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Research Article

# Morphological characterization of *Sclerotinia sclerotiorum* causing fruit rot of papaya and its management using biopesticides

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## ABSTRACT

Papaya is an important fruit crop and susceptible to several post-harvest diseases at every stage of its life cycle. Papaya fruit rot caused by *Sclerotinia sclerotiorum*, is a serious postharvest disease that results in significant losses both in storage and on the market. Papaya fruit that was gathered from several Lahore fruit marketplaces was the subject of the studies. Based on physical and cultural traits, diseased papaya fruits were separated and identified. The virus developed mycelial colonies on PDA media that were sparse and creamy white. These colonies ultimately became fluffy and light brown. Sclerotia began to appear after three days of mycelial growth, and after seven days, they were fully visible in the shape of rings or distributed across the mycelial surface of the Petri plate. This allowed for the calculation of the frequency of isolated fungi. It was recorded maximum percentage of *S. sclerotiorum* (58%) was recorded followed by *Alternaria alternata* (21.5 %) and *Aspergillus niger* (10.0 %), while *Penicillum spp*. was least isolated fungi with a mean value of 5.00%. The assessment of different plant extracts including (*Cascabela thevetia, Euphorbia milii, Moringa oleifera, Plumeria rubra, Quisqualis indica, Syzygium aromaticum*) was done with Agar Well Diffusion Technique under CRD design. The findings showed that of the medicinal plants that were extracted using methanol and ethanol, *Moringa oleifera* and *Syzygium aromaticum* showed significant results at higher concentrations and showed maximum zone of inhibition while *Cascabela thevetia* was least effective against *S. sclerotiorum*.

**Keywords:** *Sclerotinia sclerotiorum*, Papaya, Fruit rot diseases, Management, Pakistan.

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## INTRODUCTION

The papaya (*Carica papaya* L.,) is an important fruit crop cultivated all over the world. It belongs to the *Caricaceae* family, which includes 48 species. Native to tropical regions of North America, papayas are herbaceous plants with a brief lifespan (Sing, 2013). After bananas, mangoes, and pineapples, papayas are the fourth most traded tropical fruit. Just ten countries produce over 75% of the papayas grown worldwide. India is the world's top producer of papayas, with Brazil, Indonesia, Nigeria, and Mexico following suit (FAO, 2017).

Papaya plantations in Pakistan's Sindh and Punjab provinces are verdant. The papayas grown in Sindh's Malir and Thatta districts are renowned for their size and mobility. Papaya is highly destructible fruits and the rate of spoilage in transit is high. Other fruits guava, mango and banana are competing well with papaya as compliments to net-returns produced to orchardists. In malir region of Karachi, two varieties of papaya (Sindh and Bombay) are grown.

Due to their high nutritional and moisture content, papaya fruits are particularly vulnerable to disease carried on by a variety of bacteria, including fungus.

Papaya losses during transportation are mostly caused by postharvest and orchard diseases, which have a significant impact on papaya productivity and quality (Chau and Alvarez, 1990). Papaya postharvest losses of 10–40% in sea shipments and 5–30% in air shipments are typical; disease-related losses varied from 1–93%, contingent on postharvest handling and packaging technique (Alvarez and Nishijima, 1987). Many postharvest infections are caused by wounds sustained during and after harvest. Mechanical damage to the produce's skin, such as cuts, insect punctures, and scratches and abrasions, significantly facilitate the infection process, especially during postharvest (Wills et al., 1989). Certain situations, such as latent infections, include vaccination before harvest, but the disease does not manifest itself until the postharvest phase (Kays, 1991). Certain fungi can directly infect fruits by penetrating their undamaged cuticles, lesions, or naturally occurring openings in their surfaces. For several infections, the first stage of invasion requires the creation of enzymes. Additionally, the postharvest phase growth of fungal infection can be influenced by the fruit's physiological age, mechanical damage, temperature, and storage conditions (Ilag et al., 1994).

Fungal pathogens precariously attack many parts of papaya plant from root to fruits. Nearby 171 different fungi attack papaya in the world. Papaya fruits are decomposable goods suffering from severe post-harvest damages. Several postharvest diseases including Rhizopus rot, Aspergillus rot, Penicillium rot, Fusarium rot, Alternaria rot, Anthracnose and fruit rot have been reported to cause spoilage of papaya fruit at different stages (Sharma, 2015). Among all mentioned diseases, fruit rot caused by *Sclerotinia sclerotiorum* has become most important disease in Pakistan. Symptoms of ripe fruit consisted of softening, discoloration, watery rot with white fuzzy mycelium, and initial sclerotium formation.

