13 Ecofriendly methods in combating *Sclerotinia sclerotiorum* (Lib.) de Bary

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Abstract

Sclerotinia sclerotiorum (Lib.) de Bary is a soil-borne pathogen capable of infecting more than 400 host plants worldwide. It is a major pathogen that plays a crucial role in reducing the yield in economically important crops. The capability of sclerotia to survive for more than 4 years becomes very difficult to manage the crop from the infection of white mold fungus. Management of sclerotinia with chemical fungicides though remains successful; accumulation of pesticide residues in the edible parts threatens the scope for export of the commodities to other countries. Most of the conventional methods are

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not effective in management of S. sclerotiorum. In the midst of these obstacles, the antagonistic fungi Coniothyrium minitans has been commercialized for management of white mold fungus in both agricultural and horticultural crops. But the efficacy of biocontrol by C. minitans is not consistent. On the contrary, recent research activities on the usage of bacterial biocontrol agents for the management of S. sclerotiorum reflects that Pseudomonas chlororaphis (PA23), Bacillus amyloliquefaciens (BS6) and Pantoea agglomerans exert multiple mode of action and lead to the suppression of carpogenic germination and mycelial growth through the production of volatile and non volatile antimicrobial antibiotics. Moreover PA23 and BS6 triggers induced resistance via the production of defense related gene products. P. agglomerans degrades oxalic acid through the production of oxalate oxidase. Strains PA23 and BS6 protected canola crop from infection of stem rot fungus under field conditions. Since, mass multiplication of bacteria remains easier than fungal biocontrol agents, above-mentioned promising strains would pave the way for the management of S. sclerotiorum in both agricultural and horticultural crops. Development of consortial formulations with multiple modes of action will lead to the genesis of suitable bacterial biocontrol agents for controlling S. sclerotiorum in different cropping systems.

1. Introduction

Sclerotinia sclerotiorum is a ubiquitous necrotrophic pathogen that attacks a wide range of cultivated and wild plant species including canola (oilseed rape), mustard, alfalfa, soybean, field-bean, lentil, field pea, and sunflower. It results in damage of the plant tissue, followed by cell death and soft rot or white mould of the crop [1]. Initially the pathogen was first reported to infect sunflower during 1861. It caused root rot, stem rot and head rot in sunflower [2]. *S. sclerotiorum* infect 64 plant families, 225 genera and in total it affects 383 plant species [1]. But, subsequent survey during 1994 reflected a further increase in the host range of the pathogen. Pathogen was able to infect 408 plant species pertaining to 75 families and 278 genera and most of them belong to Dicotyledonae subclass of Angiospermae [3]. It causes head rot of sunflower [4,5] leaf blight of canola [6], pod rot of dry bean [7,8], blossom blight of alfalfa [9,10] and lettuce drop [11]. Most of the plants susceptible to the necrotrophic pathogen belong to Solanaceae, Cruciferae, Umbelliferae, Compositae, Chenopodiaceae and Leguminosae [12]. Flax, resistant to *S. scelrotiorum* became susceptible during the year 2000 in Manitoba and Saskatchewan [13]. Increase in host range of *S. sclerotiorum* narrows down the opportunity for disease management using either crop rotation or resistant varieties. This warrants for the development of eco-friendly management strategies for controlling the infection of white mold pathogen in different crop plants.

2. Economic importance

Sclerotinia sclerotiorum is omnipresent and has a very wide host range and causes economic losses in crops such as oilseeds, pulses, forage legumes, vegetables and ornamentals. There was severe yield loss due to the infection of Sclerotinia in vegetables such as lettuce, celery, potato and cabbage [1]. Average crop loss of drybean due to *S. sclerotiorum* was 30%, with individual field loss of 92% in Nebraska [14]. Yield loss of soybean in United States, Brazil, China, Argentina, India, Canada, Paraguay, Indonesia, Italy and Bolivia by *Heterodera glycines*, *Septoria glycines*, *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* in 1998 was 28.5×10^6 t, valued at U.S. 6.29×10^9 [15]. Crop loss of soybean in Canada during 2000 due to sclerotinia stem rot was estimated as 0.9% accounting for $7.2 million [16]. White mold of soybean is a devastating disease in Canada, northern US, Argentina and China. The annual loss in Canada was 6 million dollars [17]. Losses due to Sclerotinia range from 5-100% in individual canola fields [18]. In central Manitoba, 76% and 52% of canola fields were affected during 2001 and 2002 respectively [19]. Yield loss in Manitoba and North Dakota due to Sclerotinia rot was around $ 16,768,955 during 2001 [20]. Loss in the production of dry bean and snap bean in United States was around $ 26 and 13 million respectively. Canola growers in North Dakota and Minnesota realized a yield loss of $ 24.5 million during the year 2000. An annual loss of $ 15 million was realized by the sunflower producers in United States due to Sclerotinia infection [21]. Annual increase in yield loss due to *S. sclerotiorum*, in different crops warrants the development of management strategies to combat the necrotroph pathogen *S. sclerotiorum.*

