

Original Research Article

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Evaluation of Plant Extracts and Fungicides against *Sclerotium rolfsii* causing Collar Rot of Lentil

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A B S T R A C T

Collar rot of lentil caused by *Sclerotium rolfsii* Sacc. is one of the destructive diseases in lentil growing areas of the Madhya Pradesh. In the present work, eight fungicides viz., Captan, Blue copper, Carbendazim, Carbendazim + Mancozeb, Mancozeb, Fipronil, Thiophanate Methyl and Pyraclostrobin and seven plant extracts viz., leaves of Neem, Ashok, Parthenium, Castor, Citrus, Bulb of Onion and Clove of Garlic were evaluated for management of collar rot of lentil under *in vitro* condition. Garlic clove extract at 15 percent concentration was found best antifungal which completely inhibited the growth and sclerotial production of *Sclerotium rolfsii*. Onion bulb extract at 10 and 15 percent concentration was also found very promising antifungal for inhibiting the growth and sclerotia production of *S. rolfsii*. Pyraclostrobin was found best fungicide which completely inhibited the radial growth and sclerotia production of *Sclerotium rolfsii*. Captan, Carbendazim + Mancozeb and Mancozeb were second next in order of toxicity resulting, 94.11, 82.96 and 66.11 percent inhibition of radial growth and 89.65, 50.13 and 49.10 percent inhibition of sclerotia production, respectively. Thiophanate methyl, Carbendazim, Blue copper and Fipronil were not found effective in inhibiting the growth and sclerotia production of *Sclerotium*

Keywords

Plant extracts,
Fungicides,
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Introduction

Lentil is recognized as one of the most nutritious pulse crops ranking next to chickpea among cool season food legumes in India. The total area covered under lentil has been 15 Lha during 2017-18. The highest ever production

of 15 Lt at 1008 kg/ha is a remarkable success. More than 90% has been realized from 05 states of Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal and Jharkhand. It contains 57-60 % carbohydrate, 24- 26 % protein, 3.2% and 1.3 % fiber (Anonymous, 2018). Lentil is used for human consumption as a protein

source of a diverse range of product and is an excellent source of vitamin A and provide fiber, potassium, vitamins and iron (Kochlar, 2009). Lentil is a valuable human food, mostly consumed as dry seeds as well as fodder, and generally grown as a crop rotation after cereals to enrich the soil by their nitrogen fixing ability (Khalequzzaman, 2016). In Madhy Pradesh, lentil is grows on residual soil moisture after post rainy season under rainfed conditions. The inclusion of lentil as a crop rotation can benefits the succeeding crops by improving the soil health through biological nitrogen fixation and carbon sequestration.

Collars rot disease caused by *Sclerotium rolfsii* Sacc., is a serious threat to lentil in Madhya Pradesh that cause mortality of the crop at seedling stage under favourable environmental conditions. Diseases caused due to *S. rolfsii* requires warm climates, occurs more frequently at high moistures and high temperatures (Al-Askar *et al.*, 2013). *S. rolfsii* control has met with very limited success in Maadhya Pradesh. This may be due to the prolific growth, extensive host range of the pathogen and having the ability to produce large number of sclerotia that may persist in the soil for several years (Sennoi *et al.*, 2013). Mycelial growth of *S. rolfsii* or the sclerotia germination can be restricted by the use of several fungicides viz., thiram, quitozene, captan, carbendazin, benomyl, oxicarboxin triadimenol, carboxin plus thiram in several crops (Yaqub and Shahzad, 2006; Khan and Javaid 2015). The use of plant extracts has been shown to be ecofriendly and effective against many plant pathogens (Thobhunluepop, 2009; Duru and Onyedineke, 2010). Most of these substances were evaluated in order to find a safe alternative control method to the human and the environment. From the above facts, these type of research work are needed in Madhya Pradesh. So the present study was carried out to assess antifungal potential of some more

fungicides and plant extracts against *invitro* growth and sclerotia formation of *S. rolfsii*.

