde, and reduction of total phenolic, carotenoid and ascorbic acid contents. The formation of sclerotia was also prevented by interfering with the cell membrane formation and altering the gene expression of the enzymes responsible for sclerotium formation. The synthesis of exopolysaccharides and oxalic acid was also suppressed leading to a reduction of its pathogenicity.<sup>63</sup>

5-Hydroxy-2-hydroxymethyl- $\gamma$ -pyrone also known as kojic acid is a metabolite derived from several strains of fungi. This natural bioactive compound has been used to protect the skin from UV radiation and make the skin lighter in the cosmetic industry. It has also been used as a food additive due to its non-toxic nature and environmental friendliness.<sup>66</sup> It has earlier been established that kojic acid inhibits the biosynthesis of melanin in the cells of humans.<sup>67</sup> This prompted its investigation for controlling *S. sclerotiorum*. In an investigation,<sup>68</sup> kojic acid displayed good antifungal activities against *S. sclerotiorum* by inhibiting the synthesis of melanin and chitin. Its antifungal activities were not affected by the change in pH but the presence



Table 3 Examples of natural antifungal agents that have been used to control the white mould pathogen

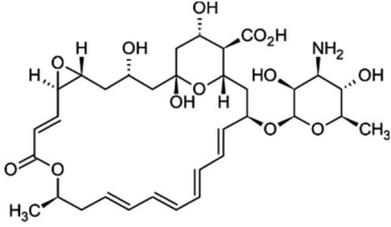
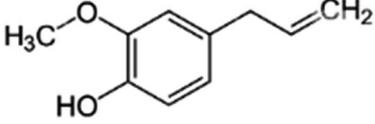
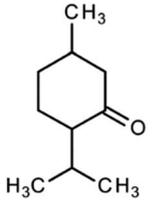
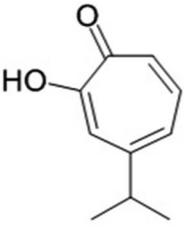
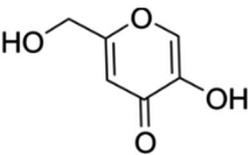
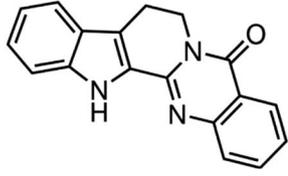
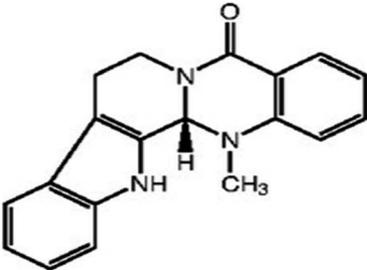
Botanical fungicides	Source	Molecular structure	Molecular weight (g mol <sup>-1</sup> )/formula
Natamycin	Bacterium <i>Streptomyces</i>		665.7/C <sub>33</sub> H <sub>47</sub> O <sub>13</sub> N
Eugenol	Sweet basil oil		164.2/C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
Menthone (and menthol)	Peppermint oil		154.25/C <sub>10</sub> H <sub>18</sub> O
Hinokitiol	Wood of the Cupressaceae family		164.20/C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
Kojic acid	Fungi ( <i>Aspergillus oryzae</i> )		142.11/C <sub>6</sub> H <sub>6</sub> O <sub>4</sub>
Rutaecarpine	'Wu-Chu-Yu'		287.30/C <sub>18</sub> H <sub>13</sub> N <sub>3</sub> O
Evodiamine	<i>Tetradium</i> plant		303.30/C <sub>19</sub> H <sub>17</sub> N <sub>3</sub> O



Table 3 (Contd.)

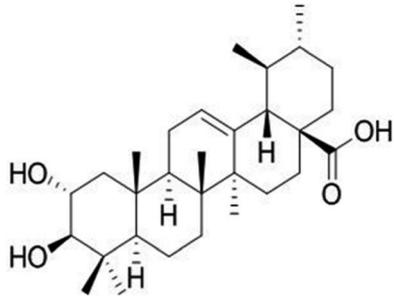
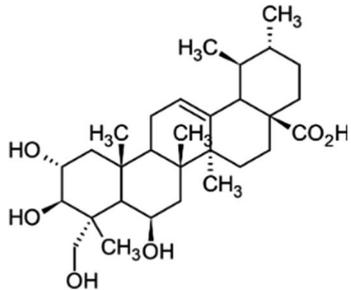
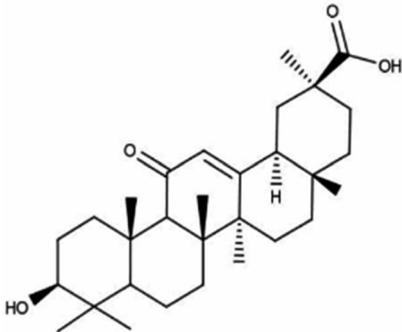
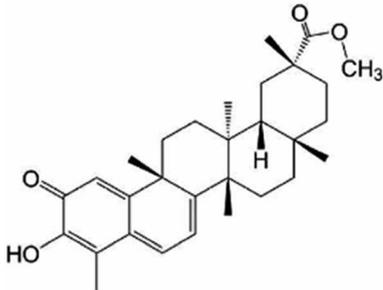
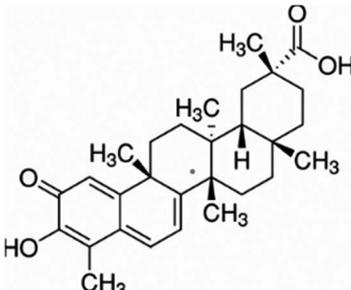
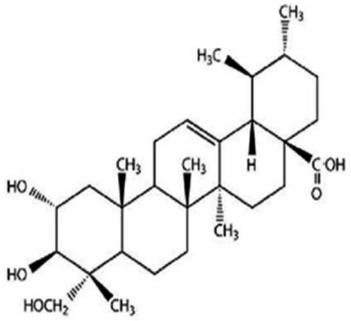
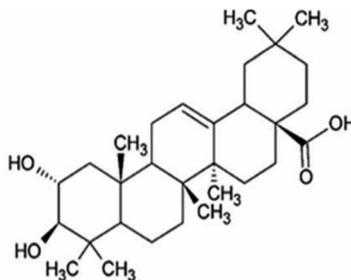
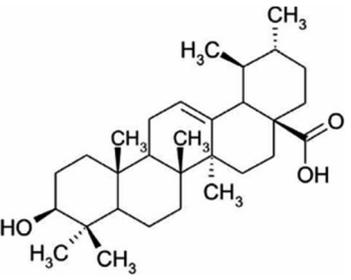
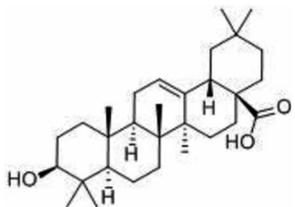
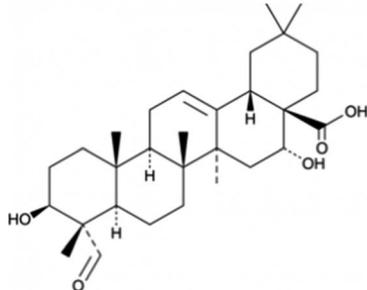
Botanical fungicides	Source	Molecular structure	Molecular weight (g mol <sup>-1</sup> )/formula
Corosolic acid	<i>Lagerstroemia speciosa</i> plant		472.70/C <sub>30</sub> H <sub>48</sub> O <sub>4</sub>
Madecassic acid	<i>Centella asiatica</i>		504.70/C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>
Glycyrrhetic acid	Root of licorice ( <i>Glycyrrhiza glabra</i> )		470.69/C <sub>30</sub> H <sub>46</sub> O <sub>4</sub>
Pristimerin	Plant families of Celastraceae and Hippocrateaceae		464.64/C <sub>30</sub> H <sub>40</sub> O <sub>4</sub>
Celastrol	Regel's threewingnut – <i>Tripterygium regelii</i> and thunder god vine – <i>Tripterygium wilfordii</i>		450.62/C <sub>29</sub> H <sub>38</sub> O <sub>4</sub>



Table 3 (Contd.)

Botanical fungicides	Source	Molecular structure	Molecular weight (g mol <sup>-1</sup> )/formula
Asiatic acid	<i>Centella asiatica</i>		488.70/C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>
Maslinic acid	<i>Salvia canariensis</i> and <i>Olea europaea</i>		472.71/C <sub>30</sub> H <sub>48</sub> O <sub>4</sub>
Ursolic acid	Thyme and rosemary		456.71/C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>
Oleanic acid	Olive – <i>Olea europaea</i> L		456.71/C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>
Quillaic acid	<i>Quillaja saponaria</i>		486.68/C <sub>30</sub> H <sub>46</sub> O <sub>5</sub>

of metal ions reduced its antifungal activities. This may be due to the formation of chelates with the metal ions. In soybean for instance, application of 7.1 mg mL<sup>-1</sup> of kojic acid completely

inhibited *S. sclerotiorum*. Apart from using kojic acid alone, its addition to commercial fungicides such as carbendazim and prochloraz boosted the antifungal activities of these fungicides.