3. Infection process

In canola (*Brassica rapa* and *Brassica napus*), and soybean (*Glycine max*) the disease manifests itself as stem rot, resulting in crop lodging and severe yield losses. Sclerotia of *S. sclerotiorum* remain viable in soil for many years. It imbibes moisture from moist soil and leads to germination of the sclerotia. Sclerotia germinate to produce apothecia (Carpogenic germination) or directly produce mycelium (Myceliogenic germination). Apothecia develop most rapidly when soils are saturated and temperatures are in the range of 10 to 20°C [22]. Fungal infection and mycelial growth is maximized in the presence of free water on the plant surfaces [22, 8]. Apothecia liberate ascospores into the air and land on the petals. Infection was initiated via the senescing petals that serve as an initial source of nutrients for the germination of ascospores landing on petals. Upon establishment the fungus deploys two main pathogenicity determinants, the secretion of oxalic acid and a battery of acidic lytic enzymes released by the advancing mycelium [23,24,8]. Stems and

petioles are infected, vascular tissues are disrupted, and stems, pods, or leaves beyond the site of infection die. As nutrients are exhausted, fungal mycelia aggregate into sclerotia that form both inside and outside the plant stem. These sclerotia then fall to the ground and over winter for years [25]. During the favorable environmental conditions resting structures germinate and initiate the disease cycle again.

4. Pathogenicity factors

Sclerotinia sclerotiorum secretes multiple pathogenicity factors. Degradation of plant cell wall, its components and tissue maceration occur by the concerted action of several extracellular lytic enzymes. Effective pathogenesis by *S. sclerotiorum* requires the secretion of pathogenicity factors like oxalic acid [26], extracellular lytic enzymes such as cellulases, hemi-cellulases and pectinases [27], aspartyl protease [28], endopolygalacturonases [29] and acidic protease [30]. These enzymes are highly active under the acidic conditions provided by oxalic acid and degrade the plant cell wall and tissues beneath it. Oxalic acid (OA) exerts a toxic effect on the host tissue by acidifying the immediate environment and by sequestering calcium in the middle lamellae leading to loss of plant tissue integrity [31,32]. Reduction in extracellular pH, activate the production of cell wall degrading enzymes [33]. OA directly limits host defense compounds by suppressing the oxidative burst [26]. In conjunction, plant cell wall-degrading enzymes, including cellulolytic and pectinolytic enzymes, cause maceration of plant tissues, necrosis followed by plant death [34]. Thus the release of an array of lytic enzymes and the oxalic acid from the growing mycelium is the pathogenicity factors that are required for the establishment of the host-parasite relationship.

5. Symptoms

Symptoms differ among host species; however, there are a number of similarities as well. Common symptoms are the appearance of water-soaked irregular spots on fruits, stems, leaves, or petioles. These spots enlarge and a cottony mycelium covers the affected area. The fungus spreads and the plant turns into a soft, slimy, water-soaked mass. The cottony mycelium produces numerous sclerotia (black seed-like reproductive structures) after host death, which is a reliable diagnostic sign of Sclerotinia infection (Table 1).

In contrast to the water-soaked symptoms, the host also exhibits dry lesions on the stalk, stems, or branches. Lesions later enlarge and lead to death of the affected plant part. Distal portions of the plant become yellow, then brown and finally lead to plant death. The girdled portion is often the base of the plant, which causes the plant to die. Sclerotia form within the stem pith cavities, fruit cavities, or between tissues (i.e., bark and xylem).