Materials and Methods

Isolate of *Sclerotium rolfsii* was recovered from diseased lentil plants collected from research farm of Jwaharlal Nehru Krishi Vishwa Vidyalaya- Jabalpur. Small pieces of infected tissues 1–2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with blade, washed well in distilled water to remove dust adhered to the infected pieces. Piece were dipped in 0.1 percent mercuric chloride solution for 30 seconds and finally washed well in three changes of sterilized distilled water. The bits were then transferred to PDA slants with the help of inoculating needle under aseptic condition and incubated at $28 \pm 1^{\circ}\text{C}$. After 48 hrs, fragments of hyphal growth from the growing tips were transferred to fresh PDA slants. Pure culture was made, following repeated hyphal tip transfer. Pure culture was maintained on PDA slants by sub culturing it at 30 days intervals. For preservation of cultures the plugged end of the culture tubes were dipped in melted wax and stored in a refrigerator at $5 \pm 1^{\circ}\text{C}$.

Seven plants extracts *viz.*, citrus leaf, neem leaf, onion bulb, parthenium leaf, castor leaf, ashok leaf and garlic clove at 5, 10 and 15% concentrations were evaluated against *Sclerotium rolfsii* by poison food technique on potato dextrose agar to for their antifungal activity against *S. rolfsii*. Extracts of plant parts such as leaf, bulb and clove were prepared by the standard method used by Gerard *et al.*, 1994. Fresh plant parts were washed with tap water followed by sterile distilled water, processed with sterile distilled water @ 1mlg^{-1} of plant tissue (1:1v/w) with pestle and mortar and filtered through a double layered cheese cloth. The filtrate so obtained formed the standard plant extract

solution. The plant extract so prepared were screened *in vitro* against *S. rolfsii* using poisoned food technique (Mortan and Straube, 1955). Stock solution 5, 10 and 15 ml were mixed respectively with 95, 90 and 85 ml of sterilized molten Potato Dextrose Agar (PDA) media to obtain 5, 10 and 15 percent concentration of plant extract.

The mixed medium was thoroughly shaken to ensure uniform mixing of extract. 20 ml of poisoned PDA was poured into sterile petriplates. Three replications were maintained for each concentration. After solidification of poisoned media, the plates were inoculated with mycelium disc (5 mm diameter) of vigorously growing pure culture colony of *S. rolfsii*.

The control petriplates in three replications were maintained using only sterile water without any plant extract but with mycelium disc (5 mm) for comparison. Plates were incubated at $28 \pm 1^{\circ}\text{C}$ and observation on radial growth after 72 hours and sclerotia formation after 21 days of the test fungus was recorded. Recorded data on radial growth and sclerotia formation was converted into percent inhibition by using following formula given by Vincent, 1947.

In order to find out a suitable fungicide for management of collar rot of lentil, seven fungicides namely Captan, Blue copper, Carbendazim, Carbendazim + Mancozeb, Mancozeb, Fipronil, Thiophanate methyl and Pyraclostrobin along with control was evaluated against *S. rolfsii* by following the poisoned food technique.

PDA poisoned with each fungicide was poured into three sterilized petriplates @ 20 ml/plate and allowed to solidify. Plates containing PDA without fungicide served as check. After solidification each petriplate was inoculated with 5 mm mycelial disc

aseptically. Plates were incubated at $28 + 1^{\circ}\text{C}$ and observation on radial growth of the test fungus was recorded after 72 hours and sclerotia formation after 21 days. Recorded data on radial growth and sclerotia formation was converted into percent growth inhibition by using following formula given by Vincent, 1947.

Results and Discussion

Out of the seven plants extracts viz., citrus leaf, neem leaf, onion bulb, parthenium leaf, castor leaf, ashok leaf and garlic clove evaluated against *Sclerotium rolfsii*, garlic clove extract could completely inhibit the growth of *S. rolfsii* at 15 percent concentration. Parthenium leaf extract was found effective to some extent as they produced 27.7 percent growth inhibition at 15 percent concentration of the extract (Table-1 and Plate. 1, 2).

