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IN-VITRO ANALYSIS OF DIFFERENT BOTANICALS AGAINST STEM AND POD ROT OF GROUNDNUT CAUSED BY *Sclerotium rolfsii* Sacc. AND THEIR SCLEROTIAL FORMATION

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ABSTRACT

Groundnut is one of the most important oilseed crop cultivated widely through the tropic and warm temperate regions. The Sclerotium rolfsii is the most dangerous soil borne pathogen and cause damages of stem and pod rot mostly. The effect of botanicals on inhibition of colony growth and germination of sclerotia of different stages was significant. Among all botanicals, Neem proved superior and recorded maximum inhibition at 3% concentration (39.17%) and 4% concentration (44.25%) followed by Garlic crude extract at -3% concentration (33.09%) and 4% concentration (39.14%), Pungam oil, Calotrophis leaf e-xtract and finally Lemon grass leaf extract. Neem oil inhibited germination of sclerotium on 7th day after inoculation (44.25%). All stages of sclerotia were inhibited to the maximum at higher concentration of Neem (Immature, partial, mature). Minimum growth period was recorded in Calotrophis treatment, where 7 day, 13 days and 16 days for immature, partial mature and mature stages of sclerotia respectively.

KEYWORD: ***Groundnut, Stem and Pod rot, Sclerotium rolfsii, Sclerotia, botanicals, Management.***

I. INTRODUCTION

Groundnut is a member of sub-family, Faboideae of the family Fabaceae. The genus *Arachis hypogea* L. had wide adaptability to varying agro-climate condition and soils, which has made its cultivation possible in most of the tropical and subtropical countries in the world. It is commercially cultivated in about 100 countries located between 40 N and 40 S.

Groundnut is essentially a tropical plant. It requires a long and warm growing season. The most favorable climatic conditions for groundnuts are a well-distributed rainfall of at least 50 - 55 cm during growing season, abundance of sunshine and relatively warm temperature. It seems that groundnut plant will grow best when the mean temperature is 21°C to 26.5°C (Thamaraikannan et al., 2009).

Groundnut is grown on wide and different variety of soil types. However, red soil, sandy loam, loamy soils and black soils with good drainage facility are best suited for this crop. Heavy and stiff clays are unsuitable for groundnut cultivation as the pod development is hampered in this type of soil.

Groundnut is called as the 'king' of oil-seeds. It is one of the most important food, oil-seeds and cash crops of our country. India is the second largest producer of groundnuts when compare to China. Groundnut is the largest Oil-seed in India in terms of production (Anonymous, 2015).

Groundnut seeds are rich source of edible oil and contain 40-50 per cent oil, 20-50 per cent protein and 10-20 per cent carbohydrate. It contains 48-50 per cent oil and is a rich source of dietary fiber, minerals, and vitamins. Seeds are nutritious and contain Vitamin E, Niacin, Folacin, Calcium, Phosphorus, Magnesium, Zinc, Iron, Riboflavin, Thiamine, Potassium etc. Groundnut oil and its protein meal constitute an important segment of world trade in oil-seeds and products. Groundnut is used for various purposes like food (raw, roasted, boiled, cooking oil), animal feed (pressings, seeds, green material, straw), and industrial raw material (Nwokolo, 1996). Shells of groundnut have great potential for commercial use. It is used as fuel, filler in cattle feed, hard particleboard cork substitute, activated carbon, etc. Groundnut straw is mainly used as animal feed and fuel and in preparation of compost (Thamaraikannan et al., 2009).

In India, groundnut is cultivating in three seasons i.e. Kharif, Rabi and summer. The groundnut is mostly grown in the central states Gujarat, Tamil Nadu Andhra Pradesh, Maharashtra and Madhya Pradesh.

In India, it is grown in 12 states in an area of 12,322 ha producing about 7355 tonnes/ha of pods. The total area under groundnut cultivation in India during the 2014-15 the total area has been covered 4768.7 ha with

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producing 7401.7 tonnes/ha with productivity of 1552 kg/ha (Anonymous, 2015). During the year 2014-2015, Gujarat has ranked first it contribution about 25 per cent of area with 48.24 per cent of production with producing of 3.57 million tonnes pods from an area of 1.23 million ha with a productivity of 2914 kg/ha (Anonymous, 2015). In India one third of the crop is cultivated in summer season. South Gujarat is the important region for summer groundnut cultivation it play important role of farmer economy.