Table 1. Symptoms of Sclerotinia infection in sunflower, canola, beans, soybean and cabbage

Crop and disease	Symptoms	Reference
Sunflower 1. Root rot	Infection of roots leads to the appearance of water soaked symptoms on the aerial parts of the plants with cottony white growth on the stem. Stems turn brittle. Decay of parenchyma and cortex tissues of root followed by root rot and death. 50–100 sclerotia could be observed either inside the stem or root tissue.	[2,35,36]
2. Stem and Head rot	Appearance of water soaked lesion on the stem or receptacle of the head. Rot extends on both directions on the stem and head leading to rotting of the head and stem. Head of sunflower appears broom like. Cottony mycelium and sclerotial bodies are produced on varying shapes on the affected portions.	[2]
Canola 1. Stem rot	Initial symptoms of stem rot appear as water soaked spots on leaves or stems. Later the lesions on the leaf extend to petiole and infect the stem. Lesions on the stem appear as a pale grey to white lesions on the stem at or above the soil surface. As disease advances it spreads to upper branches including pods. Finally stem girdles at the point of infection, leading to wilting and death of the plant. Black sclerotial bodies are produced on or inside the hollow stem.	[37]
Beans 1. White Mold	Symptom develops as pale colored, water-soaked lesions after full bloom on blossoms as white cottony growth. Leaves, stems and pods in contact with the colonized blossoms are infected. Infected tissue turns dry and has a chalky or bleached appearance. Pathogen produces black sclerotia on the affected host tissues. Finally the infected plants die off with severe yield reduction.	[22]
Soybean 1. Stem rot	Blossom infection leads to the development of water-soaked symptoms on stem or pod, which often results from infected flowers. Few days after infection diseased stem are killed and become tan and eventually dries and shred. Infected plant parts have signs of the fungal pathogen as white, fluffy mycelium during humid conditions and sclerotia on the surface of the stem.	[38]
Cabbage 1. Head rot	Symptoms first appear as water soaked spots on lower or upper cabbage leaves. Water soaked spots enlarge, infected tissue becomes soft, and outer leaves begin to wilt. As the disease progress a white cottony growth becomes evident on the leaves. Finally the entire cabbage head would be covered with white cottony growth followed by the development of sclerotia on the head.	[1]

6. Eco-friendly strategies for the management of *S. sclerotiorum*

Management of *S. sclerotiorum* is a major challenge faced by plant pathologists. Management is difficult, inconsistent and uneconomical due to the presence of wide host range and long-term survival of the resting structures [39,2]. Since no single method can effectively control *S. sclerotiorum*, the best approach to control the pathogen is by the integration of various eco-friendly measures.

6.1 Site selection

Cultivation of susceptible crops to *S. sclerotiorum* will lead to the build up of inoculum density in the field. The degree of field infestation by *S. sclerotiorum* ranges from 0 to 85% [40]. Hence, the knowledge on the level of infection by *S. sclerotiorum* will help in the selection of field with reduced infection of the pathogen. Cultivation of resistant cultivars in the field with reduced inoculum pressure of *S. sclerotiorum* will help to minimise the loss encountered by the infection of pathogen.

6.2 Zero tillage and crop rotation

Integration of zero-tillage with crop rotation will reduce the risk of the crop from the attack of the necrotrophic pathogen *S. sclerotiorum.* The sclerotial bodies are seen near the top 2-3 cm of soil [41,40]. Carpogenic germination of the sclerotia occurs in the upper 5 cm soil profile [42,19]. They deteriorate faster by the attack of mycoparasites that dwell in the top soil [40]. But if the soil is ploughed the resting structures are buried deeper in the soil and have the capability to survive for several years. A significant negative relationship was found between sclerotial viability and depth of burial, and between sclerotial viability and populations of colonizing bacteria under zero-tillage condition [19]. Thus, the inoculum load of the sclerotia could be reduced well through zero tillage and there by infection of host plants by the pathogen could be minimized.

Cultivation of non-host crops to *S. sclerotiorum* will result in the reduction of inoculum load [24]. But the pathogen has more than 400 plant species as its host for survival. Three to four years of crop rotation didnot reduce the incidence of stem rot of canola [43]. A minimum of 5 year rotation of two non host crops of *S. sclerotiorum* is essential to decrease the infection level by the pathogen [2].