Onion bulb, castor leaf, neem leaf, citrus leaf and ashok leaf extracts were not very promising as they produced only 3.3, 4.3, 5.2, 6.5 and 16.6 percent inhibition, respectively, at 15 percent concentration. At lower concentrations i.e. 5 and 10 percent growth inhibition due to onion bulb, castor leaf, neem leaf, citrus leaf and ashok leaf extract were less than 11 percent.

Garlic clove extract produced 85.77 and 100 percent inhibition of sclerotia production at 10 and 15 percent concentration, whereas onion bulb extract produced 100 percent inhibition of sclerotia production of *S. rolfsii* at both 10 and 15 percent concentration.

Citrus, castor, neem and parthenium leaf extracts were found very promising as they produced 66.28, 80.02, 64.60 and 72.60 percent inhibition, respectively at 15 percent concentration. At lower concentrations i.e. 5 and 10 per cent growth inhibition due to

citrus, castor, neem and parthenium leaf extracts were also observed. The degree of inhibition increased correspondingly with increasing concentrations of the plant extracts. Percentage of inhibitions was high at 15% concentration than 10% concentration, except *S. rolfsii* treated with 10% neem oil. Highest growth inhibitions were recorded in *S. rolfsii* treated with onion bulb extract.

In general, fungal biomass was gradually decreased with an increase in extract concentration. The present results are in conformity with earlier workers.

Banakar, 2017 reported that garlic clove extract and onion bulb extract at 15% concentration showed 100% inhibition of growth and sclerotia production of *Sclerotium rolfsii* causing foot rot of tomato.

Most of the medicinal effects of garlic are due to those sulfur compounds (thiosulfinate and sulfides), products of conversion of alliin from garlic by the enzyme alliinase. (Vlase *et al.*, 2010, Stajner *et al.*, 2008). Okereke and Wokocha, 2006 reported that, the inhibition of damping-off disease of tomato incited by *S. rolfsii* was highest with soil drenching with neem seed (62.4%) followed by ginger (57.4%).

Suleiman and Emua, 2009 reported that 40 percent concentration of ginger extract completely inhibited the mycelial growth of *Pythium aphanidermatum* causing root rot of cowpea which support our present study.

The results of the fungicidal trials revealed that Pyraclostrobin was found best fungicide which completely inhibited the radial growth and sclerotia production of *Sclerotium rolfsii*. Captan, Carbendazim + Mancozeb and Mancozeb were second next in order of toxicity resulting, 94.11, 82.96 and 66.11 percent inhibition of radial growth and 89.65, 50.13 and 49.10 percent inhibition of sclerotia

production, respectively (Table-2 and Plate 3, 4).

Thiophanate methyl, Carbendazim, Blue copper and Fipronil were not found effective in inhibiting the growth and sclerotia production of *Sclerotium rolfsii*. Bhatt (2015) also reported that Mancozeb and Captan completely inhibited *S. rolfsii* growth at 125 and 250 66 ppm. Mancozeb and Captan were found very effective in present study also. Khan and Javaid 2015 reported that Thiophanate methyl at 250 ppm significantly decreased radial growth of *S. rolfsi* over control. However, in present study Thiophanate methyl was not found effective in inhibiting the growth and sclerotia production of *Sclerotium rolfsii*.

Sheoraj *et al.*, 2005, while working on collar rot of Lentil caused by *Sclerotium rolfsii*, reported that carbendazim completely controlled the pathogen which is contrary to the present study in which Carbendazim was not found effective in inhibiting the growth and sclerotia production of *S. rolfsii*.

However, Bhuiyan *et al.*, 2012 reported that Carbendazim was not effective in inhibiting the radial growth of *Sclerotium rolfsi* which support the present finding.