It is difficult to control *sclerotinia sclerotiorum* because of the long- term constancy of sclerotia and irregularity of infection. It is important to match the management arrangement with the growth stage of the host species while assimilating multiple management strategies in addition to cultural practices, pesticides and biological control. Utilizing an aggregation of management access concurrently offers the best opportunity to prevent the elimination from the pathogen *sclerotinia sclerotiorum* (Aysan, E. and S. Demir, 2009).

Medicinal plants are utilized nowadays as an environmentally friendly and safe substitute for synthetic fungicides due to their potent antifungal action against a wide variety of fungus. Plant extracts have been shown in several investigations to exhibit antifungal action against a variety of phytopathogenic fungus (Panti and kolte, 2006). Therefore, the present research was aimed to isolate *Sclerotinia sclerotiorum* and to find some novel biopesticides for its management.

## MATERIALS AND METHODS

### Isolation of *Sclerotinia sclerotiorum* from diseased fruits

The diseased papaya fruit showing the typical symptoms were collected from sdiffernt fruit markets. The pathogen was isolated from an infected specimen exhibiting characteristic symptoms on Potato Dextrose Agar (PDA) media. The plates were incubated at 25 ± 2 C temperature for 24-48 hours. The purified isolates were then kept on PDA slants and utilized in additional studies.

### Preparation of plant extracts

The medicinal plants (*Cascabela thevetia, Euphorbia milii, Moringa oleifera, Plumeria rubra, Quisqualis indica, Syzygium aromaticum*) were taken from the Department of Botany, University of Lahore. All of the plants were cleaned with tap water, dried out, and crushed with liquid nitrogen, and extracted (for 48 hours) using methanol and 100% ethanol in a soxhlet device (Ndukwe et al., 2006). At temperatures below 50°C, the solvents were extracted individually using a rotary evaporator (Heidolph, VV2000) operating at decreased pressure. Up to the time of analysis, the resultant crude extracts were kept at 20°C. Dimethyl sulphoxide (DMSO) was used for making stock solutions and extract dilutions in sequence (Ambrozin *et al.,* 2004).

### *In vitro* antifungal assay of medicinal plant extracts

Using the agar well diffusion technique, the antifungal activity of a few chosen medicinal plants was evaluated against an isolated pathogen (Perez *et al.,* 1990). PDA medium was produced for this use, and 20 ml of the medium was added to each of the 9 cm petri dishes. Using a sterile corn borer, four wells measuring 6 mm in diameter were created in each petri plate following solidification. Using a micropipette, various doses (5, 15, 25, and 50 µg/mL) were produced in DMSO and added to each well. Using a sterile loop, a purified fungal colony (5 mm) was selected and put in the center of each petri dish and were incubated at 25°C for 2 days.

Three replications of each of the seven treatments were used in the experiment. The same concentration of DMSO

that was used to evaluate the extracts was employed in the control experiment. The extracts formed inhibition Zone against the fungal pathogens was assessed after they were dissolved in DMSO.

Dia. of Inhibition Zone = $\frac{\text{diameter of sample} - \text{diameter of control}}{\text{diameter of control}}$ (Aznita *et al.,* 2011)

**Statistical analysis**

The computer program MSTAT-C was used to statistically evaluate data collected (Russel and Eisensmith, 1983). The overall significance of the data was tested using ANOVA procedures, and the differences between the treatment means were compared using the Least Significant Difference (LSD) test (P≤ 0.05).

## RESULTS

**Cultural and morphological characteristics of the fungus:**

Fruit fragments with infection and sclerotia on the lesion surface were used to isolate pathogens, and they produced creamy white mycelial colonies on PDA media. The hyphae were septate-branched, multinucleate, hyaline, and were 3.5–4.5 µm in diameter. On PDA media, the virus generated sparse, creamy white mycelial colonies that developed into fluffy, light brown colonies over time. The whole (90 mm) Petri plate media was covered by mycelial colonies after 7 days. After a period of 72 hours, the first signs of sclerotia were seen. These appeared in seven days, either in the shape of rings or dispersed over the Petri plate's mycelial surface. Sclerotrophia were originally silvery white, but as the fungal culture grew older, they began to take on a dark brown or black hue. Their sizes ranged from 3.5 to 8.7 x 2.5 to 5.5 mm with spherical, oval, or cylindrical shapes (Table 1).