6.3 Seed treatment

Sclerotinia sclerotiorum survive in infected seeds as dormant mycelia in testae and cotyledons for 3 years or longer [44]. When infected seeds were

sown, 88-100% failed to germinate. Seedlings from infected seeds subsequently died from white mold at an early stage. Seeds that failed to germinate were rotted by *S. sclerotiorum*, and three to six sclerotia were formed in place of each seed [44]. These sclerotia could become a source of inoculum. Captan and thiophanate-methyl used in seed treatment were 100% effective in eradicating the fungus from the infected seeds [45].

6.4 Resistant cultivars

Sclerotinia sclerotiorum has a wide host range in pathogenicity [46]. The source of genetic resistance is limited and has hampered the development of resistant genotypes. Many researchers formerly believed that resistance to *S. sclerotiorum* did not exist. Since OA play a vital role in the establishment of pathogenicity, attempts made to degrade OA will enhance resistance against *S. sclerotiorum* by increasing the production of H_2O_2 mediated through oxidative burst. More recently, however, field resistance was observed in several crops against *S. sclerotiorum* [46]. In beans, some Great Northern and Black Turtle Soup varieties have disease resistance that are inherited quantitatively [47]. Unfortunately, since commercial white beans have many specific traits, attempts made to use these materials in breeding commercial white bean cultivars for white mold resistance have met with limited success. More recently, tolerance to white mold was discovered in the white bean ExRico 23 in Ontario [48]. The resistance was associated with its tolerance to oxalic acid secreted by the white mold fungus [49]. ExRico 23 was registered for commercial planting in Ontario and has since gained worldwide acceptance as a cultivar and as a main source for genetic resistance in white bean breeding [49]. Bean plants with partial physiological resistance, upright stature, narrow canopy and indeterminate growth had the capability to avoid the infection of *S. sclerotiorum* [50,51] 1993). Canadian short season soybean 80, transformed with wheat germin gene, that codes for oxalate oxidase (OXO) was resistant to the infection of *S. sclerotiorum* in both greenhouse and field testing [17]. Seven genotypes of common bean (NY6020-5; PC-50; M0162; G122; L 192; 19365-25 and B7354) had broad partial resistance to *S. sclerotiorum* [52]. Although complete resistance has not been identified in canola (oilseed rape), partial field resistance has been identified the Chinese variety Zhongyou 821 [53, 54]. Zhongshuang No. 9, a cultivar claimed to be better than Zhongyou 821 was reported in 2003 [55].

6.5 Biological control

In the light of present day concern about the environment, human health and development of resistance among the plant pathogens due to the continuous use of fungicides, biological control of plant diseases emerge as an attractive alternative for the management of plant diseases. Biological control

is the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man [56].

6.5.1 Antagonists

Sclerotinia sclerotiorum being a destructive pathogen, it over winters as sclerotia in the soil or on the plant debris. Soil microbial community plays an important role in reducing the inoculum build up of the pathogen. Among the microbes, both fungi and bacteria play a vital role in degrading the sclerotial bodies. Activity of microbes are in its peak near the soil surface. Diurnal fluctuation of soil temperature, moisture and relative humidity lead to the development of cracks on the sclerotial rinds. It results in leakage of cell constituents and get parasitized by the antagonistic microbes dwelling in the soil. The mycoparasitic fungi and bacteria associated with parasitized sclerotia include *Coniothyrium minitans*, *Trichoderma* spp., *Gliocladium* spp. *Sporidesmium sclerotivorum*, *Fusarium*, *Hormodendrum*, *Mucor*, *Penicillium*, *Aspergillus*, *Stachybotrys*, and *Verticillium* [24,57,58]. Among them, *C. minitans* and *Gliocladium virens* have shown practical potential for biological control of *S. sclerotiorum* [59].