Other natural compounds that have effectively controlled the white mould fungus are the hexane, butylalcohol, heptane and methanol extracts of different parts of *Dactyloctenium aegyptium*;<sup>69</sup> a natural bioactive compound known as kojic acid;<sup>70</sup> a series of new quinoline derivatives (cryptolepine alkaloid);<sup>71</sup> two alkaloids (rutaecarpine and evodiamine) of the isolates of *Evodia rutaecarpa* which is a traditional Chinese herbal medicine<sup>72</sup> and ten pentacyclic triterpenoids including corosolic acid, madecassic acid, glycyrrhetic acid, quillaic acid, oleanic acid, ursolic acid, maslinic acid, asiatic acid, pristimerin and celastrol.<sup>73</sup> The structure, molecular weight and sources of these natural acids are shown in Table 3. The use of these natural antifungal agents caused disruption in the enzyme activities, initiated the accumulation of reactive oxygen species leading to mycelial abnormalities in the pathogen, distortion of its mitochondria and/or inhibition of either germination or formation of sclerotia. In short, they alter the functions of the mitochondria, cell walls, cell membrane, DNA and RNA. The mechanisms of action of some of these natural antifungal agents are shown in Fig. 3.

One of several advantages botanical fungicides have over synthetic fungicides is environmental friendliness. Unlike the synthetic fungicide that could persist in the environment leading to environmental pollution, botanical fungicides are biodegradable since they are products from nature.<sup>75</sup> In addition, they are selective and effective and can impact multiple mechanisms of action since plant extracts contain multiple bioactive components that can act simultaneously. They are not

expensive unlike synthetic fungicides, and they are readily available. The fact that they contain an array of chemical families diversifies the molecular and biochemical targets towards fungi. Therefore, there is a delay in the resistance phenomenon.<sup>75,76</sup>

### 2.3 Use of nanomaterials to control *Sclerotinia sclerotiorum*

Nanotechnology is a field that involves the synthesis of nanoparticles and utilising nanoparticles to create new materials. Materials are manipulated at the molecular and atomic levels and extremely small sized nanoparticles are employed in applications such as environmental remediation, catalysis, engineering, medicine, and agriculture.<sup>77</sup> Nanoparticles have also been found to be effective in fighting the pathogens of crops by releasing metal ions, photocatalysis, interference with RNA synthesis, inhibition of DNA replication, inhibition of enzyme activities, disruption of energy transduction, generation of reactive oxygen species and/or disruption of the cell wall/cell membrane.<sup>78,79</sup> Their action also depends on their size, shape and concentration when applied.<sup>78</sup> As also pointed out by Ray *et al.*<sup>80</sup> the mechanism of action of nanoparticles against pathogens can be broadly classified into five. The first is protein oxidation, membrane oxidation and energy production leading to enhanced and distinctive antibiotic activity. The second is the release of toxic ions by the nanoparticles which leads to genotoxicity and cell death in the pathogen. The third is the induction of oxidative and cellular stress on cell pathogens

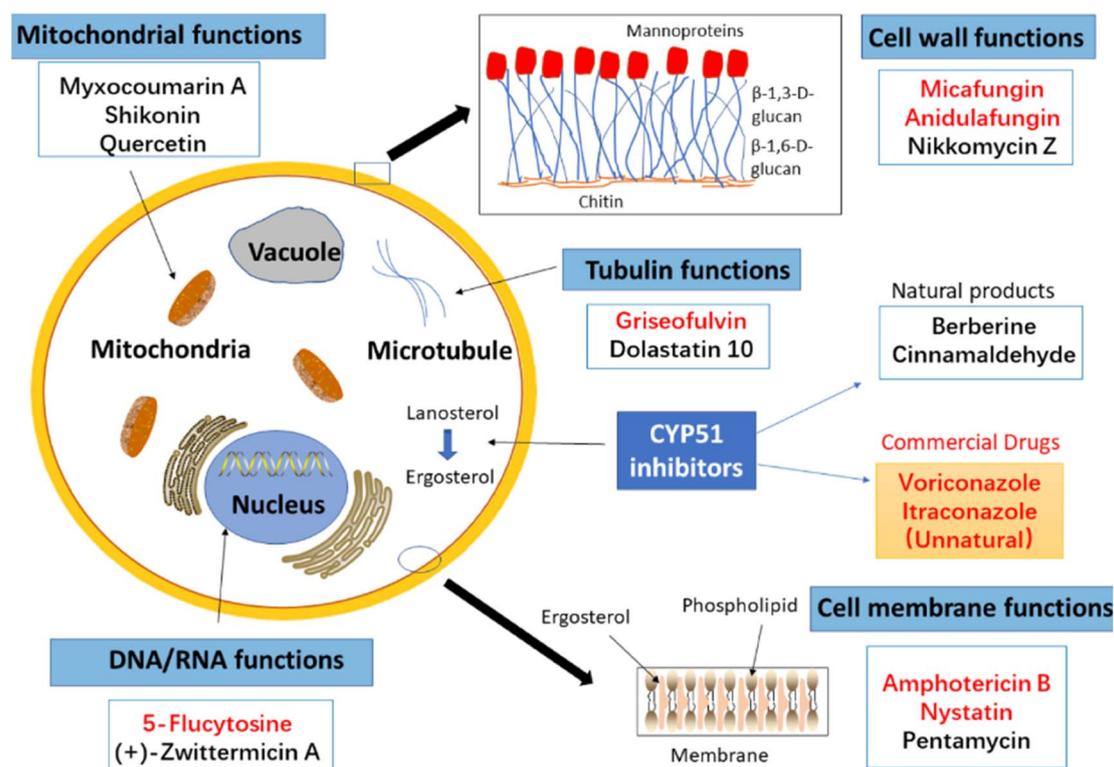


Fig. 3 Some antifungal agents from natural sources with some fungal cells that they target. Reproduced from ref. 74. Elsevier (Open Access under Creative Commons Agreement).



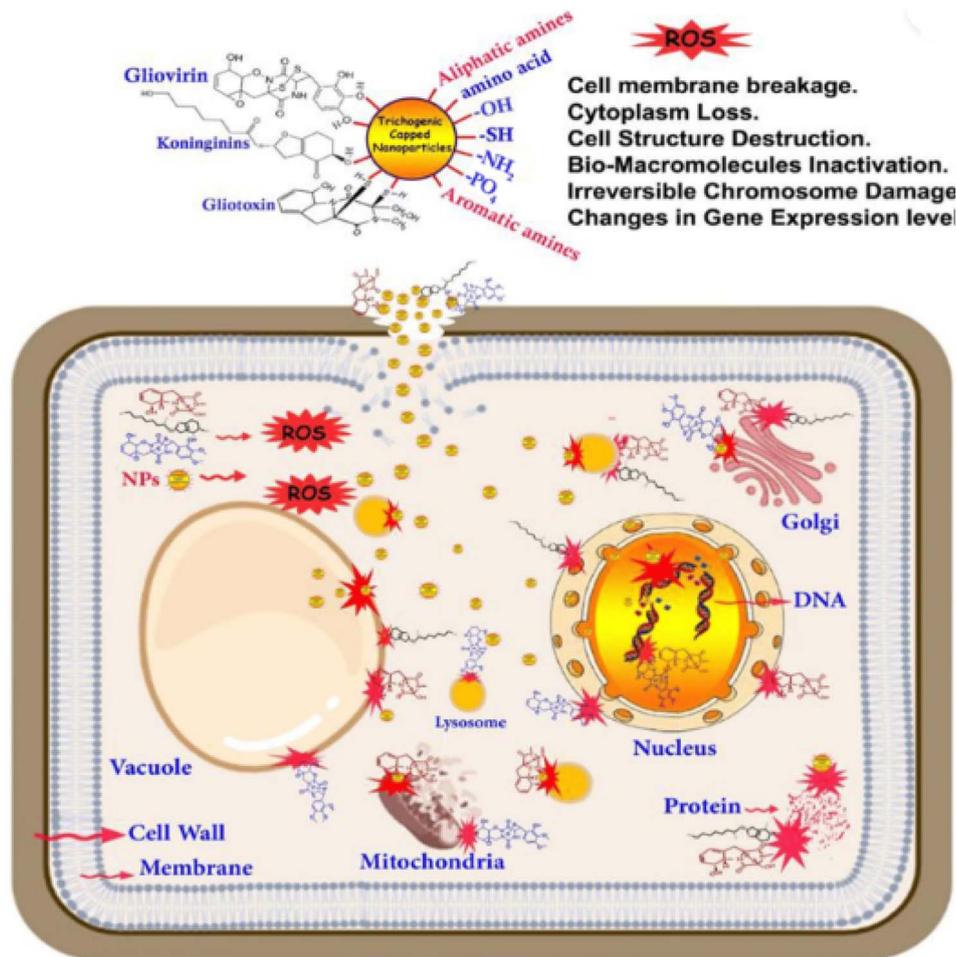


Fig. 4 Schematic illustration of the mechanism of antifungal activity of nanoparticles. Reproduced from ref. 81. MDPI Open Access.

through the reactive oxygen species released by the nanoparticles (Fig. 4). The fourth is the damage caused by the nanoparticles on the nutrient absorption and membrane transporter system. The last is the alteration of the activity and permeability of the membrane proteins initiated by the toxic ions released from the nanoparticles.

