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Evaluation of water soluble soil amendments against *Sclerotium rolfsii* Sacc. causing stem and pod rot of groundnut under *in vitro* condition

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Abstract
Groundnut (*A. hypogaea* L.), the king of oilseeds is an auxiliary food crop with greater economic and nutritive value. India is the largest producer of groundnut and in India, Gujarat ranked first in terms of area and production of groundnut. In Gujarat, the Saurashtra region covers a wide area under production and is known as the heart of Gujarat as well as of India for groundnut production. In the present study, the pathogen associated with stem and pod rot of groundnut was isolated from different groundnut growing areas of Saurashtra and confirmed by morphological observation as *Sclerotium rolfsii. In vitro* efficacy of seven water soluble soil amendments was tested against the pathogen. Among all the soil amendments tested, four of them *viz.*, farmyard manure, vermicompost, cotton seed cake and castor cake showed complete inhibition of mycelial growth at all tested concentrations. Additionally, goat manure and karanj cake showed 77.69 and 57.44 percent mean inhibition of mycelial growth, respectively. However, goat manure and karanj cake gave complete inhibition of radial growth at concentrations of 5, 7.5 and 10 percent. The lowest inhibition was recorded by neem cake with 49.20 percent mean mycelial growth inhibition.

Keywords: *Sclerotium rolfsii* (pod rot), Groundnut, *in vitro*, soil amendments

Introduction
Groundnut (*A. hypogaea* L.), the king of oilseeds is an auxiliary food crop grown in warm temperate, tropical and subtropical climates around the world. Groundnut is grown in about 120 countries in the world. Asia is one of the major region producing groundnut in the world. The most important countries in the world producing groundnut are India, China, USA, West Africa, Sudan and Nigeria etc. Amongst all oilseed crops, groundnut accounts for more than 40-50 percent in the area and 60 to 70 percent in production in India while, Gujarat ranked first in terms of area and production of groundnut. In Gujarat, the Saurashtra region covers a wide area under production. Hence, Saurashtra is known as the heart of Gujarat as well as of India for groundnut production.

Groundnut improves soil fertility by fixing atmospheric nitrogen through root nodules which adds about 12 to 40 kg N/ha. All parts of the groundnut plant can be used for a variety of purposes. The seeds contain 25 percent highly digestive protein. The biological value of the groundnut protein is among the highest of the vegetable protein and equals that of casein. It is also rich in energy (567 calories per 100 g) as well as calcium, phosphorus, magnesium, zinc and iron. It is also a good source of all B vitamins except B_{12}. The kernels compose of antioxidants and fine-quality amino acids like thiamin, riboflavin and nicotinic acid that are essential for growth and development.

As groundnut has greater economic and nutritive value, many elements restrict the productivity of groundnut including abiotic and biotic stresses. Among biotic stresses, yield loss of groundnut crop due to stem and pod rot disease caused by *Sclerotium rolfsii* Sacc. has become a major constraint in the Saurashtra region of Gujarat state. Colonies of *S. rolfsii* can be readily distinguished on infected plant material or artificial media by gross morphological characteristics. Rapidly growing, silky-white hyphae tend to aggregate into rhizomorphic cords (Aycock, 1966; Harlton *et al.*, 1995) [3, 4]. In culture, agar media are rapidly (2-3 days) covered with mycelium, including aerial hyphae. The pathogen requires a warm climate and occurs more frequently at high moistures (80%) and high temperatures *i.e.* 27-35 °C (Al-Askar *et al.*, 2013) [1]. In *Kharif* groundnut, the disease is emerging as a major threat to groundnut production by which heavy loss is recorded when favorable climatic conditions prevail.

Looking at the high disease incidence, the versatile nature of the pathogen and economic damage due to stem and pod rot, the present studies were undertaken to manage the disease through an alternate eco-friendly approach by using organic water soluble soil amendments.