6.5.2 Fungal antagonists

Coniothyrium minitans occurs naturally in soil as a mycoparasite of *S. sclerotiorum*. It was instrumental in the decline of viable sclerotia of *S. sclerotiorum* during crop growth and thereby suppresses the ascospores release [60,61]. *C. minitans* was first isolated from sclerotia of *S. sclerotiorum* in 1947. It was found to be associated with different soils and on several sclerotia-forming fungi [60,61]. It parasitizes sclerotia, destroys it and reduces airborne ascospore infections. Soil application of *C. minitans* to different host crops reduced the sclerotial viability by destroying the propagation units [62]. *C. minitans* is also effective under a wide range of temperature and soil humidity [63]. In general, the use of biocontrol agents is restricted to controlled environments because they need stable environmental conditions for successful establishment in the infection court so as to prevent the infection of the pathogen [64]. Although it has been claimed to be active under a wide range of environmental conditions it has failed in controlling the pathogen in Inner Mongolia soils in China (Liu Zhengping, personal communication). However, it suggests the scope for using *C. minitans* for field-grown crops [65,66].

Soil application of *C. minitans* as mycoparasite was effective in reducing the incidence of Sclerotinia wilt in sunflower by parasitizing the sclerotia produced in the soil and in the plant system [67,68]. Though *C. minitans* was successful in parasitizing the sclerotial bodies, it was unable to prevent the

secondary spread of the actively growing mycelium [68]. The carpogenic germination of sclerotial bodies of *S. sclerotiorum* was reduced by the mycoparasites *C. minitans* and *Talaromyces flavus* [69]. Though *T. flavus* suppressed the carpogenic germination of the sclerotia, it was inferior to *C. minitans*. Combined application of both *T. flavus* and *C. minitans* was not found to exert any synergistic or additive effect in the suppression of sunflower wilt [65]. Consecutive application of fungal antagonist is a pre-requisite to suppress the establishment of pathogen in the infection court. Soil application of either the above two antagonists continuously for two years suppressed sunflower wilt up to three years. But the crop raised during the subsequent year without the application of antagonist resulted in being susceptible [65]. Continuous monoculture of sunflower increased the natural population of *C. minitans* and *Trichoderma* spp., which in turn reduced the severity of sunflower wilt under field conditions [70]. The build up of antagonistic fungal flora during monoculture would increase the degradation of the sclerotia and thereby reduce the inoculum potential of the pathogen.

In general, irrespective of the host, disease severity of *Sclerotinia* increases only during bloom stage. Hence, protection of the petals of the susceptible crops by pre-colonization of the senescing petals with antagonist will favour the multiplication of antagonists and there by could prevent the establishment of ascospores on the infection court. Spraying spore suspension of *C. minitans* performed better in suppressing the white mold of dry bean [71]. Suppression of white mold was due to the effective colonization of senescent petals by *C. minitans* [72]. Though *C. minitans* performed better in controlling *S. sclerotiorum*, its performance was found to decline when the environmental conditions favor disease development [73] and its consistency was not stable compared to the application of benomyl under field conditions [74]. Treatment of sunflower seeds infected with *S. sclerotiorum* with conidia of *C. minitans* through film coating completely suppressed apothecial production of sclerotia and killed sclerotial bodies [75]. Incorporation of *C. minitans* A69 into potting mixture at the rate of 10^6 spores/g of potting medium or soil was effective (60-85% control) in controlling Sclerotinia rot of lettuce, cabbage and beans [76]. Recent research on *C. minitans* [77] has led to the development of a commercial biopesticide named "Contans". Application of "Contans" recorded 60% disease suppression on oilseed rape in a 2-year trial, but their experimental design was based on macroplots surrounded by guard areas to prevent major influences of invading external ascopores [78]. Earlier tests in microplots of oilseed rape proved a reduction of soil inoculum; however, this neither led to disease control nor to a yield improvement [66]. The reasons generally attributed are *S. sclerotiorum* ascospore dispersal occurs over long distances [79] and even a reduced number of sclerotia in a field can cause significant infection and yield loss [80] thus strongly affecting adjacent

plots [78]. Davies (1986) found [80] that the presence of only a few apothecia in the field might still result in relatively high disease incidence levels. Hence, there is a need for research into biocontrol of *S. sclerotiorum* on canola, specifically, on limiting petal infection by ascospores. Therefore studies on foliar applied biocontrol agents are worthwhile [81]. This is even more important in the case of Sclerotinia-canola system as the ascospores generally infect the plants at the flowering stage by infecting the petals. The bio-fungicide Contans WG, a product of *C. minitans* consists of $1x10^9$ viable conidia per gram of product. Incorporation of the product as a spray into the upper 5 cm soil layer colonizes the sclerotia in the soil and decays the sclerotia within 3 months after application. It is effective against the white mold fungus infecting vegetables, ornamentals, oilseed rape and beans [82].