In this section, the nanomaterials that have been used as antifungal agents against *Sclerotinia sclerotiorum* and discussed are shown in Fig. 5.

**2.3.1 Use of silver nanoparticles to control *Sclerotinia sclerotiorum*.** One of the common metallic nanoparticles that has been used as antifungal agents against *Sclerotinia sclerotiorum* is silver. Researchers have used diverse methods to synthesize silver nanoparticles that were used for this purpose. In one investigation, *Trichoderma harzianum* filtrate was used as an eco-friendly means of producing spherical shaped silver nanoparticles having an average size of 31.13 nm. The 100  $\mu\text{g mL}^{-1}$  assay of the synthesized silver nanoparticles was found to inhibit the formation of sclerotia not only for *Sclerotinia sclerotiorum* but also for *Sclerotium rolfisii* with a maximum inhibitory efficiency of 96.4%.<sup>82</sup> This finding confirmed the previous report that the silver nanoparticles made from *Trichoderma*

*harzianum* extract inhibited mycelial growth and sclerotium germination.<sup>83</sup>

Another study examined the effect of capping on the *Trichoderma harzianum*-derived silver nanoparticles against *Sclerotinia sclerotiorum*. It was observed that the capped silver nanoparticles contained chitinase and acid protease, the hydrolytic enzymes NAGase,  $\beta$ -1,3-glucanase, protein bands and functional groups of biomolecules from *Trichoderma harzianum*. The uncapped nanoparticles were not effective against the pathogen, but the capped silver nanoparticles were effective against *Sclerotinia sclerotiorum* which showed that capping played a significant role in the activities of silver nanoparticles, though there was no significant difference in the cytotoxicity activity of the capped and uncapped silver nanoparticles.<sup>84</sup>

*Fusarium culmorum* strain JTW1 has also been used as a source of stable, highly crystalline, spherical, and approximately 16 nm silver nanoparticles. Apart from the fact that the size of the silver nanoparticle produced *via* this method is smaller than the size obtained when *Trichoderma harzianum* filtrate was used, with 32  $\mu\text{g mL}^{-1}$ , there were zones of growth inhibition of 3.41 mm.<sup>85</sup> Gupta and Saxena similarly used enzyme amylase that is present in *Aspergillus oryzae* MTCC No.



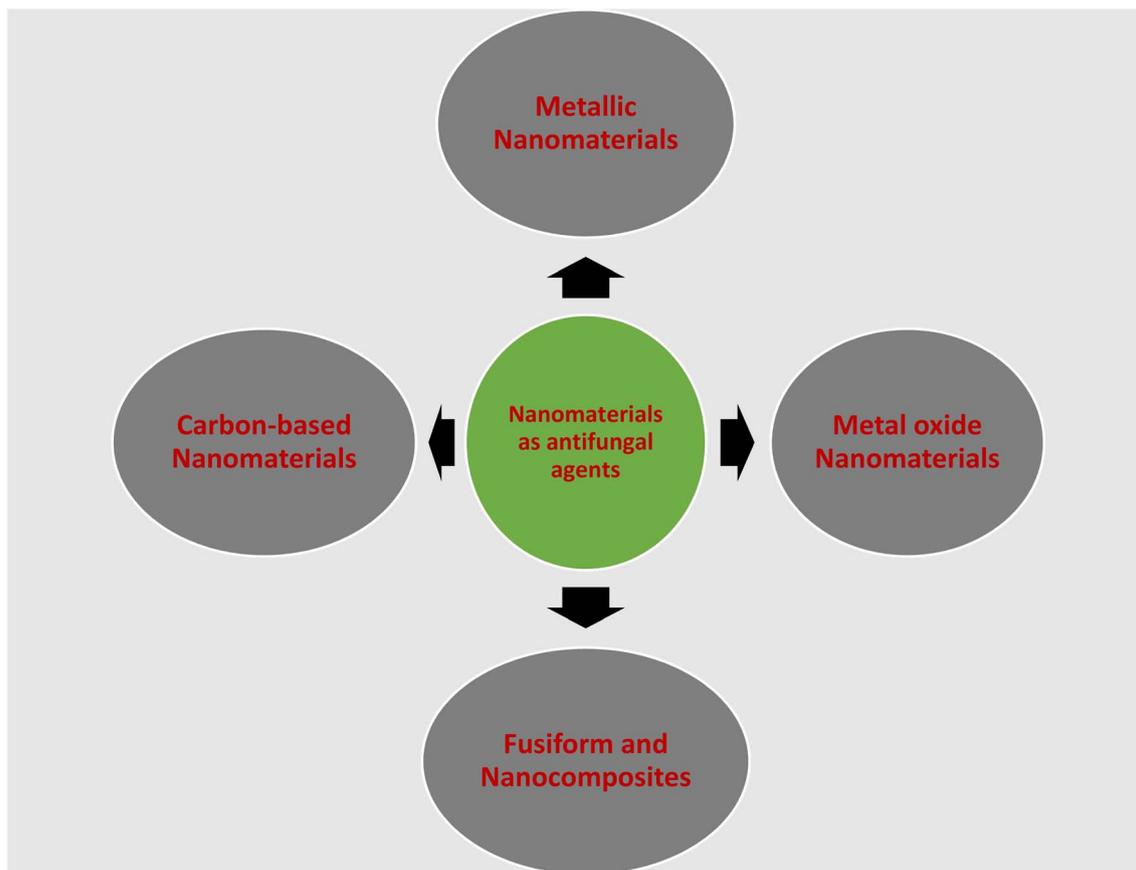


Fig. 5 Nanomaterials that have been used as antifungal agents against *Sclerotinia sclerotiorum*.

3107 for reducing silver salt to silver nanoparticles. There was complete inhibition of *Sclerotinia sclerotiorum* when a  $100 \mu\text{g mL}^{-1}$  concentration of the silver nanoparticles was used.<sup>86</sup>

Spherical silver nanoparticles that were produced from the filtrate of gliotoxin-producing *T. virens* HZA14 demonstrated 100, 93.8 and 100% activities against myceliogenic germination of sclerotia, sclerotial formation and hyphal growth. These performances were observed when the concentration of the nanoparticle assay was  $200 \mu\text{g mL}^{-1}$ . As revealed by the EDS and SEM analysis, there was direct interaction between the cells of the fungi and the nanoparticles leading to fissure and micro-pore formation on the cell wall of the fungi and fragmentation of its lamella.<sup>87</sup> Mitta *et al.* utilized *Tinospora cordifolia* leaf extract to reduce silver nitrate to spherical silver nanoparticles with a particle size of 50 nm. The bioactive compounds in the extract were also used as capping agents for the nanoparticles. The capped silver nanoparticles were effective against *Sclerotinia sclerotiorum* (MTCC 8785).<sup>88</sup>

A warm water extract of *Chlorella vulgaris* and algae *Chroococcus dispersus* was also used to reduce silver nitrate to silver nanoparticles having particle sizes lower than 55 nm. The antifungal activities of the assay of the produced silver nanoparticles were investigated using the disc diffusion method against *Sclerotinia sclerotiorum* and (*Alternaria alternata*, *Helminthosporium* sp., *Rhizoctonia solani*, *Fusarium oxysporum* and

*Fusarium solani*). The results obtained were compared to the performance of generic antibiotics (streptomycin, ampicillin, and gentamycin). It was observed that the antifungal activities of the silver nanoparticles were nine fold better than those of the antibiotics.<sup>89</sup> Generally, the inhibitory performance of silver nanoparticles is active against three common sclerotium-forming species (*S. minor*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*). However, the inhibitory effect against hyphal growth of *Sclerotinia sclerotiorum* is more than that of *S. minor* while the hyphal growth inhibition is the greatest in *Rhizoctonia solani*. Conversely, sclerotial germination growth inhibition against *Sclerotinia sclerotiorum* is the most pronounced out of the three sclerotium-forming species.<sup>90</sup> Specifically, the first article on the use of nanoparticles for controlling *Sclerotinia sclerotiorum* was written in the year 2009 and there is a significant increase from 2018 onwards as shown in Fig. 6. Overall, more than 51 research articles have been published on the control of the pathogen using different nanomaterials.