Collar rot of lentil caused by *Sclerotium rolfsii* Sacc. is one of the destructive diseases in lentil growing areas of the Madhya Pradesh. Garlic clove extract at 15 percent concentration was found best antifungal which completely inhibited the growth and sclerotial production of *Sclerotium rolfsi* followed by Onion bulb extract at 10 and 15 percent. The degree of inhibition increased correspondingly with increasing concentrations of the plant extracts. Pyraclostrobin was found best fungicide which completely inhibited the radial growth and sclerotia production of *Sclerotium rolfsii* followed by Captan and Carbendazim + Mancozeb

Table.1 Effect of plant extracts on radial growth and sclerotia formation of *Sclerotium rolfsii*

| Name of Botanicals | Local name | Parts used | Radial growth (mm) of target pathogen after 3 DAI* | | | Percent growth inhibition | | | No. of sclerotia formed after 21 DAI* | | | Percent growth inhibition | | |
|---------------------------------|------------|------------|--|-------|-------|---------------------------|------|------|---------------------------------------|-------|-------|---------------------------|-------|--------|
| | | | 5% | 10% | 15% | 5% | 10% | 15% | 5% | 10% | 15% | 5% | 10% | 15% |
| <i>Allium sativum</i> | Garlic | Clove | 52.1 | 40.0 | 00.0 | 42.1 | 55.5 | 100 | 386.3 | 98.3 | 0.0 | 44.09 | 85.77 | 100.00 |
| <i>Parthenium hysterophorus</i> | Parthenium | Leaf | 74.8 | 71.0 | 65.0 | 16.8 | 21.1 | 27.7 | 309.3 | 209.6 | 189.3 | 55.23 | 69.66 | 72.60 |
| <i>Polyalthia longifolia</i> | Ashok | Leaf | 85.6 | 80.5 | 75.0 | 4.8 | 10.5 | 16.6 | 305.6 | 214.6 | 202.3 | 55.77 | 68.94 | 70.72 |
| <i>Citrus limon</i> | Citrus | Leaf | 86.5 | 85.0 | 84.1 | 3.8 | 5.5 | 6.5 | 290.6 | 269.3 | 233.0 | 57.94 | 61.02 | 66.28 |
| <i>Azadirachta indica</i> | Neem | Leaf | 87.0 | 85.8 | 85.3 | 3.3 | 4.6 | 5.2 | 309.6 | 254.0 | 244.6 | 55.19 | 63.24 | 64.60 |
| <i>Ricinus communis</i> | Castor | Leaf | 87.5 | 87.6 | 86.1 | 2.7 | 2.6 | 4.3 | 215.0 | 172.0 | 138.0 | 68.88 | 75.10 | 80.02 |
| <i>Allium cepa</i> | Onion | Bulb | 90.0 | 90.0 | 87.0 | 0.0 | 0.0 | 3.3 | 245.6 | 0.0 | 0.0 | 71.40 | 100.0 | 100.0 |
| Control | -- | -- | 90.0 | 90.0 | 90.0 | | | | 691.0 | 691.0 | 691.0 | | | |
| CD (0.05) | | | 1.290 | 2.034 | 1.476 | | | | 2.960 | 2.643 | 2.309 | | | |

*Average of 3 replications

Plate.1 Effect of plant extracts at 15% concentration on radial growth of *Sclerotium rolfsii*