Groundnut plants suffer from many diseases caused by fungi, viruses, bacteria and nematodes diseases caused yield reduces. However, only a few diseases are economically important in India, which includes fungal disease like early leaf spot (*Cercospora arachidicola*, Hori.), late leaf spot *(Phaeoisariopsis personata*, (Berk. and Curt.) V. Arx), rust (*Puccinia arachidis*, Speg.), collar rot (*Aspergillus niger*, Van Tieghem), stem rot (*Sclerotium rolfsii* Sacc.), root rot (*Macrophomina phaseolina* (Tassi.) Goid.), aflaroot (*Aspergillus flavus* (Link) ex. Fries). Root knot nematode (*Meloidogyne arenaria* (Neal) Chitwood), virus diseases like peanut bud necrosis and peanut clump, peanut strip, peanut mottle, peanut stem necrosis (Ghewande and Reddy, 1986; Grover 1981; Reddy et al., 2002).

Most of soil borne diseases like, stem rot, root rot, collar rot and pod rot, among all the soil borne diseases, stem rot caused by *S. rolfsii* Sacc. is an important pathogen which is attacking different growth stages of groundnut plants.

Stem rot disease caused by *Sclerotium rolfsii* Sacc. was first reported by Rao (1969) in Vijayawada district of Andra Pradesh, India. Today, control of this fungal disease is the subject of many research projects involving chemical, bioagents, soil amendments and cultural modifications, etc. (Anonymous, 2001).

Sclerotium rolfsii Sacc. (Teleomorph stage=*Athelia rolfsii*, Curzi Tu and Kimbrough) has been reported to cause stem rot, root rot, sclerotial wilt, (Chohan, 1974) and stem and pod rot (Mehan et al., 1995). Stem rot also known as southern blight and also white mould is a devastating soil borne disease in the India. Stem rot has been observed, where moisture, relative humidity and temperature conditions are sufficiently high to allow the growth and survival of *S. rolfsii*. The fungus has extensive host range, prolific growth rate and ability to produce large number of sclerotia that may persist in soil for several years (Punja, 1985).

The genus Sclerotium Saccardo includes asexual fungi that form small, spherical sclerotia (tan to dark-brown) comprised of three distinct layers (an outer rind, middle cortex and inner medulla) and sterile mycelia, with no known sexual or conidial state. Several fungi which previously belonged to form-genus Sclerotium have since been placed in more appropriate genera with the discovery of associated teleomorph *S. rolfsii* Sacc. first reported on tomato by Rolfs in Florida in year (1892) is a serious fungal pathogen. It causes different plant and same disease of Southern Blight on peanut, tomato, watermelon, cowpea, sugar beet, rice and wheat. It has a wide host range as it affects different plants specious belonging to nearly 100 families (Anonymous, 2013).

The low productivity in groundnut is attributed to many and various production constraints. Among these particularly stem rot of groundnut disease play a major role in reducing the yield of groundnut.

The stem and pod rot caused by *S. rolfsii* Sacc. had become major constraints and potential to reduces Summer groundnut production in South Gujarat region. Stem rot disease is most important disease in this area which cause around 25-30 per cent yield loss. At present, all popular cultivars of groundnut are susceptible to stem rot pathogen. Under continuous Rice-Groundnut cropping system endemically the diseases regularly re-occurs causing severe damage in south Gujarat. Looking to versatile nature of pathogen and rice based cultivation system, stem and pod rot cannot be manage successfully by single method. The only way in absence of resistant cultivars is to follow integration of different method to manage the disease at satisfactory level. This includes the feasible integration of cultural practices, chemicals, bio-control agents, to identify most important susceptible stage and management at proper time is main fact of research work.

Objectives

1. Collection, isolation, identification and pathogenicity of *Sclerotium rolfsii* Sacc.
2. In vitro testing of bio-agents against *Sclerotium rolfsii* Sacc.

II MATERIALS AND METHOD

2.1 Collection, isolation, identification and pathogenicity of *Sclerotium rolfsii*

2.1.1 Sample collection

The disease sample of groundnut plant with typical showing stem rot symptoms, soil from infected field and directly sclerotia picked from infected stem were collected from the Uttaranchal collage of agricultural sciences., Uttaranchal University., Dehradun., Uttarakhand., PCP field during 2018-19. The infected plant samples, soil and sclerotia brought into the laboratory and subjected to microscopic examination and for further studies.

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2.1.2. Isolation of pathogen

2.1.2.1. Direct isolation:

Nichrome wire / needle duly sterilized were used and the fungus growth / sclerotia from infected stem were directly transferred in to plates containing PDA media under aseptic condition and plates were incubated at 27 ± 2ºC for optimum growth.

2.1.2.2 Sterilization of equipment and culture media

All the glassware used in laboratory studies viz. test tubes, Petri plates and flasks of Corning/Duran make were immersed in chromic acid solution (Potassium dichromate 80g, sulphuric acid 400 ml and water 300 ml) for 24 hrs, then thoroughly cleaned with tap water and finally rinsed with sterile water. Then glassware's were sterilized in a hot air oven at 1800C for one hour. The laminar flow was sterilizes with 95 per cent alcohol and U.V. light radiation. The inoculation loop and needle were sterilized by direct heating (incineration) over spirit lamp flame and dipped in absolute alcohol.

