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Scanning Electron Microscopic observation of mycelium and Sclerotium of stem rot of groundnut causing Sclerotium *rolfsii*

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Abstract: *Sclerotium rolfsii* was affect the many crops including wild and cultivated crops and make the destructive yield losses. It producing resting structure (sclerotia) survive in the soil upto 14 years. Which producing mycelium colonized on the any plant part which attached to the soil and disintegrate the tissues followed by reduce the economic quality of crops. These pathogens produce the mustard shape brown colour sclerotia and hyaline septate mycelium, which characters varied based on the isolates where collected. This septate mycelium penetrates the stem region, these penetrated mycelium stem region were observed on the Scanning Electron Microscope. Which sclerotia and mycelium also observed on the SEM.

Keywords: Groundnutstem rot, Sclerotium rolfsii, SEM, Sclerotia and Mycelia growth.

1. Introduction

Sclerotium rolfsii is harmful soil borne pathogen. It affects the wide range of crops and which through the creation of appressoria, a pathogen can directly enter unharmed host seedlings. The disease can also enter through natural openings such as lenticels and stomata, and it spreads in both directions from the point of entry. Smith et al. (1986) found that within 24-48 hours after inoculation, hyphae from germinating sclerotia ramify over numerous host tissues. The persistence of the pathogen in the soil and the large variety of hosts frequently hinder the efficacy of stem rot disease management. Scanning Electron Microscopy was used to better understand the early infection phase and plant pathogen interactions involved in tolerance and susceptibility in groundnut challenged with *Sclerotium rolfsii* (SEM).

2. Materials and Methods

2.1. Scanning Electron Microscope (SEM) analysis

Stem rot infected groundnut The collar region was sampled at 24-hour intervals for four days after inoculation, as indicated by Nandi *et al.* (2013). The samples were sectioned with a fine edged razor and dried in a hot air oven at 0.2 to 0.5 mm thickness 50^0C during four days using double-sided

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sticky tape, the samples were affixed to SEM (Scanning Electron Microscope) aluminum stubs and sputter-coated with gold particles. Prior to SEM, the gold particles were ionized using an ion coater. The mycelia expansion was documented in inoculated as well as control samples of groundnut (healthy) of variety, VRI 2. The images were captured with a Scanning Electron Microscope. Besides, to investigate the parasitism of *S. rolfsii* mycelium by effective *T.longibrachiatum*[T(SP)-20], The pathogen was injected at a consistent distance from the edge of the Petri dish using a 9 mm mycelial disc cut from the leading edge of a Trichoderma colony. A 1 cm2 agar block was removed. Mycelia samples from the contact zone were fixed with glutaraldehyde and osmium tetroxide vapours (3:1) for 24 hours, air-dried for 48 hours, and then analyzed (Solimanet *et al.,* 2016), Thillaigovindan, N. (2022). Madurai Kamarajar University, Madurai, coated the treated samples with gold sputter and analyzed them with a SEM (TESCAN VEGA3 SBH model).

3. Results and Discussion

For the morphological confirmation, the mycelia and sclerotial structure of *S. rolfsii* were observed in the Scanning Electron Microscope. The sclerotial germination, septate mycelium and clamp connection were observed in the Stereoscopic microscope. The mycelium of the *S. rolfsii* colonized the stem region of groundnut plant, which was clearly seen under Scanning Electron Microscope. Mycelium was found to penetrate the parenchymatous cells and degenerated the tissue. Chet (1975), as well as Zarani and Christias (1997) observed the structural development and sclerotia formation of *S. rolfsii* through by Scanning Electron Microscope (SEM). They found that the Mycelia often forms sclerotia when they completely cover the agar surface. Erental et al (2008) observed that the main process for sclerotia formation was the branching and fusing of S. sclerotiorum hyphae. On the fourth day of culturing, Ordóez-Valencia et al. (2015) observed the formation of sclerotia primordial in SEM, and the colour of the sclerotia changed from white to dark on the eighth day. On the thirdDAI, Nandi et al. (2013) discovered mycelia growth of S. rolfsiiseenin cowpea xylem vessels. Recently, Rajasekhar et al. (2019) reported that SEM observations revealed direct penetration of S. rolfsii hyphae through the cuticle and subsequent development in both inter and intracellular layers 48 hours after inoculation in susceptible groundnut variety Narayani. It also colonised fungal mycelium completely within 72 hours of inoculation, causing tissue collapse in susceptible genotypes. In comparison, the mycelia structure was not observed in resistant genotype of ICGV 86590 (Fig.1).

Figure 1. Scanning Electron Microscopic view of mycelium and sclerotium of *Sclerotium rolfsii* and mycelial colonization and groundnut stem portion

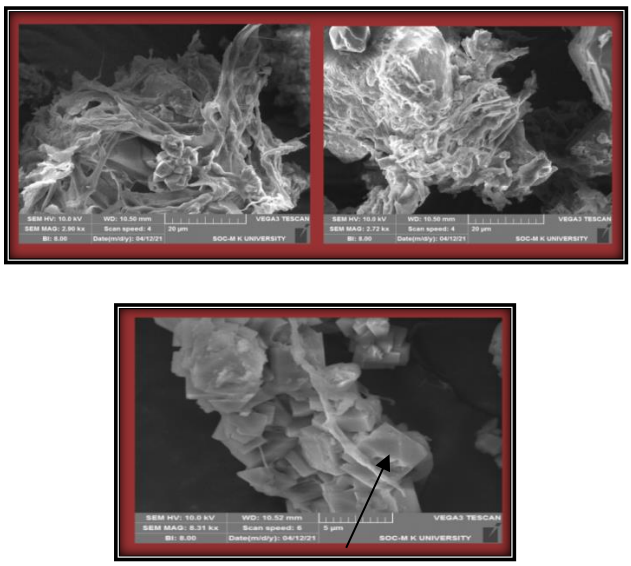

Figure 2. Mycelial colonization on groundnut stem portion

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