Study the inhibitory effect of Mustard seed powder on the growth of *Sclerotium rolfsii*

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**ABSTRACT**

Groundnut is an important crop in all over the world. The cultivation of groundnut is limited by stem and pod rot caused by *Sclerotium rolfsii*. Worldwide the groundnut yield loss due to stem rot is 10 to 25 percent. The fungus, *S. rolfsii* has a wide host range. In the present study *S. rolfsii* was exposed to mustard seed powder (MSP) at different concentrations (20, 50, 100 and 200 mg) under in vitro conditions. This bioassay was conducted using two types of methods; (a) Continuously exposing to single MSP and (b) fresh MSP was replenished every 24 hours. Cent per cent inhibition of *S. rolfsii* was observed at 100 and 200 mg of MSP in both the methods. But, 50 mg concentration was also inhibited growth of *S. rolfsii* up to 93.10% after 72 hours. All results are calculated that MSP has inhibitory effect against *S. rolfsii* it may be used as future to reduce soil borne diseases.

**Keywords:** *Sclerotium rolfsii*, mustard powder, mycelial growth

**INTRODUCTION**

Groundnut is grown on nearly 23.95 million ha worldwide with the total production of 36.45 million tons and an average yield of 1520 kg/ha in 2009. China, India, Nigeria, USA and Myanmar are the major groundnut growing countries. Developing countries in Asia, Africa and South America account for over 97% of world groundnut area and 95% of total production [1]. *Sclerotium rolfsii*, a deuteromycetous fungus is a soil borne facultative parasite having very wide host range which causes pre-emergence rot, collar rot, stem rot and wilt in groundnut. The cultivation of groundnut is restricted by stem and pod rot caused by *S. rolfsii*. Worldwide the groundnut yield loss due to stem rot is ten to 25 percent and the chemical methods are very expensive and will not provide complete protection from the pathogen. There is worldwide acceptance to the use of ecologically safe, environment friendly methods of protecting crops from the plant pathogens. Glucosinolates compounds that occur in agronomically important crops may represent a viable source of allelochemical for the control of various soil borne pests [2]. *Brassica* species contain glucosinolates (GSL), which, upon tissue disruption, are hydrolyzed in the presence of water by an endogenous myrosinase enzyme into numerous compounds, notably toxic isothiocynates (ITC). Insecticidal, nematicidal, fungicidal and phytotoxic effects are often associated with tissues of cruciferous and *Brassica* plants. The detrimental effect of pure ITC to certain fungi has long been known and the potential of *Brassica* crops to control soil borne pests and pathogens mainly attributed to these compounds. This process is termed as “bio-fumigation” [2].
MATERIALS AND METHOD

Isolation and maintenance of pathogen
Groundnut (*Arachis hypogaea* L.) plants showing stem rot symptoms were collected from Regional agriculture research station (RARS) Kadiri, Anathapur district of Andhra Pradesh, India. The isolation of the pathogen from diseased plants was performed on Potato Dextrose Agar (PDA) medium and identified according to their morphology and colony characteristics [3]. The pathogenicity of the isolated *S. rolfsii* was studied in a pot culture experiment as described by Singh and Thapliyal [4].

An experiment was carried out under *in vitro* conditions with minor modification of the procedure of Rahmanpour *et al.* [5]. MSP was prepared using pestle and mortar with commercially available mustard seeds just before the experiment.

**Method (a):** In this method different amounts of MSP viz., 20, 50, 100 and 200 mg MSP was added in the aluminum foil which was kept in the upper lid of the Petri plate. In order to hydrolyze the glucosinolates (GSLs), sterile distilled water (10 µL mg⁻¹) was added to the MSP. Twenty ml of PDA medium was poured in 90 mm (4.50 cm) sterilized Petri plates and allowed to solidify. Mycelial disc of 5 cm from three day old culture of the pathogen *S. rolfsii* was inoculated at the center of the Petri plate. The base of the Petri plate was placed on the top of the upturned lid, which contained the MSP. Petri plates without MSP served as control. The plates were incubated at 28 ± 2°C for 5 days.

**Method (b):** In another method the fungal cultures was exposed to volatiles for 5 days by replacing fresh MSP every 24 hours also similarly without replacement of MSP which was initially kept in the aluminum foils. Six replications were maintained for each treatment. The data for the growth of *S. rolfsii* was measured at 24, 48, 60, 72 and 96 hours and per cent inhibition of mycelial growth calculated using the following formula [6].

The per cent inhibition was measured using the formula:

\[ \text{I} = \left( \frac{C - T}{C} \right) \times 100 \]

Where, I= Per cent inhibition of mycelial growth,
C= Colony diameter in control (cm),
T= Colony diameter treatment (cm)

RESULTS AND DISCUSSION

**Table no: I. Bio-assay of volatiles released from MSP replaced with fresh MSP every 24 hour interval**

| Time duration | MSP Concentration | Radial growth of *S. rolfsii* (cm) |
|---------------|-------------------|----------------------------------|
|               | 20 mg | 50 mg | 100 mg | 200 mg | Control |
| 24 Hours      | -     | -     | -     | -     | 0.72 (100)* |
| 48 Hours      | 0.47  | 0.34  | 0.0   | 0.0   | 2.23 (100) |
| 72 Hours      | 0.66  | 0.41  | 0.0   | 0.0   | 3.50 (81.10) |
| 96 Hours      | 1.9   | 0.42  | 0.0   | 0.0   | 4.25 (55.00) |

SEm = CD at 5%

\[ F_1 = 0.032 \] 0.064
\[ F_2 = 0.036 \] 0.071
\[ F_1 \times F_2 = 0.073 \] 0.143