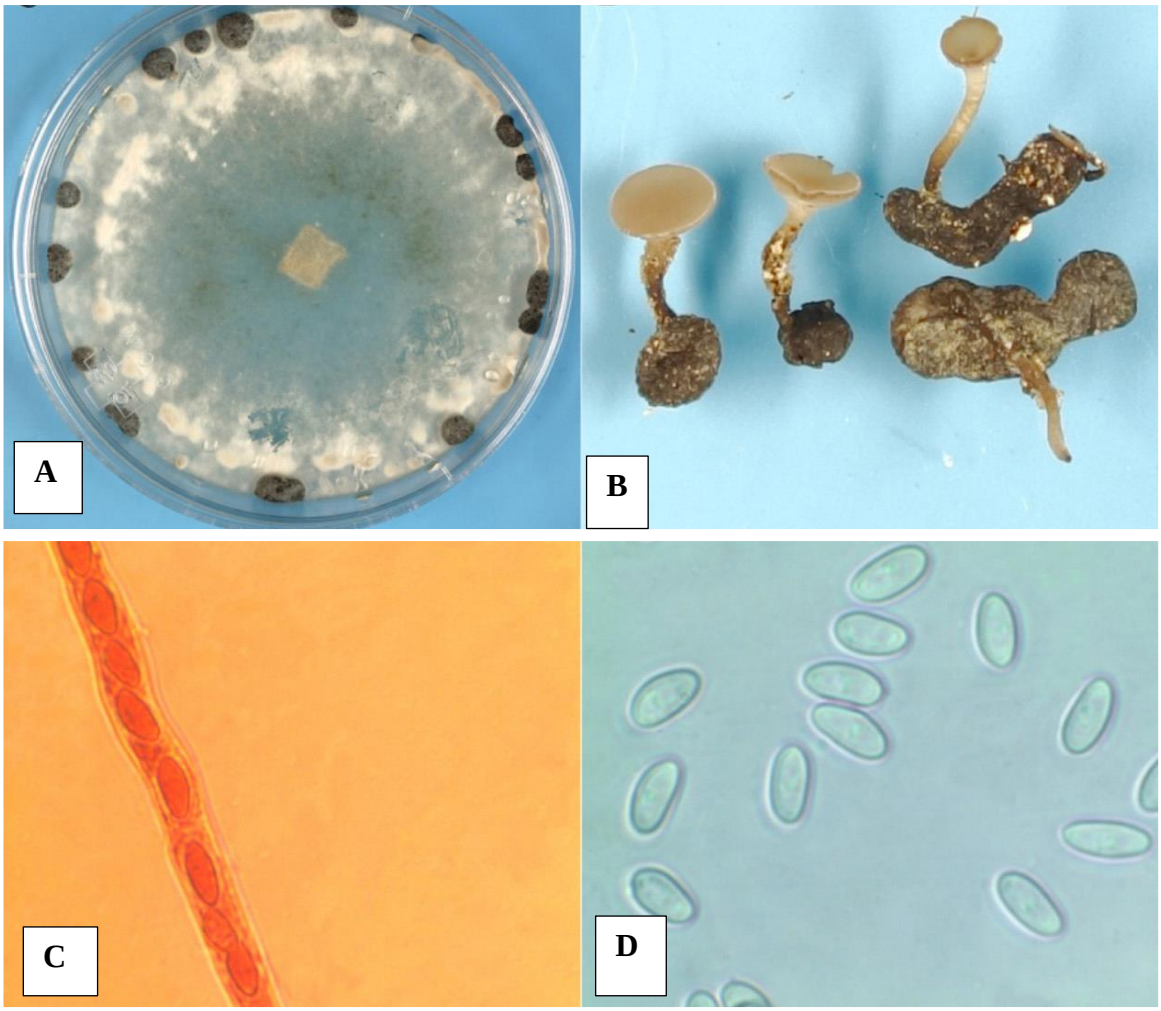


Figure 1. A, Three-week-old colony and black sclerotia of S. sclerotiorum growing on potato dextrose agar medium; B, Apothecia; C, Ascus containing 8 ascospores; D, Ascospores.