Materials and Methods

Collection and isolation of *S. rolfsii*

The plant samples, soil and sclerotia were collected from farmers' fields during the survey and the fungus was isolated into a pure culture on Potato Dextrose Agar (PDA) using the standard isolation technique. The flame-sterilized forceps were employed to directly pick the white mycelial growth of fungus and dark brown, mustard seed like sclerotia from the stem area of the groundnut plant that displayed symptoms of stem and pod rot infection. The sclerotia were surface-sterilized using a 0.1% $HgCl_2$ solution for five to ten seconds followed by three to four washes with sterilized distilled water to eliminate the traces of disinfectant. The sterilized sclerotia were then blotted and dried. The developing white mycelial strands originating from the infected tissues or sterilized blotted dried sclerotia were aseptically transferred into Petri plates containing Potato Dextrose Agar (PDA) medium. The Petri plates were incubated at 27 ± 1 °C to achieve optimal fungal growth. A total of fifty isolates of *S. rolfsii* were isolated and designated as JND-1 to JND-10 for Junagadh district, PB-1 to PB-10 for Porbandar, RJK-1 to RJK-10 for Rajkot, GS-1 to GS-10 for Gir Somnath and JMN-1 to JMN-10 for Jamnagar district. Based on the pathogenicity of all fifty isolates of *S. rolfsii* in terms of percent disease incidence, the isolate with the highest percent disease incidence was identified as a highly virulent isolate which was further used for an *in vitro* study to test the efficacy of various soil amendments.

In vitro evaluation of soil amendments

Seven water soluble soil amendments with four concentrations *viz.*, 2.5, 5.0, 7.5 and 10.0 percent (Table 1) was evaluated against the highly virulent isolate of *S. rolfsii* (JMN-2) under *in vitro* condition by using the method described by Kuldhar and Suryawanshi (2017) [5].

To obtain amendment extracts, 100 grams of each water soluble amendment powder was added to a 250 ml conical flask containing 100 ml of distilled water. The flasks were allowed to stand for overnight at room temperature and these mixtures were first filtered through double-layered muslin cloth and added to another flask. After that, the resulting mixture was obtained by filtering the flask content through Whatman's No.1 filter paper using a funnel and 100 ml conical flask. The final extracts obtained were clear and the concentration of the standard was cent percent. The specific quantity of extract was added to a conical flask containing 100 ml of melted sterile PDA medium at the time of pouring it into Petri plates to obtain the required concentration. The flask containing the poisoned medium was vigorously shaken to ensure a uniform mixture of amendment extract and 20 ml of the medium was poured into sterilized Petri plates. After the medium solidified, the plates were inoculated in the center by placing a 5 mm diameter mycelial disc which was cut aseptically using a cork borer from a 7-10 days old pure culture of *S. rolfsii*. The inoculated plates were then incubated at 27 ± 1 °C. The growth of the test fungus on non-poisoned PDA served as a control. The colony diameter was measured at the point of maximum growth of the pathogen in the control plate. The recorded observations were then used to calculate the percentage of mycelial growth inhibition using the formula given by Vincent (1947) [8].

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

Where,
I = Percent inhibition of mycelial growth
C = Average diameter of mycelial colony in control treatment (mm)
T = Average diameter of mycelial colony in treated plate (mm)

Table 1: Details of water soluble soil amendments evaluated against *S. rolfsii* under *in vitro* condition

Tr. No.	Soil amendments	Concentration (%)			
		1	2	3	4
T_1	Farm Yard Manure	2.5	5.0	7.5	10.0
T_2	Vermicompost	2.5	5.0	7.5	10.0
T_3	Goat manure	2.5	5.0	7.5	10.0
T_4	Karanj cake	2.5	5.0	7.5	10.0
T_5	Cotton seed cake	2.5	5.0	7.5	10.0
T_6	Neem cake	2.5	5.0	7.5	10.0
T_7	Castor cake	2.5	5.0	7.5	10.0

