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RESEARCH ARTICLE

# *In-vitro* Assessment of Fungicides and pH Levels on the Mycelial Growth and Sclerotia Production of *Sclerotium rolfsii* Sacc. causing White Rot of Onion in Manipur

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## ABSTRACT

An onion white rot fungicide, Sclerotium rolfsii Saccardo, was tested in Manipur. The fungicides were tested on PDA using the poisoned food approach. The effect of pH on potato dextrose broth was assessed by weighing dry mycelium and counting sclerotia generated by the fungus. The white rot pathogen was completely inhibited by three fungicides: Hexaconazole (500 and 1000ppm), Propiconazole (500 and 1000ppm), and Mancozeb (1000 and 2000ppm). S. rolfsii produced 0.62g dry mycelial weight and 1069 sclerotia in pH 4.0. At pH 5.0, the greatest mycelial growth was 0.76g, followed by 0.72g at pH 6.0. The most sclerotia were generated at pH 7.0, followed by pH 6.6 and 6.0.

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## INTRODUCTION

Onion is an cool-season crop and belongs to family *Amaryllidaceae.* Onion is known for its high nutritional value containing water (89.11g), protein (1.1g), carbohydrate (0.34g), fiber (1.7g), total sugar (4.24g), vitamin C (74mg), potassium(146mg), phosphorus (29mg), calcium (23mg), magnesium (10mg) and sodium (4mg) per 100g of onion bulb (Pareek et al., 2017). It has a rich source of various

organo-sulphur compounds. These compounds are responsible for their flavour (Mahajan et al., 2017). Onion contains a range of oxidative enzymes, namely, quercetin, S alkaneylcystiene sulfoxide, superoxide dismutase, catalase, peroxidise and glutathione peroxidase (Stajner and Varga, 2003; Dini et al., 2008; Nile et al., 2017).

In Manipur, onion suffers from various diseases. White rot of onion caused by *Sclerotium rolfsii* Saccardo is a severe disease that has devastated onion cultivation. The disease has been observed at commercial onion growing locations in the valley districts of Manipur. The disease becomes evident only late in the crop season, near harvesting period, when the leaves start yellowing, drooping, blighting and wilting (Mathur and Sharma, 2002). The harvested onion bulbs are soft, watery and rots accompanied by white cottony mycelium and small white, brown and black colour sclerotia. The fungus has a wide range of hosts, including cereals, solanaceous crops, legumes, crucifers, cucurbits, flowers and even weeds (Aycock, 1966; Punja, 1985; Punja, 1988). The fungus is well known for producing oxalic acid (Punja and Jenkins, 1984; Paramsivan et al., 2013) in addition to several enzymes, including endo-polygalacturonase, endo-pectimethyl polygalacturonase and cellulase (Punja et al., 1985). The fungus produces sclerotia as resting structure for its survival (Punja, 1985; Xu et al., 2008). This pathogen also survives as dormant mycelium in infected plants and attacks plants' collar region (Mullen, 2001). The sclerotial wall contains melanin pigment and a considerable amount of non-hydrolysable residue, lipids, and ash, making sclerotia tolerant to biological and chemical degradation (Chet et al., 1967). There is also variability in morphology, cultural, physiology and pathogenicity among isolates of *Sclerotium rolfsii* (Sarma et al., 2002; Shukla and Pandey, 2008; Kumar et al., 2014). Therefore, the management of *S. rolfsii* causing white rot of onion in Manipur with fungicides that inhibit mycelial growth, as well as sclerotia production, is very crucial because the fungus is soilborne, polyphagus and the sclerotia remain viable for a long period (Punja; 1985, Kator et al., 2015).

## MATERILAS AND METHODS

Soaked in 1 percent sodium hypochloride solution for 10 minutes, the infected onion bulbs were recovered. PDA was prepared and fungicides were added to 50ml molten PDA, mixed well, and placed onto three sterilised petriplates. A 90mm petriplate was filled with molten PDA media and allowed to cool. The sterilised pieces were dried and inoculated on potato dextrose agar for 4 days at 28oC. The pathogen was purified by hyphal tip cut and re-isolated on PDA solid state. Throughout the research time, the pathogenic culture was kept on PDA and sub-cultured to fresh PDA.