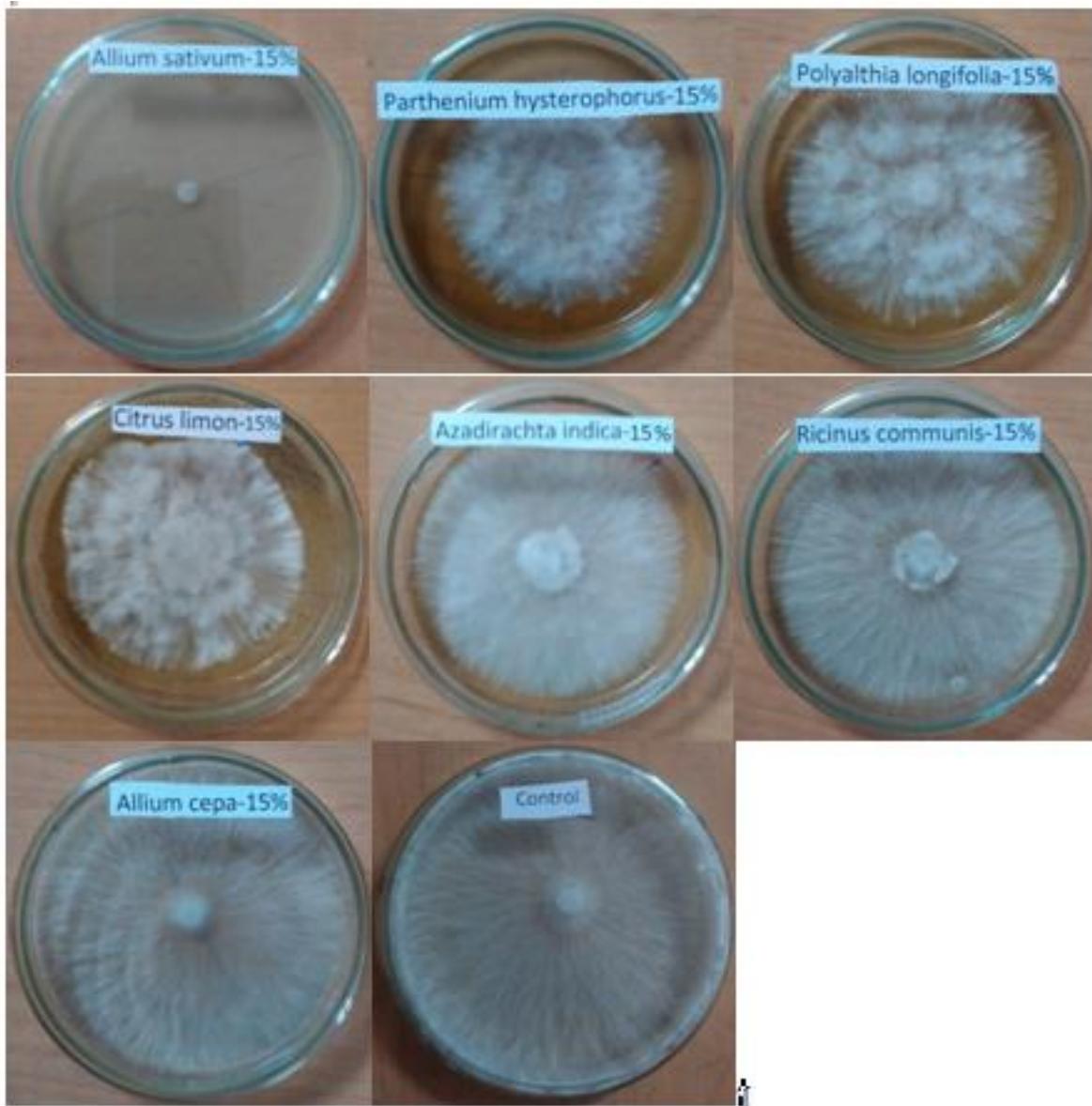


Plate.2 Effect of plant extracts at 15% concentration on sclerotia formation of *Sclerotium rolfsii*