Success of biocontrol agents depends on the environmental conditions that favor its proliferation and establishment in the infection court. Change in air temperature by 4^oC or the change in relative humidity by 5% affects the performance of the fungus *Alternaria alternata*, *Dreschlera sp.*, *Fusarium graminearum* and *Myrotheccium verrucaria* isolated from anthroplanes of bean and rapeseed in suppressing *S. sclerotiorum* [83]. Instead the antibiotic producer *Epicoccum purpurescens* was not influenced by the change in environment. Foliar application of spore suspension of *E. purpurascens* effectively controlled white mold of bean [10]. These findings emphasize that the antibiotic producers are not influenced by the environmental changes. Spray application of *E. nigrum* (an aggressive saprophytic colonizer) during flowering on the vines of kiwifruits prevented the establishment of infection process of the ascospores of *S. sclerotiorum* and thereby prevented the occurrence of fruit rot in New Zealand kiwi fruit [84]. *Trichoderma harzianum* (Th38) and *E. purpurescens* exhibited mycoparasitism against *S. sclerotiorum* [85]. The disease suppression was due to the effective saprophytic colonization of petals by the antagonistic fungi. Soil incorporation of sclerotial parasite *Sporidesmium sclerotivorum* was effective up to five years in controlling Sclerotinia stem rot of soybean, a major problem in USA [86].

6.5.3 Bacterial antagonists

Our planet is enriched with biodiversity, especially of prokaryotes. Bacterial antagonists like plant growth promoting rhizobacteria are exploited for the management of both foliar and soil borne pathogens of various economically important crop plants. Several bacterial antagonists such as *Bacillus*, *Pseudomonas* and *Agrobacterium* species are commercialized, for their potential role in disease management. But, research on the use of bacterial antagonists for the management of white mold fungus still remains to be explored and poorly studied. Strains of *Bacillus* spp. were frequently isolated

from the sclerotia of *S. sclerotiorum* from North Dakota in the USA. It adversely reduced the germination of infected sclerotia. Examination of infected sclerotia revealed that the integrity and colour of medulla was adversely affected [87]. Spraying of *B. cereus* strain alf-87A reduced the incidence of basal pod rot of pea caused by ascospore infection of *S. sclerotiorum* [88]. Foliar application of *B. subtilis* reduced white mold of white bean under field conditions [89]. However, results were not consistent with *B. subtilis* to control white mold of bean between fields [73]. Fifty three per cent of sclerotial bodies of *S. sclerotiorum* recovered from the soils of North Dakota were infected by *Bacillus* species. It increased degradation and reduced germination of the sclerotia [90]. *Erwinia herbicola* and *Bacillus polymyxa* inhibited the growth of *S. sclerotiorum in vitro* [91]. Bean plants pre-treated by *E. herbicola* in controlled environment had less disease severity than control plants [92], but its performance was not effective enough to suppress the disease spread under field conditions.

Antagonistic *Pseudomonas* spp. (DF41) and *P. chlororaphis* (PA23) inhibited the germination of ascospores of *S. sclerotiorum* causal agent of stem rot of canola [93]. Delivering of DF41 and PA23 on to petals increased bacterial population after 24 h and later decreased between 96 and 120 h after application. Significant differences in disease severity were found with respect to timing of ascospore applications in the control treatments (ascospores only). One isolate completely suppressed disease when co-applied with ascospores, while only minor suppression occurred when applied 24 or 48 h after. Results from all studies indicated that PA23 and DF-41 are effective biocontrol agents against *S. sclerotiorum* of canola [93]. A four-year study has shown that PA23 and DF41 have a wide scope for the management of canola stem rot under field conditions [94,95, Fernando et al., unpublished data].

Pantoea agglomerans isolated from leaves and flowers of canola produce oxalate oxidase and degrade oxalic acid produced by *S. sclerotiorum*, the pathogencity factor required for the successful establishment of the host-parasite relationship [93]. Pre colonization of infection court such as blossoms and leaves by *P. agglomerans* would be highly successful in preventing the infection process.

