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Study the Inhibitory Effect of Mustard Seed Powder on the Growth of *Sclerotium rolfsii*

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A B S T R A C T

Stem and Pod rot caused by *Sclerotium rolfsii* is one of the major constraint affecting the seed and oil production in groundnut growing areas of the world. An attempt was made to manage the disease by holistic approach. In vitro evaluation of different concentrations of mustard 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 over the control. The test concentrations in this experiment successfully inhibited the growth of *S. rolfsii*. Hence it is necessary that these treatments be tested under field conditions before they can be exploited in a commercial set up.

Introduction

Groundnut (*Arachis hypogaea* L.) is also known as peanut, is a legume that ranks 6th among the oilseed crops and 13th among the food crops of the world (Icrisat, 2017). Groundnut is grown on nearly 4.76 million ha in India with the total production of 7.40 million tonnes and an average yield of 1552 kg/ha in 2014-15 (India Stat, 2017). 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 (Icrisat, 2017). The crop suffers with many fungal, bacterial and viral diseases resulting in huge yield losses. Among the fungal diseases, stem and rot or Southern blight disease caused by *Sclerotium rolfsii* is one of the major concern and causing the economic losses in Groundnut (Deepthi and Reddy, 2013).

Sclerotium rolfsii, a deuteromycetous soil borne facultative parasitic fungi having extensive host range which causes pre-emergence rot, collar rot, stem rot or wilt in at least 500 species in 100 families and the most common hosts are the legumes, crucifers, and cucurbits (Aycock, 1966; Domsch *et al.,*1980; Farr *et al.,* 1989). Worldwide the groundnut yield loss due to stem rot is 25 to 90 percent and the control of the disease was not achieved (Grichar and Bosweel, 1987; Adiver

2003; Rodriguez Kabana *et al.,* 1975). But the management of disease by using chemical fungicides and synthetic plant growth regulators are very expensive and disturb the ecosystem and will not provide complete protection from the pathogen. The long term effect were overlooked hence, boom of one time become bane for the ecosystem degradation. At the present context, it has become indispensable to look for sustainable crop protection management approaches for disease management and the present work is an effort to this direction.

A crucial factor in the management of diseases caused by these pathogens is to reduce their inoculum level below the critical threshold level before a susceptible crop is planted. Since the 1950s, chemical soil disinfestations have commonly been used for this purpose. Soil fumigants, especially Methyl Bromide (MB) is the most effective soil fumigant used by farmers around the world for this purpose. Today, there is the need for diversified options and alternatives to fill different roles across the soil borne pest and disease management spectrum. Apart from various synthetic chemical alternatives, numerous nonchemical tactics have also been explored, field authenticated and in some cases implemented commercially. For certain situations, biofumigation and soil solarization are among the most useful of the non-chemical disinfestations methods (Prasad *et al.,* 2015). 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 (Angus, *et al.,* 1994). *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" (Angus, *et al.,* 1994).

Materials and Methods

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. Infected stem tissues were surface sterilized with 0.1% $HgCl_2$ (1 g/lit) for 1miunte followed by three subsequent washing with sterilized distilled water in aseptic condition. The sterilized pieces were then transferred aseptically under laminar airflow on sterilized Petri plates containing 20 ml potato dextrose Agar (PDA) medium. The Petri plates were incubated in biological oxygen demand (BOD) at 28±2°C temperature for optimum growth. The fungal hyphae developing from the infected tissues were sub-cultured aseptically on PDA media containing in Petri plates. Thus, pure culture was obtained by hyphal tip method and microscopically examined for identification and it was further purified by using single sclerotial body. The culture was maintained on PDA slants for further investigations.

Identification of the pathogen causing stem rot of groundnut was carried out by studying the cultural and morphological characters were recorded right from initiation of mycelial growth till the period of 15 days. The morphological characters *viz.*, mycelia growth and sclerotial formation, its size,

shape and colour were studied under low power magnification (10X) from 10 days old culture of *S. rolfsii* and were compared with identification key described in "Illustrated Genera of Imperfect Fungi" (Barnett and Hunter, 1998). The pathogenicity test of the pathogen was also carried out in pots by stem inoculation technique as described (Patil *et al.,* 1977).

An experiment was carried out under *in vitro* conditions with minor modification of the procedure of Rahmanpour *et al.,* (2009) in two different methods. Mustard seed powder (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 (Vincent, 1927).

The per cent inhibition was measured using the formula:

$$I = \frac{C - T}{C} \times 100$$

Where,

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

Results and Discussion

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.

Studies of Rahamanpour *et al.,* (2009) 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.

Table.1 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)*	(100)	(100)	(100)	--
48 Hours	0.47	0.34	0.0	0.0	2.23
	(78.90)	(84.75)	(100)	(100)	--
72 Hours	0.66	0.41	0.0	0.0	3.50
	(81.10)	(88.30)	(100)	(100)	--
96 Hours	1.9	0.42	0.0	0.0	4.25
	(55.00)	(90.10)	(100)	(100)	--

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

Table.2 Bio-assay of volatiles released from MSP

Time duration	MSP Concentration Radial growth of S. rolfsii (cm)				
	20 mg	50 mg	100 mg	200 mg	Control
24 Hours	-	-	-	-	0.72
	(100)	(100)	(100)	(100)	--
48 Hours	1.05	0.60	0.00	0.00	2.23
	(53.00)	(73.00)	(100)	(100)	--
72 Hours	2.69	2.04	0.0	0.0	3.50
	(23.14)	(41.71)	(100)	(100)	--
96 Hours	2.91	2.20	0.0	0.0	4.25
	(31.52)	(48.23)	(100)	(100)	--

SEm±		CD at 5%
F_1 =	0.056	0.110
F_2 =	0.063	0.123
$F_1 \times F_2$ =	0.126	0.247

* Figures in parenthesis are per cent inhibition over control

The effect of mustard seed meal on linear growth of soybean root-rot and wilt fungal pathogens was observed by Fayzalla *et al.,* (2009). 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.,* (2002). Seed meal of *Brassica* species suppresses the growth of *Pythium ultimum*, *Rhizoctonia solani* (Charan and Sams (1999)) and *Fusarium sambucinum* (Mayton *et al.,* 1996). Chung *et al.,* (2002) 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.,* (2006) reported that seed meal of mustard was fungicidal to five soil borne pathogens. Further, Robert Larkin and Griffin (2007) 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 erythrospetica*, *Pythium ultimum*, *Sclerotinia sclerotiorum* and *Fusarium sambucinum* which are in agreement with our results.

Smolinska *et al.,* (1997) 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 (Goud *et al.,* 2011). 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.

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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