**2.3.2 Use of other metallic nanoparticles for controlling *Sclerotinia sclerotiorum*.** Although silver nanoparticles have been extensively investigated against *Sclerotinia sclerotiorum*, other metallic nanoparticles have also been investigated. For example, titanium nanoparticles were biosynthesized by using *Trichoderma harzianum* as the stabilizing agent. The nanoparticle suspension produced an inhibitory effect on the



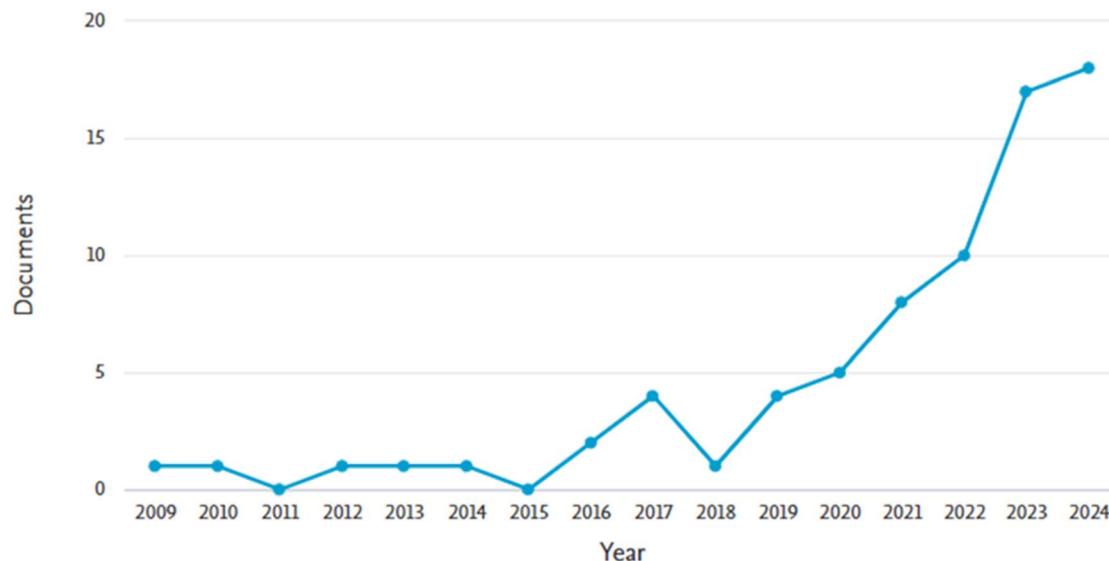


Fig. 6 Research trends on the use of nanoparticles for eliminating *Sclerotinia sclerotiorum* as shown by the number of published documents from 2009–2024 on the Scopus database. Accessed on November 12, 2024.

mycelial growth of *Sclerotinia sclerotiorum*. Also, the formation of new resistant structures was more inhibited with the assay of titanium nanoparticles than when an extract of *Trichoderma harzianum* was used. Similarly, the chitinolytic activity of the nanoparticles was clearly better than that of *Trichoderma harzianum*. The evaluation of the toxicity of the titanium nanoparticles showed that agriculturally important microorganisms were not affected by the as-prepared titanium nanoparticles. The only notable impact was a decrease in the number of bacteria involved in the nitrogen cycle. Besides, there was no genotoxicity as shown by 3T3 and V79-4 cell lines. In fact, when its effect on soybean was investigated, it was observed that silicon nanoparticles had no biochemical and morphological effects on soybean after application.<sup>91</sup> This further buttressed the fact that titanium metal nanoparticles are safe and effective in controlling fungal disease in soybean.

Apart from titanium nanoparticles, copper nanoparticles have also been investigated as antifungal agents against *Sclerotinia sclerotiorum*. Sadek *et al.*<sup>92</sup> compared the antifungal performance of copper sulphate solution and sulphur nanoparticles to that of copper nanoparticles. To achieve this, *in vivo* and *in vitro* applications of these materials in cucumber at varied temperatures were carried out by these researchers. The findings showed that copper sulphate displayed the lowest antifungal activities against *Sclerotinia sclerotiorum*. Even when the concentration of the copper sulphate nanoparticles assay was raised to 4000  $\mu\text{g mL}^{-1}$ , the antifungal activities were still comparatively lower than when 100  $\mu\text{g mL}^{-1}$  assay of the copper nanoparticles was used. Notably, the sulphur nanoparticles showed better antifungal performance than copper nanoparticles. For the two nanoparticles (sulphur and copper), as the concentration of the nanoparticles increased, the antifungal activities also increased. Also, the temperature impacted the antifungal activities of these nanoparticles. As the temperature

was decreased, there was a significant drop in the fungal growth. Sulphur nanoparticles also caused lower cytotoxic effects compared to copper nanoparticles. Even though there are sulphur and copper in copper sulphate solution, the antifungal activity of the solution is far less than that of sulphur and copper nanoparticles.

Selenium is a beneficial element to plants which has also been employed as a good antifungal agent against *Sclerotinia sclerotiorum*. It has been experimentally proven that when between 0.1 and 0.5  $\text{mg kg}^{-1}$  of selenium nanoparticles are applied to soil, the diseases caused by *Sclerotinia sclerotiorum* are reduced in oilseed rape. This is because the presence of selenium nanoparticles increases the transpiration rate, stomatal conductance, the antioxidant system of leaves and the net photosynthetic rate. As revealed by the metabolome analysis, the presence of selenium boosts the amino acids and energy of the oilseed rape leaves infected by *Sclerotinia sclerotiorum*.<sup>93</sup> In another investigation, there were soluble protein leakage and severe damage to the hyphae of *Sclerotinia sclerotiorum* when the soil used to plant rape straw was treated with selenium. This led to an increase in the oxalic acid secretion and a decrease in cell-wall degrading enzymes in the pathogen with an overall increase in the *Sclerotinia sclerotiorum* fungal inhibition.<sup>94</sup>

In a different study,<sup>95</sup> nano-silicon was found to inhibit the growth of the mycelium of the pathogen depending on the dose of the nanoparticle applied. The disease index was significantly reduced by a maximum value of approximately 52% when the nano-silicon was used for the field trial on *Brassica napus*. This was linked to the promotion of minerals such as calcium, potassium and silicon when silicon nanoparticles were applied while at the same time suppressing the absorption of sodium. Besides, the lignin and soluble sugar levels were boosted when silicon nanoparticles were applied unlike the level of cellulose



that was reduced in the stems and the leaves of *Brassica napus*. This is clearly shown in the diameter of lesions with and without silicon nanoparticles.

**2.3.3. Control of *Sclerotinia sclerotiorum* using metal oxide nanoparticles.** One of the most common metal oxides that has been used for controlling *Sclerotinia sclerotiorum* is zinc oxide nanoparticles. In green synthesis of zinc oxide nanoparticles, several extracts have been used as stabilizers and/or reducing agents. One of such investigations utilized cell filtrate of a strain of *Trichoderma harzianum*. The produced zinc oxide was used as an antifungal agent against *Sclerotinia sclerotiorum* and it was observed that the inhibitory properties of the nanoparticle are dose-dependent.<sup>96</sup> Bulk zinc oxide also inhibited the fungi, but the concentration required is more than the concentration required for when zinc oxide nanoparticles were used. Al-Tememe *et al.* confirmed this by subjecting eggplants to bulk zinc oxide and zinc oxide nanoparticles under both greenhouse and laboratory conditions. Both bulk zinc oxide and zinc oxide nanoparticles inhibited *Sclerotinia sclerotiorum* but the best inhibition was observed at 10 mg L<sup>-1</sup> with bulk zinc oxide compared to 7.5 mg L<sup>-1</sup> for zinc oxide nanoparticles.<sup>97</sup>