2.2 Preparation of medium

2.2.1 Potato dextrose agar medium

For all laboratory experimental studies, the standard potato dextrose agar (PDA) medium was used for culturing the *Sclerotium rolfsii*. The composition of PDA used is given below.

Peeled potato - 200 g
Dextrose - 20 g
Agar-agar - 20 g
Distilled water - 1000 ml

2.7.2 Botanicals

2.9. Effect of neem and pongam oil against mycelial growth and sclerotial production of *Sclerotium rolfsii*.

Two medicinal oil viz. Neem oil and pongam oil was evaluated against the pathogen in-vitro to examine the inhibitory effect of mycelial growth and sclerotial production. For evaluation of antifungal activities of the oil, two concentration i.e. 3.0 & 4.0 % were taken. Desired concentrations obtained by adding appropriate amount of oil to PDA in petri plates. Four replication were maintained in each treatment. PDA without oil served as control. Each plate was inoculated with a 5 mm diameter mycelial disc taken from 4 days old culture of *sclerotium rolfsii* grown on PDA. The observations were recorded for mycelial growth regularly after inoculation and sclerotia were counted 15 days after inoculation.

Percent inhibition of mycelial growth was calculated by the following formula,

$$\text{Inhibition \%} = C–T/C \times 100$$

Whereas,

C = Diameter of fungus colony (mm) in control plate
T = Diameter of fungus colony (mm) in treated plate

2.8. In-vitro evaluation of medicinal plants leaf extracts against mycelial growth and sclerotial production of *Sclerotium rolfsii:*

Antifungal activity of three medicinal plants leaf extracts were studied under in-vitro condition by using respective plant leaf dextrose agar medium. The following medicinal plants viz., garlic crude extract (*Allium sativum*), Aak *(Calotropis procera*), lemongrass extract (*Cymbopogon flexuosus*) were used and evaluated by poisoned food technique method. Medium without extract was used as control.

Percent inhibition of mycelial growth was calculated by the following formula,

$$\text{Inhibition \%} = C–T/C \times 100$$

Whereas,

C = Diameter of fungus colony (mm) in control plate
T = Diameter of fungus colony (mm) in treated plate

The leaf parts of three different plants belonging to three different families namely Asclepiadaceae, Amaryllidaceae and poaceae were collected for the studies to find out their extract effect on germination of *S. rolfsii*.

For extraction of botanicals, the leaves of the botanicals collected were washed in tap water and finally passed through sterile distilled water. Hundred grams of the sample was crushed in pestle and mortar by adding 100 ml of sterile distilled water (1:1 w/v). The extracts were strained through two layers of muslin cloth. Later, these extracts were centrifuged for 15 minutes at 500 revolutions per minute to separate the plant debris and to get clear supernatant of plant extract. The supernatant thus obtained was used as the standard stock extract (100per cent). The stock extract was finally made up to required concentrations, viz., 3.0 and 4.0 per cent by adding 97 and 96 ml of stock extract to 95.0 and 90.0 ml of potato dextrose agar medium respectively. The plant extracts

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were added to the potato dextrose agar medium at molten state after sterilization. The medium was shaken thoroughly for uniform mixing of test extract. Twenty ml of mixture was poured into sterile Petri-plates, three replications were maintained for each concentration of botanical. Potato dextrose agar medium without plant extract served as control. 5mm size of *S.rolfsii* disc were placed on the Petri-plates containing the medium and plant extract. These inoculated plates were incubated at 27±1ºC. Observations of growth of *sclerotium rolfsii* were recorded. Per cent inhibition of germination of sclerotia over control was calculated according to Vincent (1947) and the results were analyzed statistically by one factorial CRD design.

3. EXPERIMENTAL RESULTS

3.3 Botanicals

3.3.1 Inhibition of germination of sclerotia

Here, the efficacy of five botanicals was tested during the experiment on germination of *sclerotium rolfsii* and details were presented in Table 1.

The effect of botanicals on inhibition of germination of sclerotia of different stages was significant. Among all botanicals, Neem proved superior and recorded maximum inhibition at 3% concentration (39.17%) and 4% concentration (44.25%) followed by Garlic crude extract at 3% concentration (33.09%) and 4% concentration(39.14%), Pongam oil at 3% concentration (28.23%) and 4% concentration (32.97%), Calotrophis leaf extract at 3% concentration (21.23%) and 4% concentration (27.24%) and finally Lemon grass leaf extract at 3% concentration (20.76%) and 4% concentration (24.52%).

Neem oil inhibited germination of *sclerotium rolfsii* on 7th day after inoculation (44.25%). All stages of sclerotia were inhibited to the maximum at higher concentration of Neem (Immature, partial, mature). At 4 per cent concentration inhibitions were 44.25 per cent mature sclerotia, respectively. At 3 per cent of concentration it was 39.17 per cent on 7th day after inoculation of *Sclerotium rolfsii*, respectively.