The three non-systemic fungicides (Captan, Mancozeb, and Blitox) and four systemic fungicides (Hexaconazole, Thiophanate methyl, Propiconazole, and Carbendazim) were used against Sclerotium rolfsii (Grover and Moore, 1962). The same sterilised PDA medium was used to test the efficiency of several fungicides. Each replication had three petriplates with a sufficient control San fungicide. Each treatment had three replications. A three-day-old culture of the fungus was injected on each plate after proper PDA solidification. The plates were incubated at 28oC inverted until the test fungus had fully grown on the control plates. Using Vincent's approach, we estimated the percent inhibition of mycelial growth (1927).

After 15 days of incubation, sclerotia generated by two fungicide concentrations were counted. Using Vincent's approach, we estimated the percent suppression of sclerotia generation (1927).

This study examined the influence of pH on growth and formation of sclerotia in the fungus. Each pH level had three replications, including control. Sterilised conical flasks with adjusted potato dextrose broth at 1210C for 20 minutes at 15lbs. Using a three-day-old potato dextrose agar culture, each conical flask was infected and incubated at 2810C. The weight of each filter paper was noted beforehand. The separate potato dextrose broths were filtered through pre-weighed filter papers and allowed to dry fully. After 15 days, the dried mycelial development of the fungus was estimated.

## RESULTS AND DISCUSSION

Morphological and taxonomic data from the monographs helped identify the fungus as Sclerotium rolfsii Saccardo (Saccardo, 1913; Mordue, 1974; Punja, 1985).

Among the studied fungicides, Hexaconazole, Propiconazole, and Mancozeb were effective against Sclerotium rolfsii, inhibiting mycelial growth and sclerotia development. All fungicides' efficacy increased with concentration. At 500ppm and 1000ppm, thiophanate methyl inhibited mycelial growth and sclerotia formation by 82.22 and 100%, respectively. Copper oxycholride and Captan are inferior non-systemic fungicides to Mancozeb. Carbendazim is inferior to other systemic fungicides in reducing mycelial growth and sclerotia formation. Both Copper oxychloride and Captan concentrations could generate sclerotia. Johnson et al. (2008) showed that 1000ppm Hexaconazole and Propiconazole totally inhibited Sclerotium rolfsii growth. In the study, Rakholiya et al. (2015) found that Hexaconazole and Propiconazole at 500ppm and Mancozeb at 1000ppm totally suppressed mycelial development and sclerotia generation of S. rolfsii (groundnut stem rot).

Propionazole 500ppm and 1000ppm and Mancozeb 1000ppm and 2000ppm showed 100% suppression of mycelial growth according to Rangarani et al. 1000ppm carbendazim was less effective. S. rolfsii isolates were entirely inhibited by Hexaconazole at 500ppm, whereas Mancozeb at 1000ppm and 2000ppm completely inhibited select isolates, according to Sharma and Dhruj (2018). Carbendazim and Captan were less inhibitory at 0.1 percent (1000ppm) concentration, according to Bhagat and Chakraborty (2013). According to Mahato et al. (2014 S. rolfsii and had no influence on mycelial growth. Arunasri et al. (2011), Manu et al. (2012), Deepthi (2014), Suneeta et al. (2017), Shirsole et al. (2019) and Sahana et al (2020).