The effect of zinc oxide nanoparticles in inhibiting *Sclerotinia sclerotiorum* has also been studied and compared with the inhibiting ability of Benomyl (a known antifungal). As shown by the diameter of the colony of *Sclerotinia sclerotiorum* grown on the potato dextrose agar and broth, after applying the nanoparticles and the fungicide, there was a 9 cm diameter after 0.3 mL within 5 days of inoculation which was better than that of the fungicide. Besides, it caused the sclerotia with sizes between 25 micrometers and 2 millimeters to disappear.<sup>98</sup> When zinc oxide was coated with ethylene glycol and used for inhibiting *Sclerotinia sclerotiorum* in lettuce, an increased quantum yield rate and net photosynthetic rate were observed. There was no phytotoxic effect on lettuce after the application of this nanoparticle. In short, the impact of the pathogen was reduced due to the application of the nanoparticles.<sup>99</sup> In seeds of chickpea treated with zinc oxide made by using the leaf extract of *Carica papaya*, compared to other fungal pathogens (*R. necatrix* and *Fusarium* species), *Sclerotinia sclerotiorum* was significantly inhibited by 59.7%. This further buttressed the fact that zinc oxide nanoparticles are effective antifungal agents against *Sclerotinia sclerotiorum*.<sup>100</sup> A similar trend was observed when zinc oxide was used as an antifungal agent in potato. There was 60.5% inhibition against *Sclerotinia sclerotiorum* compared to 55.2% and 37.5% that were reported for inhibition against *Fusarium* spp and *Rosellinia necatrix* respectively.<sup>101</sup>

Apart from zinc oxide nanoparticles, other metal oxide nanoparticles have been reported for combating *Sclerotinia sclerotiorum*. One of these is titanium oxide synthesized by using shell extracts of pawpaw. Varied concentrations of the nanoparticle were used to preserve the germs of pea from *Sclerotinia sclerotiorum*. There was a 60.5% inhibition ratio of the pathogen and increases in catalase activities and superoxide dismutase. Besides the mechanisms of inhibition, there was increased plant and root growth, enhanced seed germination, and improved antioxidant enzyme activities and root interval.<sup>102</sup>

Iron oxide nanoparticles have also been used to control the pathogen. They are particularly used for the plants' investigations. This is because iron is an important micronutrient in plants that helps with boosting the chlorophyll, protein carbohydrates and plant growth.<sup>103</sup> Bilesky-José *et al.* used *Trichoderma harzianum* as a stabilizing agent for synthesizing iron oxide nanoparticles. The as-synthesized nanoparticles displayed an inhibitory effect on *Sclerotinia sclerotiorum* without hindering the growth of the plants. In addition, the mitotic index and cell viability of the plants were not altered by the application of iron oxide nanoparticles compared to the controls.<sup>104</sup> *Trichoderma harzianum* has also been used as both a stabilizing agent and a reducing agent for the synthesis of copper oxide that was used for controlling *Sclerotinia sclerotiorum*. Copper oxide had no effect on the size of the mycelia of the pathogen but there was alteration in the size and distribution of the sclerotia due to the application of copper oxide nanoparticles.<sup>96</sup>

In another research study, copper oxide nanoparticles applied to green bean pods induced an inhibition against *Sclerotinia sclerotiorum*. In response to the application of copper nanoparticles, high amounts of phaseollin, 6- $\alpha$ -hydroxyphaseollin, phaseollidin, coumestrol, kievitone, and phytoalexins were detected. There was also an increase in the transcriptomic levels of pvgluc, PvGIP, PR1, DOX and pk20 with non-genotoxic impact on the DNA of the green bean. As a result of the inhibition of the pathogen by copper oxide nanoparticles, the shelf life of the green bean pods was extended to 21 days when the storage temperature was maintained at 7  $\pm$  1 °C.<sup>105</sup> Silicon oxide capped with a network containing phenol and copper has also proven to be effective in controlling *Sclerotinia sclerotiorum* in oilseed rape plants. The nanoparticles were found to be environmentally safe and efficient as revealed by the toxicity test carried out on zebrafish.<sup>106</sup>

**2.3.4 Control of *Sclerotinia sclerotiorum* using carbon-based nanomaterials.** Carbon compounds have also been reported as antifungal materials against *Sclerotinia sclerotiorum*. One of the carbon materials that has been investigated is chitosan (nanoparticles, low molecular weight, and high molecular weight). In the study carried out by El-Mohamedya *et al.*, the *in vitro* evaluation of the pathogen in green bean, potato and tomato showed that the nanoparticle form of chitosan (whether it is of high or low molecular weight) at a very low dosage of 0.1% and 0.05% displayed a complete inhibition of mycelial growth of eighteen phytopathogenic fungi. Some of these pathogens are *Sclerotium* spp., *Alternaria solani*, *Phytophthora infestans*, *Macrophomina phaseolina*, *F. oxysporum*, *Fusarium solani* and *Rhizoctonia solani*. In contrast, the bulk forms of chitosan (whether it is of high or low molecular weight) were not effective against all the pathogens until the dosage was increased to 1.0%. At this dosage, there was total inhibition of all the pathogens. This showed that chitosan (both high molecular and low molecular weight), whether in the bulk form or nanoparticle form, is a good antifungal agent, but the nanoparticle form will function effectively even at a very low dosage unlike the bulk form of chitosan.<sup>107</sup> Instead of chitosan alone as an antifungal agent, the nanoencapsulation of the



combination of chitosan, graphitic carbon nitride and hydroxyapatite has been investigated as the antifungal agent. With a mixing weight ratio of 2:1:5 for hydroxyapatite, graphitic carbon nitride and chitosan respectively, there was an encapsulation efficiency of 82%. This combination had an *in vitro* minimum inhibitory concentration of 187.5  $\mu\text{g mL}^{-1}$  against *Sclerotinia sclerotiorum* that is present in kiwifruit. On artificially inoculated fruit, higher doses of 375  $\mu\text{g mL}^{-1}$  were required for the effective control of the same pathogen.<sup>108</sup>

The need to reduce the toxicity of mancozeb which is a commercial antifungal agent led to the fabrication of chitosan-gum acacia polymers loaded with mancozeb. When the loading of mancozeb was 1.0  $\text{mg mL}^{-1}$ , there was 100% inhibition of *Sclerotinia sclerotiorum* through the mycelium inhibition method. The disease-control efficiency against stem rot diseases in pathogen-treated plants was  $60.2 \pm 1.4\%$ . However, commercial mancozeb also showed 100% inhibition against *Sclerotinia sclerotiorum*, but the toxicity is comparatively higher than the one loaded on the chitosan-gum acacia polymers as shown when applied on Vero cell lines.<sup>109</sup> This same group of researchers loaded mancozeb on chitosan-carrageenan combinations. Mancozeb was entrapped and loaded on the composite carbon-based material. With 1.5 ppm loading of nanomancozeb, there was 100% inhibition of *Sclerotinia sclerotiorum*. The nano formulation also showed lower toxicity compared to commercial mancozeb only.<sup>110</sup>

Another formulation is the poly(lactic acid) and cellulose nanocrystal made into spherical beads and combined with methoxylated sucrose soyate polyols and used along with a commercial antifungal against *Sclerotinia sclerotiorum* (Lib.). The strategy involves loading the commercial antifungal on the nanoformulated material prior to application on the plant. To demonstrate the functionality of this strategy, a model fungicide (azoxystrobin) was loaded on the prepared nanoformulation with canola being the model plant. The design is unique because there is controlled porosity in the cellulose nanocrystals allowing diffusion of the fungicides to the environment *via* the beads. The material is also cheaply available and biocompatible and has good water dispersibility, the presence of several hydroxyl functional groups that can interact *via* hydrogen bonds, large surface area and a high aspect ratio. This nanoformulation loaded with fungicides was preferred to ordinary commercial fungicides as commercial fungicides do not persist for a long period of time on the plant before being washed away due to exposure to air flow, rain, and other environmental conditions.<sup>17</sup> Without a delivery system like this nanoformulation, less than 0.1% of the applied fungicides get to their biological targets while the remaining are lost through runoff, leaching, degradation, photolysis, and volatilization.<sup>111</sup> This is not only economically wasteful for farmers, but it also affects non-target beneficial organisms.<sup>17</sup>

Graphene oxide is another carbon-based material that has been used to suppress the growth of *Sclerotinia sclerotiorum*. Treatment of rapeseed seedlings with 15  $\text{mg L}^{-1}$  graphene oxide or treatment of the seeds with the same concentration significantly inhibited the growth of *Sclerotinia sclerotiorum* compared to the control samples. At this concentration, the growth of

rapeseed was not affected.<sup>112</sup> Hydrophobic interactions between polyhexamethylene biguanide and fenhexamid fungicides mixed in a ratio of 7 to 10 at 60 °C resulted in stable, spherical nanoparticles. There was a synergistic inhibitory effect on *Sclerotinia sclerotiorum* compared to when only a polyhexamethylene biguanide or fenhexamid fungicide was used under the same conditions. The introduction of polyhexamethylene biguanide into the fenhexamid fungicide did not increase the cytotoxicity of fenhexamid as revealed by *Vicia faba* genotoxicity evaluations.<sup>113</sup>