Similar trend was observed in all concentrations on 7th day after inoculation of Garlic crude buld extract, Pongamia seed oil, Calotrophis leaf extract and Lemon grass leaf extract on germination of *sclerotium rolfsii* (Table 1).

3.3.2 Growth period

Observations on time interval required for further germination of sclerotial bodies (ungerminated) which were treated with different botanicals are furnished in Table 2.

Incase of Neem treatment, the germination of immature bodies took place at 7 day, partial mature bodies at 14 day and for mature bodies at 18 day. In case of Garlic crude extract immature bodies 8 day, partial mature bodies 15 days and mature bodies 20 days. Pongamia treatments, the growth period required for immature bodies 6 days, partial mature bodies 17 days. Calotrophis leaf extract treatments, immature bodies 7 days, partial mature bodies 13 days and mature bodies 16 days. Finally Lemon grass leaf extract immature bodies 9 days, partial mature bodies 18 days and mature bodies 20 days.

Minimum growth period was recorded in Calotrophis treatment, where 7 day, 13 days and 16 days for immature, partial mature and mature stages of sclerotia respectively.

Table 1: Effect of boanicals in inhibiting the germination of *sclerotium rolfsii*

S.No	Concentration	Colony diameter* (mm)				
		Neem Oil	Pungam Oil	Garlic Crude Blub Extract	Calotrophis Leaf Extract	Lemon Grass Leaf Extract
1	0.3	50.79	59.92	55.86	65.82	67.83
2	0.4	46.55	55.96	50.82	60.79	63.02
3	Control	83.5	83.5	83.5	83.5	83.5
SEm±		0.29	0.31	0.26	0.22	0.45
CD at 5%		1.17	1.25	1.03	0.88	1.82

* Mean value of five replication

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S.No	Concentration	Percent Inhibition*				
		Neem Oil	Pongam Oil	Garlic Crude Bulb Extract	Calotrophis Leaf Extract	Lemon Grass Leaf Extract
1	0.3	39.17	28.23	33.09	21.23	20.76
2	0.4	44.25	32.97	39.14	27.24	24.52
3	Control	0	0	0	0	0
SEm±		0.23	0.35	0.27	0.14	0.84
CD at 5%		1.48	2.29	1.78	0.93	2.79

* Mean value of five replication

Table 2: Growth period for different stages Sclerotial bodies on different botanical

S.No	Botanicals	Number of days required for Sclerotial formation		
		Immature bodies	Partial mature bodies	Mature bodies
1	Neem oil	7	14	18
2	Garlic crude extract	8	15	20
3	Pongam oil	6	14	17
4	Calotrophis leaf extract	7	13	16
5	Lemon grass leaf extract	9	18	20

4. DISCUSSION

4.1 Botanicals

4.1.1 Inhibition of germination of sclerotia

Here, the efficacy of five botanicals was tested during the experiment on germination of *Sclerotium rolfsii* and details were presented in Table 7.

The effect of botanicals on inhibition of germination of sclerotia of different stages was significant. Among all botanicals, Neem proved superior and recorded maximum inhibition at 3% concentration (39.17%) and 4% concentration (44.25%) followed by Garlic crude extract at 3% concentration (33.09%) and 4% concentration(39.14%), Pongam oil at 3% concentration (28.23%) and 4% concentration (32.97%), Calotrophis leaf extract at 3% concentration (21.23%) and 4% concentration (27.24%) and finally Lemon grass leaf extract at 3% concentration (20.76%) and 4% concentration (24.52%).

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Similar trend was observed in all concentrations on 7th day after inoculation of Garlic crude bulb extract, Pongamia seed oil, Calotrophis leaf extract and Lemon grass leaf extract on germination of *sclerotium rolfsii.*

4.2 Growth period

Incase of Neem treatment, the germination of immature bodies took place at 7 day, partial mature bodies at 14 day and for mature bodies at 18 day. In case of Garlic crude extract immature bodies 8 day, partial mature bodies 15 days and mature bodies 20 days. Pongamia treatments, the growth period required for immature bodies 6 days, partial mature bodies 17 days. Calotrophis leaf extract treatments, immature bodies 7 days, partial mature bodies 13 days and mature bodies 16 days. Finally Lemon grass leaf extract immature bodies 9 days, partial mature bodies 18 days and mature bodies 20 days.

Minimum growth period was recorded in Calotrophis treatment, where 7 day, 13 days and 16 days for immature, partial mature and mature stages of sclerotia respectively.

5. CONCLUSION

- Among botanicals, Neem was most effectively inhibited the sclerotial germination (44.25%) and delayed the period of further germination of sclerotium was presented after 18th -days.

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