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Pathogenic variability of *Rhizoctonia bataticola* and *Sclerotium rolfsii* isolates of Groundnut (*Arachis hypogeae* L.) in Andhra Pradesh

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ABSTRACT: Groundnut is an important oil seed leguminous crop which is affected by several fungal, viral and phytoplasmal diseases. Among the fungal diseases, soil borne diseases *viz*., dry root and stem rot incited by *Rhizoctonia bataticola* and *Sclerotium rolfsii* respectively are the most important as they possess the major threat to the crop in all the stages of crop and causes higher yield loss. The soil borne fungal pathogens survives in the soil for many years in the form of microsclerotia and sclerotia and causing soil borne fungal infections when the conditions are favorable. The current study was conducted to know the pathogenic variability (virulence) among these pathogens. Dry root rot and stem rot affected samples were collected from the major groundnut growing areas of Rayalaseema region of Andhra Pradesh and isolated the pathogens. A total of 44 *Rhizoctonia bataticola* and 40 *Sclerotium rolfsii* isolates were isolated and purified and tested the pathogenicity under glasshouse conditions at ARS, Kadiri by following the sick soil inoculation method with mass multiplied pathogen(s) inoculum on sorghum grains. The results revealed that the isolate of GNRb-2 of *Rhizoctonia bataticola* and GNSr-25 of *Sclerotium rolfsii* isolate were found significantly more virulent with maximum of 89.4 and 95.8 percent disease incidence respectively and the isolates GNRb-5, GNRb-17, GNRb-29 of *R. bataticola* and GNSr-24 of *S. rolfsii* were recorded the least percent disease incidence with 13.3 and 13.3 respectively, when compared with other isolates tested. Further the isolates were categorized into the non-pathogenic, weekly pathogenic (WP), moderately pathogenic (MP, strongly pathogenic (SP) and highly/ aggressively pathogenic (HP/AP) based on their percent disease incidence (PDI) on the susceptible host cv. Kadiri-6.

Keywords: Groundnut, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, Pathogenic variability.

INTRODUCTION

Groundnut (*Arachis hypogeae* L.) is an important oilseed legume belongs to the family Fabaceae (Pasupuleti *et al*., 2013). It is a major oilseed, food and fodder crop grown in the temperate, tropical and sub-tropical climates in the world. Groundnut is a major oil seed crop cultivated in India. Its seeds are rich source of oil (35-56%), proteins (25-30%), carbohydrates (9.5-19.0%), vitamins (E, K and B) and minerals (P, Ca, Mg and K) (Gulluglu *et al*., 2016 and Hawaladar *et al*., 2021). Groundnut crop despite of growing in all seasons, it is hampered by the diseases such as seedling rot, leaf spots (early and late), rust, stem rot, pod rot, dry root rot, bud necrosis, stem necrosis and nematodes. Among them the soil borne diseases *viz*., dry root rot and stem rot are major constrains for groundnut production in India. The dry root rot is incited by *Rhizoctonia bataticola (*Pycndial stage: *Macrophomina phaseolina*) and the stem rot caused by the *Sclerotium rolfsii* Sacc. (teleomorph: *Athelia rolfsii*) are infect the crop at any stage from seedling to maturity stage and causes the heavy loss to the growers.

MATERIALS AND METHODS

A. Isolation, purification and identification of the pathogens

For isolation of dry root rot and stem rot pathogens, groundnut plants showing the typical symptoms were collected from various locations *viz*., Anantapuramu, Chittoor, YSR Kadapa, Kurnool and SPSR Nellore and were packed in labelled paper bags and brought to the laboratory.

The dry root rot and stem rot pathogens *viz., Rhizoctonia bataticola* and *Sclerotium rolfsii* respectively were isolated from groundnut plants showing the typical symptoms on infected portions (stem, root, pods etc.) by tissue segment method using

Potato Dextrose Agar (PDA) medium (Ranagaswami, 1972). The infected portions of the plant were excised with a sterilized blade into small bits of 1 cm and these bits were surface sterilized by dipping in 1% sodium hypochlorite (NaOCl) for 60 sec, followed by three serial washings with the sterile distilled water (SDW) to remove the traces of NaOCl and placed on the sterilized filter paper to remove the excess moisture and place them on PDA medium. The petri dishes were incubated at 28 ± 2 °C for growth observations.