The use of covalent organic frameworks for controlling the pathogen has also been reported. In particular, zeolite imidazole framework-8 has been investigated due to its easy decomposition at acidic pH, its adjustable nano-scaled size, its large surface area and its good stability. In an investigation, a model pesticide, pyraclostrobin, was loaded on the zeolite imidazole framework made from dimethylimidazole zinc. The release rate of the combination was found to be pH dependent. In a pot experiment, addition of 0.1  $\mu\text{g mL}^{-1}$  of the nanoformulated material led to the inhibition of *Sclerotinia sclerotiorum* by 9.4%. However, when the experiment was repeated *in vitro*, there was 16.4% inhibition of *Sclerotinia sclerotiorum*. This shows that both the pot experiment and *in vitro* investigations showed inhibition of the pathogen by the nanoformulation and there was slow release of the highly efficient nanomaterial.<sup>114</sup>

**2.3.5 Control of *Sclerotinia sclerotiorum* using nanocomposites.** A nanocomposite comprising metal/metal oxide and carbon-based materials has also been studied as an antifungal agent against *Sclerotinia sclerotiorum*. Huang *et al.* investigated the use of a controlled-release delivery system of the fungicide into the target crop to minimize wastage and secure the environment. This was carried out by loading the carbendazim fungicide on a nanocomposite made from mesoporous selenium, methyl orange and trimethylammoniumpillar [5]arene. When applied to rape leaves and maize, the methyl orange in the composite shed because of protonation while the remaining part of the nanocomposite dissolved due to the acidic nature of the environment, leading to rapid release of the fungicide. As revealed by the *in vitro* experiment, there was 1.74-fold fungicide release when the pH was maintained at 4.5 compared to the release under neutral pH conditions. This system caused a drop in the reducing sugar and dry biomass of *Sclerotinia sclerotiorum*. It is therefore a good strategy to control the fungal pathogen.<sup>115</sup>

Apart from the use of nanocomposites as delivery systems, they have also been used as antifungal agents against *Sclerotinia sclerotiorum*. In a study, polyethylene glycol and diethylene glycol were separately composited with copper-doped zinc oxide. The two nanocomposites were tested against *Sclerotinia sclerotiorum* *via* foliar spray on lettuce and it was found that they showed antifungal activities of 260 and 278  $\mu\text{g mL}^{-1}$  against *S. sclerotiorum*, respectively. Their application also led to an increase in phenolic compound contents and antioxidant activities of the lettuce plants. Overall, the nanocomposite was effective against the fungal pathogens.<sup>116</sup>



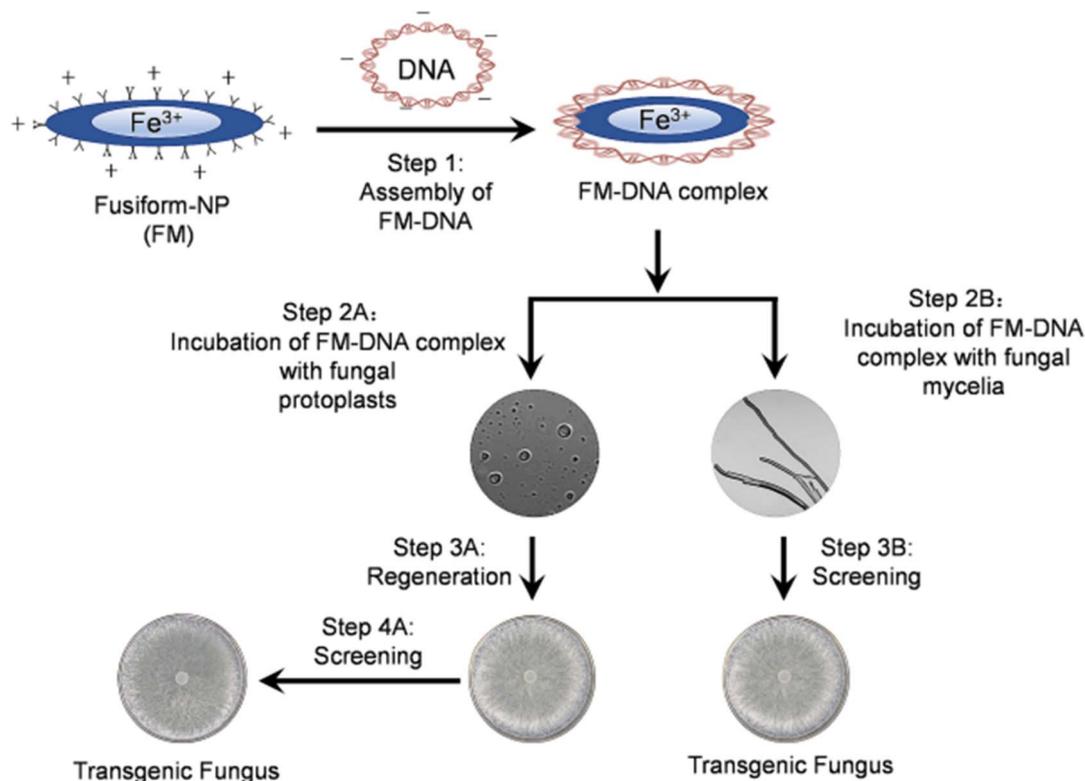


Fig. 7 Schemes of the transformation route in *S. sclerotiorum* using Fusiform nanoparticles.<sup>118</sup> Springer Nature-Open Access.

**2.3.6 Control of *Sclerotinia sclerotiorum* using fusiform nanoparticles.** Morphological modification of the nanoparticles can be done to get a fusiform shape. This is to enhance the ability of the nanoparticle to penetrate the cell wall of the organism easily. In an investigation, fusiform nanoparticles were made from 2,6-diaminopyrimidine and iron trichloride. Briefly, 0.02 mol of iron oxide was dispersed into a known volume of deionized water in the presence of argon to eliminate air interference. 2,6-Diaminopyrimidine was added and stirred at 50 °C for 24 h for polymerization to take place. The impurities were removed and the products obtained were dialysed for 1 day.<sup>117</sup> These nanoparticles were used as carriers for effecting transgenic transformation in white mould pathogens. The presence of the nanoparticle caused the transformation of the mycelium without the common protoplast preparation step.<sup>118</sup> The process is also advantageous as it displayed high-throughput and high efficiency. The mechanism involves the formation of a complex with the DNA of the pathogen, incubation of the complex, regeneration and screening as illustrated in Fig. 7.

### 3 Revisit of biological methods of controlling *Sclerotinia sclerotiorum*

#### 3.1 Use of antagonistic micro-organisms to control *Sclerotinia sclerotiorum*

The sclerotia of *S. sclerotiorum* have been used as the source of some biological strains to serve as mycoparasites for controlling

the same fungus (*S. sclerotiorum*). Examples of fungal strains isolated from *S. sclerotiorum* and used for controlling *S. sclerotiorum* are *T. asperellum* TRI 15, *Cl. rosea* TNAU-CR 02 and *Co. minitans* LU112 plants.<sup>119–121</sup> The isolation of viruses from *S. sclerotiorum* for biocontrol of *S. sclerotiorum* is not very common. However, the use of mycoviruses (viruses that infect fungi) such as mycovirus SsNSRV-1, DNA mycovirus SsHADV-1, and deltaflexivirus SsDFV2 has been reported.<sup>122–124</sup> In the case of bacterial biocontrol agents, the common sources are soil and plants.<sup>119</sup> In this section, the fungi and bacteria that have been used for controlling *S. sclerotiorum* will be discussed.