Similarly bacterial strains DF200 and DF209 isolated from canola, produced antifungal organic volatile compounds such as benzothiazole, cyclohexanol, n-decanal, dimethyl trisulfide, 2-ethyl 1-hexanol, and nonanal under *in vitro* conditions. These compounds inhibited sclerotia and ascospore germination. In addition it also inhibited the mycelial growth of *S. sclerotiorum* both under *in vitro* and in soil tests [96]. Augmentation of soil with DF200 and DF209 under field conditions would lead to the dissipation of these volatiles into soil and could suppress the carpogenic germination of sclerotial bodies and could prevent the release of ascospores. Hence,

development of consortial formulations that produce volatile, non-volatile antibiotics and oxalic oxidase could protect the crop better from the infection of white mould fungus.

Bacillus strains isolated from canola and wheat plants showed antifungal activity to *S. sclerotiorum in vitro.* Pre treatment of canola petals with *Bacillus* strains 24h before ascospores inoculation reduced disease severity than non-bacterized plants. Reduction in disease severity might be due to the pre-colonization of the petals and thereby inhibit ascospore germination [97]. Apart from pre-colonization, several *Bacillus* spp., also produced the antibiotic Zwittermicin-A that may be antifungal to *S. sclerotiorum* [98]. Spray application of *Bacillus amyloliquefaciens* strain BS6 (10^{-8} cfu ml^{-1}) on to petals at 30% bloom stage reduced Sclerotinia stem rot on canola by 60%. HPLC results indicated that the disease suppression was correlated to the induction of defense related secondary metabolites in canola leaves that suppressed the ascospore germination of *S. sclerotiorum* [99].

Pseudomonas chlororaphis strain PA-23 controlled ascospore germination, and stem rot of canola incited by *S. sclerotiorum* in both greenhouse and field studies. Antibiotics extracted from PA23 caused inhibition of sclerotial and spore germination, hyphal lysis, vacuolation, and protoplast leakage in a number of plant pathogens, including *S. sclerotiorum*, *Pythium aphanidermatum*, *Macrophomina phaseolina*, *Rhizoctonia solani, Sclerotium rolfsii*, *Fusarium oxysporum*, *Alternaria solani* and *Botryodiplodia theobromae* [100]. Inhibitory action of PA23 may be due to the synthesis of phenazine and pyrrrolnitrin [95]. Presence of these antibiotics was confirmed by sequencing the PCR products and through BLAST search in the Gen bank [101]. These results suggest that strain PA23 could be exploited for the management of various soil-borne pathogens including *S. sclerotiorum.* Studies on the management of white mold fungus with bacterial antagonists clearly indicated that there are potential bacterial antagonists that have the ability to degrade the oxalic acid, the pathogenicity factor. And several strains are bestowed with multiple modes of action to counteract the infection caused by stem rot pathogen under the canola ecosystem. As these strains perform well in the canola system it may perform well in other crops. Development of formulations with these bacterial strains will take care of several pathogens encountered during various stages of life cycle of the crop growth, which may help the farming community.

7. Other methods

Augmentation of soil with either organic or inorganic compounds leads to the proliferation of microbes that have the potential to suppress the germination of sclerotial structures present in the soil. Soil application of compost inhibited carpogenic germination of *S. sclerotiorum* and reduced

Sclerotinia infection in carrot [102]. Microbes in the compost might be responsible for the inhibition of sclerotial germination. Soil solarization is a method followed in different parts of the world for the management of soil-borne pathogens. Amending soil with S-H, CF-5 mixtures promoted the growth of *Trichoderma* sp, soil-borne bacteria and actinomycetes. It controlled carpogenic germination of *S. sclerotiorum* [103,104,105,106]. Solarization of soil with transparent thick plastic sheets of 60 micron thickness over two months period under field conditions reduced the incidence of lettuce drop caused by *S. sclerotiorum* by 76% [107]. Timely sowing, field sanitation, burning of stubble deep plowing followed by crop rotation with irrigated rice cultivars and seed treatment with benomyl (0.1%) was found effective in managing Sclerotinia rot in Indian mustard [108].