* Figures in parenthesis are per cent inhibition over control

In the present experiment all the concentrations of MSP tested were significantly effective in inhibiting the mycelial growth of *S. rolfsii*. In both the methods at 100mg and 200mg concentrations 100 per cent growth of *S. rolfsii* was inhibited. But also growth of *S. rolfsii* was not detected at all the concentrations tested in both the methods at 24 hours after incubation whereas control plates shown the growth of 0.72 cm radial growth at same incubation period. Whereas in method (b) at 48, 72 and 96 hours there was 78.9 to 84.75 per cent, 81.1 to 88.30 per cent and 55 to 90.10 per cent inhibition observed when the MSP was increased from 20 to 50 mg was maintained with the fungal
cultures were exposed to volatiles for 5 days with replacement of fresh MSP every 24 hours when 20 and 50 mg were used respectively. A highest inhibition of 90 per cent was observed after 96 hours when 50 mg concentration MSP was used in method (b) results are represented in table I.

However the growth of S. rolfsii was more when the MSP was not replaced every day. Whereas in method (a) maintained without replacement of MSP which was initially kept in the aluminum foils for five days the growth of S. rolfsii was more compare to exposure to the fresh MSP for every 24 hours. The results obtain in this at 20 and 50 mg concentration are 53.00, 73.00 per cent inhibition, 23.14, 41.70 per cent inhibition, 31.52, 23.00 per cent inhibition at 48, 72 and 96 hours of exposure results are presented in table II. The growth observed was up to 2.20 cm (23%) in 50 mg MSP used treatment at the end (96 hours) of the experiment. Both the experiments gave complete inhibition in growth of S. rolfsii at 100 and 200 mg concentration of MSP.

Table no: II. Bio-assay of volatiles released from MSP

| Time duration | MSP Concentration | Control |
|---------------|-------------------|---------|
| 24 Hours      | 20 mg             | 0.72    |
|               | 50 mg             | (100)   |
|               | 100 mg            | (100)   |
|               | 200 mg            | (100)   |
|               | Control           | --      |
| 48 Hours      |                   |         |
|               | 2.05              | 0.60    |
|               | 0.00              | 0.00    |
|               | 2.33              | --      |
| 72 Hours      |                   |         |
|               | 2.69              | 2.04    |
|               | 0.00              | 0.00    |
|               | 3.50              | --      |
| 96 Hours      |                   |         |
|               | 2.91              | 2.20    |
|               | 0.00              | 0.00    |
|               | 4.25              | --      |
|               | (31.52)           | (48.23) |
|               | (100)             | (100)   |
|               | Control           | --      |

F figures in parenthesis are cent inhibition over control

Studies of Rahamanpour et al. [5] showed that the growth rate of the fungal colony Sclerotinia sclerotiorum over 5 days was significantly inhibited initially, rates recovered to reach those of the control over a 2 to 4 day period, depending on the amount of MSP supplied. All the treatments with MSP had statistically similar growth rates 72 hours after application.

The effect of mustard seed meal on linear growth of soybean root-rot and wilt fungal pathogens was observed by Fayzalla et al. [7]. Mustard seed meal proved to be effective for controlling the pathogen and resulted in decreasing the linear growth of the pathogen Rhizoctonia solani at all levels (5, 10 and 25 mg plate\(^{-1}\)) as compared with the control. The results are in conformity with those of Noble et al. [8]. Seed meal of Brassica species suppresses the growth of Pythium ultimum, Rhizoctonia solani [9] and Fusarium sambucinum [10]. Chung et al. [11] proved that the volatile substances in the ground seed of mustard showed the strongest fungicidal effect on R. solani through comparing three Brassica species for volatile compounds in hydrated ground seeds. Kirkegaard et al. [12] reported that seed meal of mustard was fungicidal to five soil borne pathogens. Further, Robert Larkin and Griffin [13] also found that in vitro assays of Indian mustard resulted in nearly complete inhibition (80-100%) of growth of soil borne pathogens of potato, including Rhizoctonia solani, Phytophthora erythroseptica, Pythium ultimum, Sclerotinia sclerotiorum and Fusarium sambucinum which are in agreement with our results.

Smolinska et al. [14] conducted experiment with the seed meal of Brassica napus (rapeseed) which produced volatile fungi-toxic compounds potentially of value in the control of Aphanomyces root rot of pea. These compounds were extremely effective in the suppression of encysted zoospore germination.

In the present investigation although the volatiles produced from brassica leaf tissue were inhibitory to S. rolfsii, colonization of the plant material continued to progress over 48 h, suggesting that the fungus may have the ability to adapt to volatiles. Despite evidence for production of volatiles as well as their ability to inhibit fungal growth in a bioassay, the pathogen S. rolfsii continued to grow in the leaf discs suggesting that it may have a mechanism for overcoming toxicity of volatiles produced [15]. Results of volatiles released from MSP showed that the growth of the pathogen was completely inhibited initially at 24 hours, but due to continuous exposure to toxic volatiles, initial inhibition was followed by adaptation and recovery of growth to control level suggesting that the fungus may have the ability to adapt to volatiles at lower concentrations.
CONCLUSION

Adaption of *S. rolfsii* to the exposure of MSP continuously for five days with replacement of fresh MSP at 24 hours interval showed effective results with the replacement of MSP for five days at low concentrations 20 and 50 mg. The concentrations 100 and 200 mg inhibited the fungus growth absolutely. However, these studies showed that the exposure of the pathogen *S. rolfsii* to the MSP volatiles to 100 mg concentration and more inhibited the growth under *in vitro* conditions. Future research should focus on determining the effects of MSP under field conditions.

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