**Table 1**. Effect of fungicides at first concentration

| S.No. | Fungicides | Concentration (ppm) | Growth (cm)* | Inhibition (%) over control | Sclerotia production* | Per cent inhibition on sclerotia production |
|---|---|---|---|---|---|---|
| T1 | Hexaconazole (5% EC) | 500 | 0.00 (0.71) | 100 | 0 | 100 |
| T2 | Copper oxychloride (50% WP) | 1000 | 8.83 (3.05) | 1.88 | 113 | 47.19 |
| T3 | Mancozeb (75% WP) | 1000 | 0.00 (0.71) | 100 | 0 | 100 |
| T4 | Thiophanate methyl (70% WP) | 500 | 1.60 (1.43) | 82.22 | 0 | 100 |
| T5 | Captan (50% WP) | 1000 | 7.75 (2.84) | 13.88 | 106 | 50.46 |
| T6 | Propiconazole (25% EC) | 500 | 0.0 (0.71) | 100 | 0 | 100 |
| T7 | Carbendazim (50% WP) | 500 | 8.33 (2.97) | 7.44 | 109 | 49.06 |
| T8 | Control | - | 9.00 (3.08) | 0.00 | 214 | 0.00 |
| | SE(d) ± | - | 0.34 (0.08) | - | - | - |
| | CD $_{(0.05)}$ | - | 0.74 (0.19) | - | - | - |

**Table 2.** Fungicides effects at second concentration

| Sl. No. | Fungicides | Concentration (ppm) | Growth (cm)* | Inhibition (%) over control | Sclerotia production* | Per cent inhibition on sclerotia production |
|---|---|---|---|---|---|---|
| T1 | Hexaconazole (5% EC) | 1000 | 0.00 (0.71) | 100 | 0 | 100 |
| T2 | Copper oxychloride (50% WP) | 2000 | 8.67 (3.03) | 3.66 | 47 | 78.73 |
| T3 | Mancozeb (75% WP) | 2000 | 0.00 (0.71) | 100 | 0 | 100 |
| T4 | Thiophanate methyl (70% WP) | 1000 | 0.93 (1.20) | 89.66 | 0 | 100 |
| T5 | Captan (50% WP) | 2000 | 6.53 (2.65) | 27.44 | 57 | 71.94 |
| T6 | Propiconazole (25% EC) | 1000 | 0.0 (0.71) | 100 | 0 | 100 |
| T7 | Carbendazim (50% WP) | 1000 | 7.23 (2.78) | 19.66 | 62 | 71.94 |
| T8 | Control | - | 9.00 (3.08) | 0.00 | 221 | 0.00 |
| | SE(d) ± | - | 0.08 (0.01) | - | - | - |
| | CD $_{(0.05)}$ | - | 0.19 (0.04) | - | - | - |

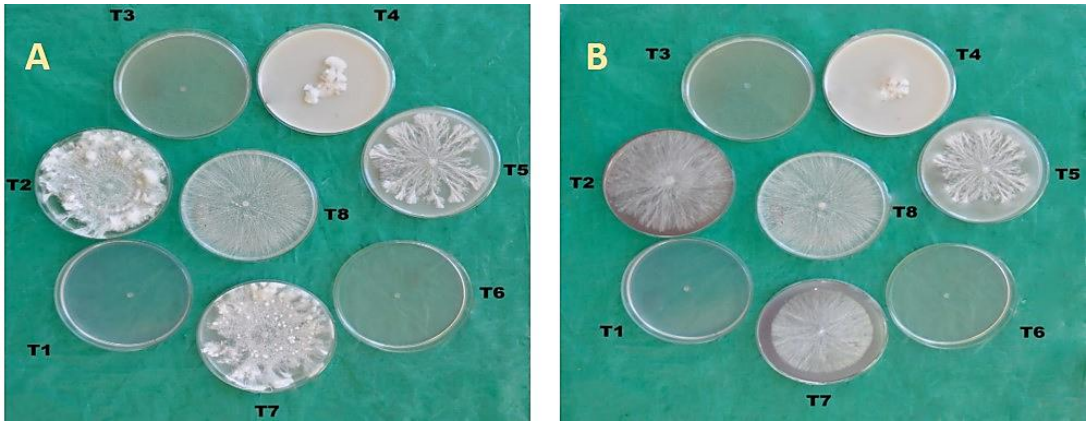


**Figure 1.** Fungicides effect on the mycelial growth of *S. rolfsii*at first (A) and second concentration (B). T1, Hexaconazole; T2, Copper oxychloride; T3, Mancozeb; T4, Thiophanate methyl; T5, Captan; T6, Propiconazole; T7, Carbendazim; T8, Control