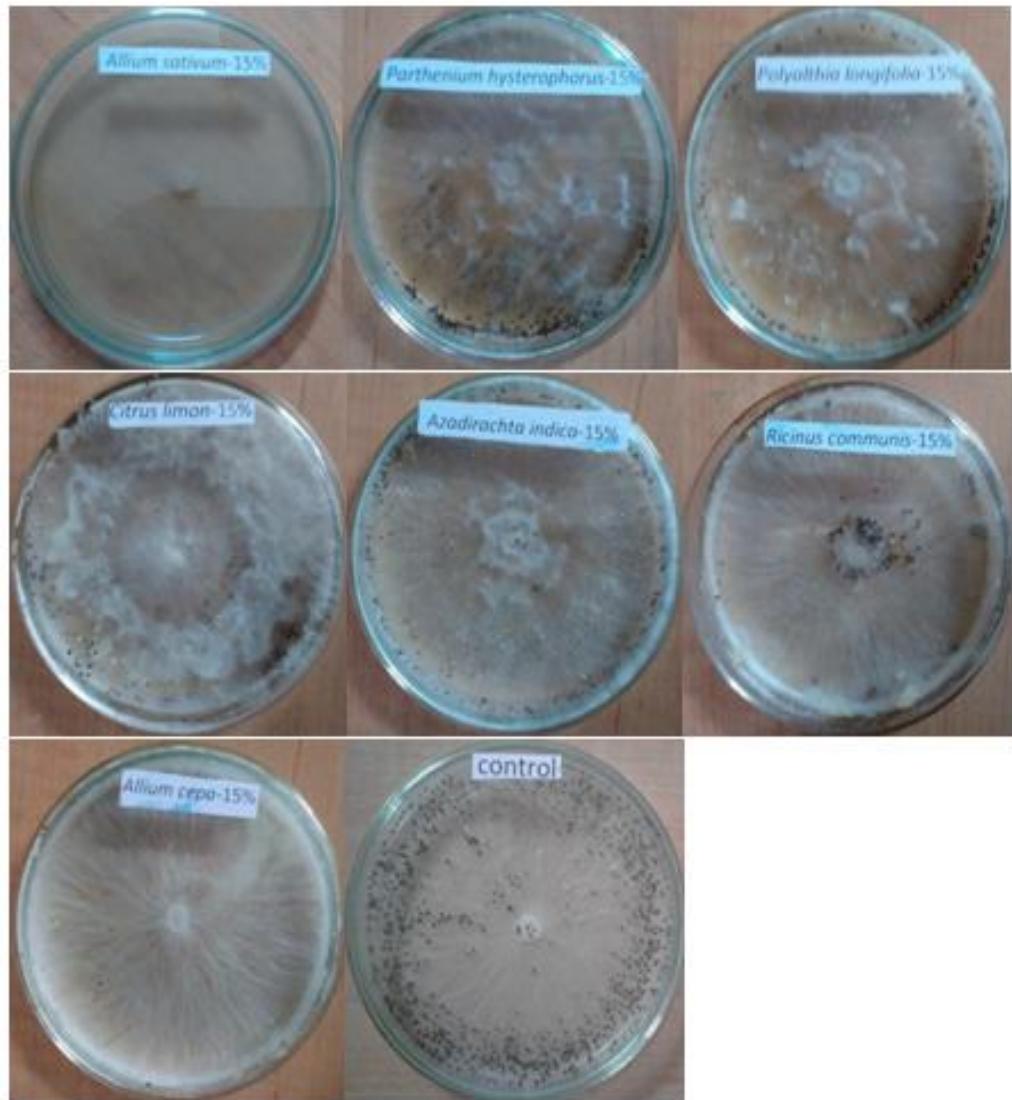


Table.2 Effect of fungicides on radial growth and sclerotia formation of *Sclerotium rolfsii*

| Name of Fungicides | Doses (gm/liter) | Radial growth (mm) after 72 hrs* | Growth Inhibition over control (%) | No. of sclerotia formed after 21DAI days | Sclerotia Inhibition over control (%) |
|-------------------------------|------------------|----------------------------------|------------------------------------|--|---------------------------------------|
| Pyraclostrobin | 0.2 | 0.0 | 100.0 | 0.00 | 100.0 |
| Captan | 2.5 | 5.3 | 94.1 | 70.0 | 89.6 |
| Carbendazim + Mancozeb | 2.5 | 15.3 | 82.9 | 305.3 | 69.6 |
| Mancozeb | 2.5 | 30.5 | 66.1 | 311.6 | 49.1 |
| Fipronil | 1.0 | 69.1 | 23.1 | 501.0 | 25.9 |
| Blue copper | 3.0 | 80.0 | 11.1 | 600.0 | 11.3 |
| Carbendazim | 1.0 | 87.0 | 3.3 | 642.6 | 5.0 |
| Thiophanate Methyl | 1.0 | 90.0 | 0.0 | 612.3 | 9.5 |
| Control | | 90.0 | | 676.66 | |
| CD (0.05) | | 1.411 | | 3.191 | |

*Average of 3 replications.