**3.1.1 The use of antagonistic fungi to control *Sclerotinia sclerotiorum*.** A cellulose-decaying fungus ascomycetous fungi, *Clonostachys rosea*, has been studied as an antagonistic organism against *Sclerotinia sclerotiorum*. *Clonostachys rosea* used for the investigation was sourced from the sclerotia of the pathogen to be controlled (*Sclerotinia sclerotiorum*). The highest mycelial inhibition found was 79.63% compared to the control. The GC-MS study of the fingerprinting in *Clonostachys rosea* showed that it contains eighteen compounds.<sup>125</sup> In a similar investigation, *Clonostachys rosea* was produced using a two-stage fermentation process from the grains of rice. The production process was altered to vary the ratio of carbon-to-nitrogen to increase the yield of *Clonostachys rosea*. There was 100% inhibition of the growth of *S. sclerotiorum* through direct parasitism. When the conidia of *Clonostachys rosea* were air-dried prior to usage, there was 76.2% mortality of white fly nymphs and there was 88% activity suppression on sclerotia.<sup>30</sup>





Table 4 Antagonistic potential of *Trichoderma* species against *Sclerotinia sclerotiorum*

Trichoderma investigated	Plant used	Methodology adopted	Key findings	Ref.
<i>Trichoderma asperellum</i> , <i>Trichoderma longibrachiatum</i> and <i>Trichoderma harzianum</i>	Egyptian clover ( <i>Trifolium alexandrinum</i> L.)	Field experiments and <i>in vitro</i> investigations	The performance of <i>Trichoderma harzianum</i> stood out as it displayed an inhibition of 80.61% compared to the control; 1/10 (1.24 cm) was the best concentration of <i>Trichoderma harzianum</i> that gave the optimal response; 66% sclerotial inhibition; reduction of disease severity from 73.5% (untreated/control) to 51.7% (treated with <i>Trichoderma harzianum</i> ); an increase in dry fodder weight and green weight compared to the control	127
<i>Trichoderma harzianum</i> , <i>Trichoderma viride</i> and other 69 <i>Trichoderma</i> spp.	Clover	Laboratory investigations involving the use of volatile and non-volatile metabolites, followed by greenhouse experiments	<i>Trichoderma harzianum</i> was more active than other isolates showing 20% sclerotium sclerotia hypha reduction; 20.37% inhibition of the growth of <i>S. sclerotium</i> mycelia was observed with 5% non-volatile metabolite isolate of <i>T. viride</i> ; 15% and 25% concentrations of <i>T. harzianum</i> displayed an inhibition of 62.22 and 73.7% inhibition with non-volatile metabolite isolate; enhancement of seeds per pod, height of plants, fresh weight of root, shoot dry weight, branch number, pod diameter and total grain weight	128
<i>Trichoderma virens</i> , <i>Trichoderma atroviride</i> , <i>Trichoderma koningi</i> and <i>Trichoderma harzianum</i>	Canola	Seeds were coated with a <i>Trichoderma</i> conidia cell suspension and <i>Trichoderma</i> mixed with soil was also used; the use of nanocides with <i>Trichoderma</i> was also investigated in the green house	<i>Trichoderma harzianum</i> showed the best inhibition against the pathogen; no statistical difference between the use of <i>Trichoderma</i> alone and <i>Trichoderma</i> with nanocides; <i>Trichoderma</i> alone boosted the growth parameters compared with <i>Trichoderma</i> with nanocides	129
<i>Trichoderma harzianum</i>	Tomato	Control of <i>Sclerotium rolfsii</i> , <i>Sclerotium cepivorum</i> and <i>Sclerotinia sclerotiorum</i> using <i>Trichoderma harzianum</i> by laboratory investigations. Mycelial growth analysis was carried out by using the Petri dish pairing method	Reduction in the phytopathogen growth rate; no effect on the total area covered by the pathogen; the three strains showed similar conidium production	130

*Aspergillus flavus* strain NJC04 isolated from kiwifruit was also used for controlling *Sclerotinia sclerotiorum* in soybean. This fungal strain competed for nutrients and space with the pathogen leading to the inhibition of sclerotial development and inhibition of the growth of mycelia. Another cause of inhibition is the formation of antifungal kojic acid. In the culture medium,  $87 \pm 4 \text{ mg L}^{-1}$  was detected. However, the reduction of the symptom of white rot depends on the number of spores per millilitres of the *flavus* strain that was applied to the soybean plant. For instance, there was 100% and 83.2% white rot symptom suppression when  $1 \times 10^8$  and  $1 \times 10^7$  NJC04 spores per mL were used respectively.<sup>31</sup> Atallah *et al.* extended the investigation beyond *Aspergillus flavus* to check the efficiency of other species of *Aspergillus* against *Sclerotinia sclerotiorum*. The species investigated are *Aspergillus pseudoalegans*, *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus japonicus*. Under greenhouse conditions, their potential to suppress white mold disease was examined. It was observed that beans treated with *Aspergillus niger* and *Aspergillus japonicus* displayed the best rate of survival when compared with the bean plants that were not treated. Another observation was that the sporulation of the *Aspergillus* isolates is a function of the temperature. This is because *Aspergillus* became inhibited as the temperature lowered which made its colour change to white. In short, in carrying out investigations involving all the tested *Aspergillus* spp., the temperature factor should be considered. Interestingly, none of the *Aspergillus* species tested was aflatoxigenic and none was infectious to the legume that was used for the investigation.<sup>32</sup>

**3.1.1.1 The use of *Trichoderma* spp. for controlling *Sclerotinia sclerotiorum*.** *Trichoderma* isolate has been used for the treatment of soil that was used for planting in a bid to control *Sclerotinia sclerotiorum*. In the study carried out by Montalvão *et al.*,<sup>126</sup> thirty-four *Trichoderma* antagonist fungal strains were used to treat soil. The *T. afroharzianum* species obtained from the soil cultivated with cotton performed better than other strains of *Trichoderma*. Though, all the strains tested led to hyphal penetration, deformation and folding against *Sclerotinia sclerotiorum* as revealed by the microscopy studies, notably, the isolate of *Trichoderma* (CEN281 and CEN126) from tomato seedlings had the best antagonistic potential against the disease. Relatedly, two indigenous strains of *Trichoderma* which originated from Brazil (*T. lentiforme* CMAA 1585 and *T. asperelloides* CMAA 1584) were investigated for controlling *Sclerotinia sclerotiorum*. The two strains were able to release volatile organic carbons by solubilizing phosphorus minerals. The released volatile organic compounds were potent enough to impair the growth of the mycelia of the pathogen in the cotton plant. They equally reduced the number of sclerotia formed and the growth rate of *Sclerotinia sclerotiorum* on the plant. Treatment of sclerotia with the conidial suspensions of the two strains led to significant inhibition in myceliogenic germination. It was however observed that the efficiency of *T. asperelloides* CMAA 1584 was clearly better than that of *T. lentiforme* CMAA 1585 in controlling *Sclerotinia sclerotiorum*. In contrast, *T. lentiforme* CMAA 1585 was more efficient as a biostimulant than *T. asperelloides* CMAA 1584 as it contributes more to the

growth of the cotton. Therefore, application of these two strains of *Trichoderma* will not only control the white rot diseases, but also contribute to the growth of cotton plants. This will reduce the amount of fungicides and synthetic fertilizers being applied to the cotton plants.<sup>33</sup> Other investigations involving control of *Sclerotinia sclerotiorum* are shown in Table 4.

**3.1.1.2 The use of *Coniothyrium minitans* for controlling *Sclerotinia sclerotiorum*.** The mycoparasitic attack of *Sclerotinia* by *C. minitans* has been reported.<sup>131</sup> The mechanism of action of this mycoparasite is the generation of enzymes such as glucanases and chitinases which have the ability to degrade the cell walls of *Sclerotinia sclerotiorum*. Molecular studies have also revealed an increase in the  $\beta$ -1,3-glucanase gene *cmg1* in *C. minitans* when it attacks *Sclerotinia sclerotiorum*.<sup>132</sup> Different plants have been used to investigate the efficiency of the mycoparasite in controlling *Sclerotinia sclerotiorum*. For instance, aerial application of this mycoparasite both *in vivo* and *in vitro* to rapeseed plants revealed that there was restriction in the growth of the mycelia of *Sclerotinia sclerotiorum*.<sup>34</sup> The efficiency of this mycoparasite has also been confirmed by using it to control *Sclerotinia sclerotiorum* in peanuts,<sup>133</sup> tomato<sup>134</sup> and cabbage.<sup>135</sup> Yang *et al.*<sup>136</sup> reported that application of this mycoparasite along with N-P-K fertilizers in rapeseed resulted in dosage-dependent mycelial growth inhibition and a maximum of 10% conidial germination inhibition in *Sclerotinia sclerotiorum*. This combined strategy is advantageous because it reduces the cost of labour and boosts the yield of the plant. Another combined strategy involves the combination of *Trichoderma* species and *Coniothyrium minitans* for controlling *Sclerotinia sclerotiorum*. In an investigation, there was 30–50% reduction in white mould disease (compared with the untreated control) when *C. minitans* LU<sub>112</sub> was combined with *T. virens* LU<sub>555</sub> and *T. hamatum* LU<sub>595</sub>. This was clearly better than the disease reduction observed when carbendazim fungicide treatment was used under similar conditions.<sup>137</sup>

**3.1.2 The use of antagonistic bacteria to control *Sclerotinia sclerotiorum*.** Bacteria have also been used as parasites against *Sclerotinia sclerotiorum*. This was demonstrated in a greenhouse when several bacterial strains were tested against *Sclerotinia sclerotiorum* from garden eggplant. Among the bacteria tested, *P. fluorescens* displayed the highest percentage inhibition efficiency compared to the control. The two bacterial strains that are close to *P. fluorescens* in performance are *P. commune* and *T. asperellum*. Either singly or when combined, the three bacteria showed inhibition of infection severity ranging from 5.55 to 66.66% compared to the control (without any bacteria). The presence of these bacterial strains contributed to the cumulative weight (and other growth parameters) of the eggplants.<sup>35</sup> *Sclerotinia sclerotiorum* inoculated on tomato has also been inhibited by *Bacillus amyloliquefaciens* PKM16 both *in vitro* and *in vivo*. There was approximately 30% sclerotial germination and mycelial growth inhibition. The treatment was also found to activate the induction of tomato plant defence enzymes (including  $\beta$ -1,3-glucanase, phenylalanine ammonia-lyase, polyphenol oxidase, catalase, and peroxidase).<sup>138</sup> Other investigations involving the control of *Sclerotinia sclerotiorum* using antagonistic bacteria are shown in Table 5.