Sclerotium rolfsii grows in all pH tested, 4.0 to 7.0. The pH of the control potato broth was found to be 6.6. S. rolfsii grew to 0.76g at pH 5.0. From pH 4.0 to 7.0, sclerotia were developed. Sclerotia (1109) were generated at pH 7.0. In pH 4.0, mycelial growth (0.62g) and sclerotia development (1069) were minimal. Richhariya (1984) showed that pH 5.0 was optimal for S. rolfsii mycelial development. Many other authors have observed that S. rolfsii can grow in a wide range of pH. The present findings agree with Muthukumar and Venkatesh (2013). They found that pH 5.0 produced the most mycelial dry weight, followed by pH 6.0. According to Chaurasia et al. (2013), pH 4.0 to 7.0 is optimal for sclerotia

formation. Sarker et al. (2013) found a pH range of 4.5 to 6.5 for dry mycelial growth of S. rolfsii. According to Zape et al. (2013), sclerotia numbers peak at pH 7.0. They found that S. rolfsii grew best at pH 4–7 (Ayed et al. 2018). Kushwaha et al. (2019) reported good sclerotia development at pH 6.0, 6.5, and 7.0. Similarly, Sri et al. (2020) found that pH 7.0 favoured S. rolfsii sclerotia production.

**Table 3.** Effect of different pH levels on the mycelial growth

| pH | Mycelial dry weight(g) * | Sclerotia production* |
|---|---|---|
| 4 | 0.62 | 1069 |
| 4.5 | 0.70 | 1070 |
| 5.0 | 0.76 | 1075 |
| 6.0 | 0.72 | 1089 |
| 7.0 | 0.60 | 1109 |
| Control (6.6) | 0.64 | `1093 |
| SE(d) ± | 0.03 | - |
| $CD_{(0.05)}$ | 0.07 | - |

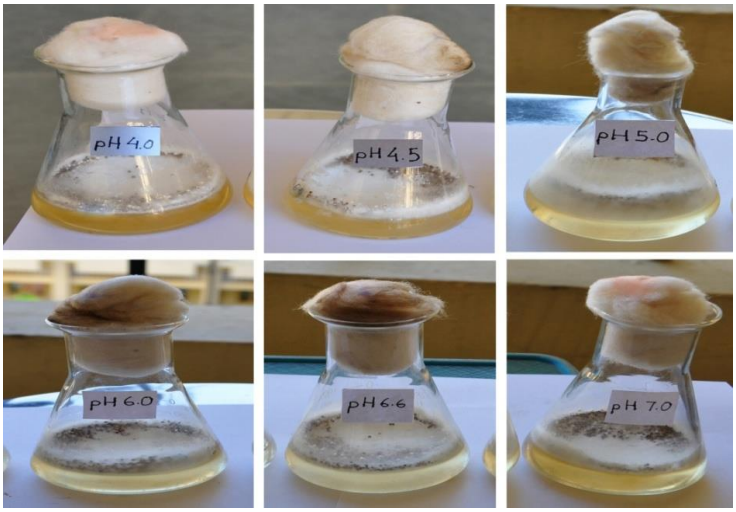


**Figure 2.** The effect of various levels of pH on the mycelial growth

## CONCLUSION

The present investigations disclosed that *S. rolfsii* causing white rot of onion in Manipur can grow and produce sclerotia in a broad range of pH levels. Among the fungicides tested, Hexaconazole, Propiconazole and Mancozeb were effective against the pathogen, and completely inhibited both mycelial growth and sclerotia production. The fungicides effective against this fungus under laboratory conditions should further be tested for their efficacy under field conditions. Since *Sclerotium rolfsii* is soilborne and survives as mycelium or sclerotia, fungicides that inhibit both mycelial growth and sclerotia production can be employed in integrated disease management strategies.

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