8. Conclusions

Management of *S. sclerotiorum* still remains as a challenging task worldwide. Since *S. sclerotiorum* has a very wide host range the techniques adopted for management does not work well. The pathogen produces resting structures that could inhabit the soil up to 5 years without loosing its viability. In addition, carpogenic germination of sclerotial structures releases millions of ascospores. The senescing petals of the crops serve as a nutrient source for the proliferation of ascospores to establish pathogenicity. Hence, management of sclerotia resting in the soil is alone not sufficient to reduce the disease. Instead the infection court such as petals and the leaves has to be protected from the ingress of ascospores.

Though cultural methods such as seed treatment, altering row, plant spacing, and cultivation of crops with non-lodging characters, application of organic amendments, soil solarization and crop rotation aid to reduce the disease, none are highly effective to develop a reliable strategy for managing Sclerotinia rot. Amid these scientific developments, several biocontrol agents of fungal origin emerge as a promising tool for the management of white mold fungus. *C. minitans* has been commercialized and marketed as Contans WG for the management of Sclerotinia rot in both agricultural and horticultural crops. However, *C. minitans* was not consistent under field conditions. The research on the exploration of bacterial antagonist for the management of white mould fungus is very limited. Instead, in recent years our lab has carried out pioneering work by using bacterial antagonists on petals for the management of *S. sclerotiorum* in canola. The bacterial antagonists such as *P. chlororaphis* PA23, *B. amyloliquefaciens* BS6 and *Pseudomonas* sp. DF41 strains has multiple mechanisms which play a vital role in suppression of Sclerotinia infection both under field and greenhouse conditions. These strains produce volatile and non-volatile antibiotics. Volatile antibiotics diffuse in to the soil and inhibit both mycelial and carpogenic germination of the resting

structures, thereby possibly reducing the inoculum potential of the pathogen. Production of multiple antibiotics by strain PA23 has broad spectrum of action against various soil borne pathogens, in addition to *S. sclerotiorum* [100]. If biocontrol agents aim only a specific pathogen, then the other pathogens might dominate and destroy the crop. In addition, these strains also proliferate well on petals of canola and protect the crop from the infection of ascospores and reduce stem rot disease [97].

Effective pathogenesis of Sclerotinia requires the secretion of oxalic acid. Degradation of oxalic acid will be a benefit for the management of Sclerotinia infection. Oxalate oxidase converts oxalic acid to carbon dioxide and hydrogen peroxide. Hydrogen peroxide helps in structural reinforcement of plant cell walls leading to cell wall thickening and arrest the penetration of pathogen [109]. Increased levels of H_2O_2 in plant tissues are toxic to microbes [110], and also it triggers lipid peroxide and salicylic acid synthesis, that play a vital role in signal transduction leading to the induction of hypersensitive reactions coupled with the synthesis of pathogenesis related proteins and phytoalexins [111].

The bacterial antagonist *Pantoea agglomerans* produce oxalate oxidase, and suppress stem rot of canola [93]. *P. chlororaphis* PA23 induce systemic resistance by inducing the defense gene products such as peroxidase, polyphenol oxidase, chitinase, glucanase, phenols and phenyl alanine ammonia lyase in different crops [106]. Owing to the benefits of antagonists, development of consortia comprising of antagonists with multiple mechanisms will protect the crop from *S. sclerotiorum* and could lead to the development of a successful candidate to manage the necrotroph pathogen. Though, recent research on biological control of white mold fungus with antagonists pave the way for preparation of a better disease management strategy, following research has to be executed for developing a potential candidate.

1. Identification of native biocontrol agents (BCA) with high competitive saprophytic ability and rhizosphere competence, which possess wide spectrum of biological suppressive activity against more than one pathogen.
2. Improvement of bio-efficacy of identified antagonists/hyperparasites.
3. Development of a biocontrol consortium, which would have wider adaptability to different ecological niches.
4. Monitoring the population stability of the BCA in relation to pathogen population and their ecological parameters that would ensure biological balance. This is essential to regulate the augmentation of biocontrol inoculum.
5. Large-scale production of inoculum and developing suitable inexpensive delivery systems.

6. Standardization of quality parameters for various biocontrol formulations, specifically, the viability of propagules and the minimum inoculum requirement based on cfu/g and their keeping quality.
7. Developing BCAs having compatibility with agrochemicals is essential to develop Integrated Disease Management (IDM) strategies.
8. Popularization of this eco-friendly technology between farming community with proper instructions for use.

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