Table 1. Cultural and Morphological features of *S. sclerotiorum*.

| Cultural and morphological parameter* | Description |
|---|---|
| Hyphae color | Hyaline |
| Diameter | 3.5-4.5 µm |
| Colony color | Creamy white to light brown |
| Colony growth rate (cm/day) | (1.2) 24h, (4.3) 48h, (6.8) 72h, (9.0) 96h |
| Type of growth | Sparse, fluffy |
| Initiation of sclerotia formation | 72 h after incubation |
| Shape of sclerotia | Whitish to blackish |
| Average size of sclerotia | Spherical to oval or cylindrica |
| Position of sclerotia in Petri plate | Appeared strewn in the plate's center or as a ring around the edge |

**Frequency of isolated fungal pathogens**

During isolations from diseases fruits, maximum percentage of *S. sclerotiorum* (58%) was recorded followed by *Alternaria alternata* (21.5 %) and *Aspergillus niger* (10.0 %). The least isolated pathogen was *Penicillum spp*. (5.00 %). Table 2.

Table 2. Overall percentage of fungi isolated from rotted papaya fruit.

| Fungi isolated | Frequency (%) |
|---|---|
| *S. sclerotiorum* | 58.0 |
| *A. alternate* | 21.5 |
| *Asprgillus niger* | 10.0 |
| *Rhizopus stolonifera* | 7.00 |
| *Penicillium spp.* | 5.00 |

**Antifungal potency of medicinal plant extracts**

Among different medicinal plants extracted in ethanol, *Moringa oleifera* at the concentration of 50 µl/ml, showed maximum inhibition zone of 3.5 mm followed by *Syzygium aromaticum* (3.26 mm and *Quisqualis indica* 2.8 mm) while *Euphorbia milii* showed least inhibition zone of 2.23 at 50 µl/ml. In case of methanolic extracts, it was observed that *Syzygium aromaticum* showed maximum inhibition zone of 3.26 mm at concentration of 50 µl/ml while *Plumeria rubra* exhibited least inhibition zone with a value of 2.56 mm at 50 µl/ml.

Table 3. Effects of different ethanolic medicinal plant extract on the mycelial growth of *S. sclerotiorum* after 7 days.

| Treat | Concentration | | | | Mean |
|---|---|---|---|---|---|
| | 5µl/ml | 15µl/ml | 25µl/ml | 50µl/ml | |
| *Euphorbia milii* | 2.83±0.026ef | 2.53±0.038hij | 2.60±0.021ghi | 2.23±0.014l | 2.55±0.066C |
| *Plumeria rubra* | 2.32±0.085kl | 2.45±0.037jk | 2.60±0.057ghi | 2.50±0.063ij | 2.47±0.041D |
| *Cascabela thevetia* | 3.00±0.021cd | 3.23±0.046b | 2.83±0.095ef | 2.73±0.021fg | 2.95±0.062A |
| *Syzygium aromaticum* | 3.03±0.018cd | 2.90±0.036de | 2.70±0.025fg | 3.26±0.067b | 2.97±0.064A |
| *Moringa oleifera* | 2.52±0.039ij | 3.13±0.036bc | 2.53±0.031hij | 3.50±0.075a | 2.92±0.127A |
| *Quisqualis indica* | 2.70±0.056fg | 2.66±0.028gh | 2.70±0.055fg | 2.80±0.064ef | 2.72±0.027B |
| Mean | 2.73±0.064B | 2.82±0.072A | 2.66±0.030C | 2.84±0.106A | |

(P>0.05).

Table 4. Effect of methanolic medicinal plant extract at different concentration on the mycelial growth of *S. sclerotiorum* after 7 days.