Results and Discussion

The results regarding percent mycelial growth inhibition by different soil amendments presented in Table 2 and Plate-I revealed that out of all the soil amendments tested four of them *viz.*, farmyard manure, vermicompost, cotton seed cake and castor cake showed complete inhibition of mycelial growth at all tested concentrations. Additionally, goat manure showed 77.69 percent mean inhibition of mycelial growth and achieved complete inhibition of radial growth at concentrations of 5, 7.5 and 10 percent, while at 2.5 percent concentration goat manure showed only 35.56 percent inhibition of mycelial growth. However, karanj cake gave 57.44 percent mean mycelial growth inhibition and showed cent percent mycelial growth inhibition at 7.5 and 10 percent concentrations except 2.5 and 5 percent concentrations that reported 2.22 and 44.07 percent mycelial growth inhibition, respectively. The lowest inhibition was recorded by neem cake with 49.20 percent mean mycelial growth inhibition. Neem cake showed cent percent radial growth inhibition at 7.5 and 10 percent concentrations.

Upon inspection of individually tested concentrations of water soluble soil amendments, it exemplified that all amendments showed a significant increase in mycelial growth inhibition with increasing concentration. Furthermore, only three amendments, goat manure at 2.5 percent, karanj cake at 2.5, 5 percent and neem cake at 2.5 and 5 percent concentrations displayed a significant difference in mycelial growth inhibition of *S. rolfsii* at all tested concentrations. In contrast, the remaining amendments exhibited complete mycelial growth inhibition at all concentrations (2.5%, 5%, 7.5% and 10%).

Previous studies also showed more or less a similar pattern with several workers, in which Rubayet *et al.*(2011) [6] revealed that among five organic amendments, mustard cake gave the best result to control the mycelial growth of *S. rolfsii* followed by tea waste. Kuldhar and Suryawanshi (2017) [5]

examined that neem seed cake extract was found most effective against
S. rolfsii with significantly least mycelial growth (19.13 mm) and highest mycelial inhibition (78.74%) which was followed by castor cake (20.07 mm and 77.70%) and karanj cake (23.93 mm, and 73.40%). However, gypsum and compost were less effective with 55.44 percent and 50.62 percent mycelial inhibition of *S. rolfsii*, respectively. Thakkar *et al.* (2018) [7] reported that among all six amendments, the maximum growth inhibition of *S. rolfsii* was recorded in castor cake at 10, 20 and 30 percent concentrations with76.74, 91.71 and 98.55 percent mycelial growth inhibition, respectively and 68.67, 18.33 and 0.00 sclerotial production, respectively. Similarly, Anitha *et al*. (2019) [2] revealed that mahua oil cake (10% concentration) and farm yard manure recorded the maximum inhibition on mycelial growth of *S. rolfsii*.

Table 2: Evaluation of water soluble soil amendments against *S. rolfsii* under *in vitro* condition

Tr.	Soil amendments	Mycelial growth Inhibition (%)				Mean inhibition
		2.5 %#	5.0 %	7.5 %	10.0 %	
T_1	Farm Yard Manure	73.29 (91.70)*	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)
T_2	Vermicompost	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)
T_3	Goat manure	36.62 (35.56)	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)	64.12 (77.69)
T_4	Karanj cake	8.39 (2.22)	41.62 (44.07)	73.29 (91.70)	73.29 (91.70)	49.15 (57.44)
T_5	Cotton seed cake	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)
T_6	Neem cake	8.94 (2.59)	19.10 (10.74)	73.29 (91.70)	73.29 (91.70)	43.66 (49.20)
T_7	Castor cake	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)	73.29 (91.70)
		Fungicide (F)		**Concentration (C)**		**F × C**
S.Em. ±		0.24		0.18		0.48
C.D. at 5%		0.68		0.52		1.37
C.V. %		1.31				

#Mean of three replications
*Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values and values of 0 and 100 in the table were replaced with 1/(4 n) and (1-1/(4 n)100), respectively. Whereas n represents the number of observations

Conclusion

Based on the present investigation, it can be concluded that soil amendments including farmyard manure, vermicompost, cotton seed cake and castor cake found highly effective on the stem and pod rot causing fungi *S. rolfsii* with cent percent growth inhibition at all tested concentrations under *in vitro* condition. Therefore effective management strategies were developed through an alternate eco-friendly approach by using organic water soluble soil amendments which showed fungicidal activity.

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