Table 5 Control of *Sclerotinia sclerotiorum* using antagonistic bacteria

Bacteria used for controlling the pathogen	Plant used	Instrumentation and technique used for the study	Key findings	Ref.
<i>Bacillus cereus</i> CF4-51	Multiple crops	Gas chromatography–mass spectrometry (GC-MS), a scanning electron microscope (SEM), quantitative reverse transcription polymerase chain reaction (qRT-PCR), and whole-genome sequencing	Volatile organic compounds (VOCs) released by CF4-51 (including 2-pentadecanone, 6,10,14-trimethyl-, 1,2-heptadecane, cyclododecane, dibutyl phthalate and bis(2-methylpropyl) ester) inhibited the formation of sclerotia and damaged the hyphae of <i>S. sclerotiorum</i>	139
<i>Pseudomonas</i> spp (18 isolates)	Potato and rice	Broth-based <i>in vitro</i> dual culturing; analysis of plant growth promoting rhizobacteria (PGPR)	Mycelial growth inhibition ranging from 68.9 to 42.6%; higher phosphorus solubilizing efficiency; inhibition of black scurf severity up to 67.59% compared to the control	140
<i>Bacillus species</i> A5F	Soybean	'One-variable-at-a-time' (OVAT) approach; high resolution liquid chromatography–mass spectrometry (HR LC-MS); a transmission electron microscope (TEM) and qRT-PCR	Significant effects on seed weight, number of pods, shoot biomass and chlorophyll content; reduction in the disease incidence of <i>S. sclerotiorum</i> infected soybean plants; ITS gene copy number reduction of 16% compared to the control	141
<i>Streptomyces albulus</i> CK-15 (using its secondary metabolite (wuyiencin))	Soybean	Seed-soaking method	Spraying wuyiencin (200 µg mL <sup>-1</sup> ) three times was the best with an inhibitory percentage of 64.0% but inferior to the 77.6% obtained when 200 µg mL <sup>-1</sup> dimethachlon was used	142
<i>Streptomyces albulus</i> CK-15 (using its secondary metabolite wuyiencin)	Soybean	<i>In vitro</i>	78.3% inhibition of mycelial growth; inhibition of the formation of sclerotia; an increase in cell membrane permeability; a decrease in the content of pectin methyl-galacturonic enzymes, oxalic acid and activities of polygalacturonase	143
Five <i>Bacillus</i> strains	Potato	HPLC; the radial growth method for antifungal evaluation; a Zeiss light microscope	Ergosterol (in the cell wall) was blocked; inhibition of sclerotial formation and 100% growth inhibition; polyene compound production by the bacterial strains	144
Three <i>Streptomyces</i> species ( <i>S. sampsonii</i> , <i>S. rochei</i> and <i>S. griseus</i> )	Green beans	Nucleotide sequencing; PCR amplification; <i>in vitro</i> and <i>in vivo</i> investigations; light microscopy and SEM	<i>S. rochei</i> showed the best inhibition among the three bacteria followed by <i>S. griseus</i> ; <i>S. sampsonii</i> was more efficient as a bioagent in reducing mycelial growth pathogen by 84.50%	145
<i>Bacillus</i> sp. FSQ1	Bean ( <i>Phaseolus vulgaris</i> )	Sequencing	Inhibition of the growth of the phytopathogen by 2.4 ± 0.2 cm through <i>in vitro</i> antagonism	146
<i>Streptomyces</i>	—	RT-qPCR and RNA-seq	ε-Poly-L-lysine is produced; inhibition of the mycelial growth with EC <sub>50</sub> values of 283 µg mL <sup>-1</sup>	147



## 4 Conclusion and future perspectives

To achieve sustainable global food security, efforts must be geared towards the fight against plant diseases. Studies on the use of gene editing strategy (usually modifying susceptible genes or effector targeted genes), transgenic strategy (such as genes from microorganisms), and spraying small RNA induced gene silence (SIGS) should be focussed on reducing the menace of white mold caused by *Sclerotinia sclerotiorum*. The limitations to the commercial utilization of gene silencing are a lack of general approaches that can be used for all plant species and off-target silencing. More research is needed to overcome these challenges. Using UV radiation for controlling the pathogen without harming beneficial microbes through mutation should be investigated. Also, the use of plants that are resistant to white mould along with the pretreatment of the seed prior to planting should be considered.

Although different natural compounds have been used for the control of white mould, miltana, cinnamaldehyde and jojoba oil have not been used for controlling *Sclerotinia sclerotiorum*. Therefore, the use of these natural compounds for controlling white mould diseases should be investigated. Apart from using ordinary nanoparticles for the control of the pathogen, combinations of different nanoparticles with other methods such as gene silencing and the use of antagonistic micro-organisms should be studied. Before this can be achieved, further research needs to be conducted to understand the best conditions that will work for the hybrid control strategies. Also, increasing the storage life of the fungicides derived from the botanical source to compete with the chemical fungicide is still a challenge which requires serious research attention.

Despite the advantages of botanical fungicides over synthetic fungicides, large scale commercialization has not been achieved. This could be due to variation of the active component of the extract with climatic conditions, speedy degradation of botanical pesticides, and low awareness among the farmers.<sup>76</sup> These challenges could be solved by focussing research on these limitations. Research studies that are urgently required in this aspect are quicker market introduction with price control, improving the awareness of their usage to farmers, establishment of regulatory guidelines, establishment of possible pathways to obtain maximum extraction of bioactive compounds from plants, and carrying out *in vivo* experiments in open fields and greenhouses to understand phytotoxicity and toxicity to other living organisms. Future research will also require understanding the shelf-life of grains stored by using these botanical fungicides. Some of these would require collaborative research between sciences, humanities and social sciences.

As discussed in this review, new strategies are emerging, but the use of nanomaterials is one of the major strategies that could significantly reduce fungal diseases in plants (particularly white mold fungal diseases caused by *Sclerotinia sclerotiorum*). However, the impacts of nanoparticles on plants must be fully understood before they would be used for antifungal applications. This is necessary to prevent the applied nanoparticles from having detrimental effects on the anatomy and

physiological attributes of plants. Even though some metallic and metal oxide nanoparticles have been investigated, ternary oxides and quaternary oxide nanoparticles have not been investigated as antifungal agents against fungal pathogens. In addition, the potency of several sulphide nanoparticles has not been studied. Besides, the use of carbon-based materials such as activated carbon, carbon nanotubes, cyclodextrins, fullerenes, gels (xerogels, aerogels, cryogels, and hydrogels), polymers, alginates, reduced graphene oxide, biochar and their composites has not been studied. Chemists and materials scientists will have significant roles to play in ensuring that these materials and nanocomposites are efficiently produced. Simultaneous control and detection of the pathogens can be done by using different nanoparticles/nanocomposites. Finally, most studies on the control of *Sclerotinia sclerotiorum* were conducted *via in vitro* methods. It is necessary to carry out these investigations using *in vivo* methods because laboratory environments are not the same as what is obtainable in the field. When this has been done, commercial applications of nanoparticles in the field for controlling fungal pathogens will be feasible.

## Data availability

All data used for analysis in this manuscript are available from the corresponding author or first author upon reasonable request.

## Conflicts of interest

There are no conflicts of interest to declare.

## Acknowledgements

Timothy O. Ajiboye gratefully acknowledges the postdoctoral funding support from the University of the Free State, South Africa. Lisa Ann Rothmann (Plant Sciences: Plant Pathology, University of the Free State, Bloemfontein, South Africa) is acknowledged for reading and correcting the manuscript.

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