



Carbon source dependent-anaerobic soil disinfestation (ASD) mitigates the sclerotial germination of *Sclerotinia sclerotiorum*

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Abstract

Though *Sclerotinia sclerotiorum* is a well-studied plant pathogen that causes significant economic damage worldwide, sustainable and environmental friendly control methods are difficult to establish due to its wide host range, cosmopolitan distribution and production of recalcitrant structures that can survive in soil for a long time. The pathogen was found causing a severe disease incidence on cabbage in 2016 in Sri Lanka. It was hypothesized that a) isolates of the recent disease outbreak display cross resistance to commonly applied fungicides and b) carbon (C) source supplemented Anaerobic Soil Disinfestation (ASD) is effective in mitigating the germination of sclerotia. *In vitro* fungicide sensitivity assays showed large variation in mycelial growth inhibition indicating high adaptability of the population towards environmental fluctuations and management practices. Signatures of cross resistance were evident. ASD was carried out using cabbage (*Brassica oleracea*) and leek (*Allium ampeloprasum*) cull piles, durian (*Durio zibethinus*) peels and grass cuttings (*Axonopus compressus*) as C sources and determined the sclerotial viability. Cabbage and leek cull piles at rates of 60–100 mg/g soil completely inhibited sclerotial germination. Maintaining anaerobic condition along with C source amendments was found to be a critical step in mitigating the sclerotial germination. GC-MS analysis of the volatiles of cabbage leaves, leeks and durian further confirmed the presence of various bioactive compounds with potential antifungal activity. Therefore, in addition to elevated microbial activity in treatments, the volatiles of C sources may have helped mitigating sclerotial germination.

Keywords Anaerobic soil disinfestation · Fungicides · *Sclerotinia sclerotiorum*

Introduction

Sclerotinia sclerotiorum is a well-studied necrotrophic soil borne fungus thriving in more than 400 plant species (Boland and Hall 1994). It is known to produce melanised hardened hyphal aggregates called sclerotia that can survive in soil for more than 8 years (Ben-Yephet et al., 1993). Long term survival ability of sclerotia is due to the presence of exterior black

protective coating that contains melanin pigments, which are highly resistant to degradation. Unfortunately, no sources of complete resistance have been found among many crops (Garg et al. 2010). Though not sustainable and effective against soil inoculum, fungicides from different chemical families have been providing a temporary solution against the pathogen (Lehner et al. 2015). Methyl Benzimidazole Carbamate (MBC) fungicides have been widely used to control *Sclerotinia* since 1980 (Chase 1992). Later, development of resistance has been reported around the world due to a single nucleotide mutation at the position 198 of the β -tubulin gene altering amino acid glutamic acid to alanine (Xu et al. 2005). In addition, change of phenylalanine to tyrosine at the codon 200 has also been reported (Duan et al. 2015). Lehner et al. (2015) reported mutation at codon 240 in β tubulin gene (L240F mutation) replacing leucine by a phenylalanine causing resistance to thiophanate methyl (a benzimidazole fungicide) in *S. sclerotiorum*.

Soil chemical-fumigation with methyl bromide has been the most effective method of controlling soil borne pathogens including *S. sclerotiorum*. However, due to number of unfavourable

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