| Treat | Concentration | | | | Mean |
|---|---|---|---|---|---|
| | 5µl/ml | 15µl/ml | 25µl/ml | 50µl/ml | |
| *Euphorbia milii* | 3.10±0.074a-d | 3.06±0.059a-d | 3.03±0.040b-e | 2.90±0.149def | 3.02±0.045AB |
| *Plumeria rubra* | 2.93±0.050c-f | 2.60±0.046gh | 2.80±0.051fg | 2.56±0.061h | 2.72±0.051C |
| *Cascabela thevetia* | 3.06±0.047a-d | 3.13±0.024abc | 3.13±0.074abc | 3.03±0.070b-e | 3.09±0.028A |
| *Syzygium aromaticum* | 3.10±0.058a-d | 2.96±0.028a-f | 3.10±0.060a-d | 3.26±0.103a | 3.11±0.043A |
| *Moringa oleifera* | 3.00±0.133b-e | 2.90±0.085def | 2.83±0.053ef | 2.96±0.083c-f | 2.92±0.044B |
| *Quisqualis indica* | 3.20±0.062ab | 2.96±0.032c-f | 3.00±0.051b-f | 2.93±0.038c-f | 3.02±0.038AB |
| Mean | 3.07±0.033A | 2.94±0.044B | 2.98±0.036B | 2.94±0.059B | |

(P>0.05).

## DISCUSSION

One of the most destructive soil-borne pathogens, *S. sclerotiorum*, is responsible for papaya fruit rot and is endangering the production of other crops worldwide (Zhao et al., 2004). Papaya fruit with naturally occurring disease was used to isolate *S. sclerotiorum* on PDA. Following the fungus's separation, the hyphal tip procedure was used to purify the isolated fungal culture. A combination of physical and cultural traits was used to identify the *S. sclerotiorum*. Without zonation, a consistently one type of fungal colony began to develop on PDA, taking 72 hours to cover the entire Petri plate in growth that ranged in color from white to gray. The perimeter of the Petri plates acquired tiny mycelial tufts after five days of fungal development. Eventually, from converted mycelial tufts, firm, black sclerotia were formed. A single sclerotium was fixed and completely encircled by a white mycelium net. Sclerotria developed in oblong to asymmetrical shapes. Hyaline and branching hyphae were observed under a microscope. Ascospores are single-celled, hyaline, elliptical to oval, and there are eight ascospores per ascus. These outcomes agree with the conclusions of a number of previous researchers. According to cultural traits, Pones et al. (1979) identified the fungus they recovered from beans, cabbage, and lettuce. In PDA, colonies of *S. scleotiorum* developed globose to irregular black sclerotia, as reported by Kim and Cho (2003) and Abdel Kader et al. (2012). The colonies were white to grey. Corresponding to this, reports of global research on the morphological and cultural aspects of *S. sclerotiorum* mycelial and sclerotial traits in a variety of crops (Zhao et al., 2104; Luong *et al.,* 2010).

The pathogen invasion and the start of the disease are supported by the ideal climatic conditions and the dropping of old blooms. According to Luong et al. (2010), the germination of sclerotia and the production of apothecia in the bean crop in Vietnam are dependent on chilly, rainy weather. Ascospore release may occur at the same time as faded flowers fall into leaf axils, which is thought to be the perfect environment for the start of a pathogen infection. For this reason, it has been noted that senescent blossoms and chilly, humid conditions are important for *S. sclerotiorum* disease in climbing beans, peanuts and chilies (Goswami et al*.,* 2012). After 10–12 days inoculation, the fungus produced water-soaked lesions and sclerotia on papayas' fruit. The fruit rot disease survey records of fungus host as well existing publications indicate that the study provided a new investigation about the problem in Pakistan.

During *In vitro* evaluation of medicinal plant ethanolic and methanolic extracts against *S. sclerotiorum* varied significantly. *Moringa oliefrera* and *Szygium aromaticum* give greatest results against *S. sclerotiorum* when extracted in ethanol and methanol at maximum concentration. Comparable results were described by different researchers that *Moringa oliefrera* give best results against *Alternaria alterrnata, Aspergillus flavus*, *Colletotrichum gloeosporioides, Colletotrichum musae, S. sclerotiorum*, *Penicillium spp.* at maximum concentrations (Maqbool et al*.,* 2010; Avasthi et al*.,* 2010; Necha et al*.,* 2008; Shivpuri and Gupta, 2001; Dar et al., 2007).

## CONCLUSION

This study has demonstrated the possibility of extracts from *S. aromaticum* and M. oliefera as viable substitutes for synthetic fungicides against *S. sclerotiorum.* The current method of managing post-harvest diseases can help reduce the dangers and threats that toxic fungicides have to the environment and the health of consumers, which is the primary factor in their acceptability and natural origin.

## COMPETING OF INTEREST

The authors declare no competing interests.

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