Plate.3 Effect of fungicides on radial growth of *Sclerotium rolfsii*

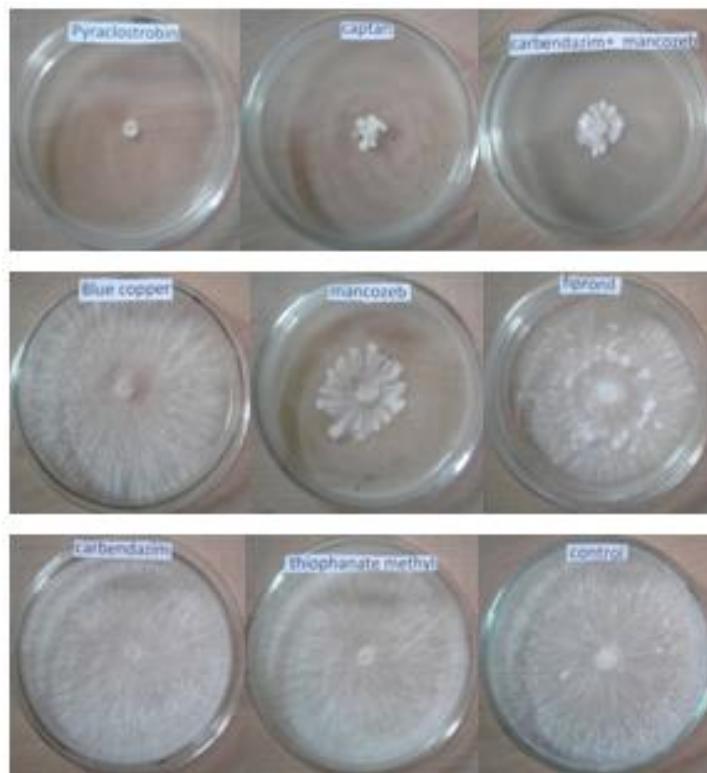
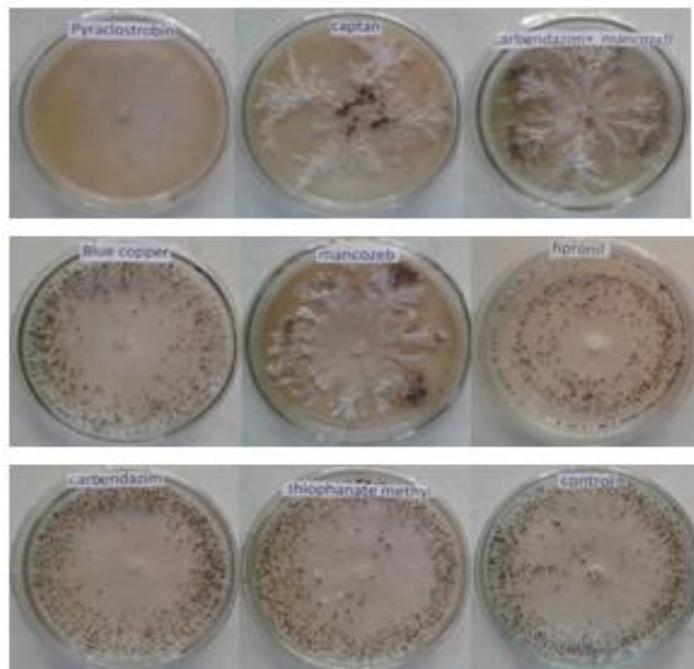


Plate.4 Effect of fungicides on sclerotia formation of *Sclerotium rolfsii*



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