The mycelial disc of 5 mm at the end of the radial growth was cut with the cork borer and transferred to the new petriplates for purification. After the purification the cultures were identified based on their morphological characters *viz*., mycelial colour (greyish black), the right-angle branching pattern on hyphae and formation of the microsclerotia in case of *Rhizoctonia bataticola.* Flat or fluffy white cottony mycelium and formation of brown or brick red coloured sclerotia in case of *Sclerotium rolfsii,* as described by common wealth mycological institute (CMI) (1970). The pure cultures of the pathogens were maintained on the PDA slants by periodical transferring.

B. Mass multiplication of Rhizoctonia bataticola & Sclerotium rolfsii

After isolation and purification of the pathogens, they were mass multiplied individually on the sorghum grains. For mass multiplication, the sorghum grains were washed thoroughly to remove inert materials and then soaked in the water for about 16-24 h, by adding 2 % of sucrose to the water. After soaking the grains were shade dried to remove excess moisture and fill in 500 or 1000 ml conical flasks and sterilize in autoclave at 121° C for 15 min at 15 pounds per square inch (Psi). The actively grown 5-7 days pure culture of pathogen(s) *viz*., *Rhizoctonia bataticola* and *Sclerotium rolfsii* discs were inoculated into each flask separately under laminar air flow chamber and flasks were incubated at room temperature 28±2° C for about 10-15 days for mass multiplication of the pathogen(s) (Fig.2a & 2b). This mass multiplied pathogen inoculum was used for further studies.

C. Pathogenicity test for Rhizoctonia bataticola & Sclerotium rolfsii

Pathogenicity test for *Rhizoctonia bataticola* and *Sclerotium rolfsii* isolates was carried out by using the soil inoculation technique in the pots (22.5 cm diameter) under greenhouse conditions using the groundnut cv.Kadiri-6 with three replications for each isolate in a completely randomized design (CRD). Sterilized soil was filled in these pots and the mass multiplied pathogen inoculum of 100 g/kg of soil was mixed thoroughly in upper 4-5 cm layer of soil with each isolate of *Rhizoctonia bataticola* and leave it for two weeks to allow the pathogen to establish in the pots (Veena *et al*., 2014).

The seeds of groundnut cv.Kadiri-6 were surface sterilized with 1 % sodium hypochlorite (NaOCl) were sown in the pots. In case of the stem rot pathogen (*Sclerotium rolfsii*) the inoculum was added at 25-30 days after sowing (DAS) in pots at base of the plants. The control was maintained without adding any pathogen inoculum. Pots were kept in the greenhouse and watered regularly in require quantities. The incidence of the dry root rot and stem rot was recorded and percent disease incidence was calculated. The pathogen was re-isolated from the diseased plants to confirm with the original isolate.

Pathogenic variability of *Rhizoctonia bataticola.* The pathogenic variability of all 44 *Rhizoctonia bataticola* isolates was carried out in the earthen pots by following the soil inoculation technique under controlled environmental conditions (Jayasimha *et al*., 2021) using groundnut cv. Kadiri-6 with three replications for each isolate by following completely randomized design (CRD).

Based on the percent incidence of dry root rot disease on groundnut cv.Kadiri-6, isolates of *Rhizoctonia bataticola* were categorized into four groups *viz*., 0%- Non-pathogenic, 1-20%- weakly pathogenic, 21-50% moderately pathogenic, 51-70%- strongly pathogenic and > 71% aggressively pathogenic (Om Gupta *et al*., 2012).

Pathogenic variability *Sclerotium rolfsii.* Pathogenic variability of all the 40 *Sclerotium rolfsii* isolates was studied by growing groundnut seeds of cv.Kadiri-6 in earthen pots by following the soil inoculation of different isolates of *S. rolfsii* at 30 DAS. The pathogenicity was recorded for each isolate separately in the pot showing the stem rot disease symptoms like wilting, drying etc. Pathogenicity of each isolate was recorded from the day of addition of pathogen inoculum *i.e* at 25-30 DAS (Basandrai *et al*., 2021).

Based on the percent incidence of stem rot disease on cv.Kadiri-6 of groundnut the isolates were categorized into four groups (0%- Non-pathogenic, 1-25 %- weakly pathogenic, 25- 50%- moderately pathogenic, 51-75% highly pathogenic and >75 % aggressively pathogenic) (Mahato and Biswas, 2017).

RESULTS AND DISCUSSION

A. Symptoms of dry root rot and stem rot diseases

The dry root in the field was observed as the complete drying or wilting of the plant within 30-45 DAS (Fig.1a) and when uproot the dry root rot infected plant the root will be splitting into different portions and appearance of the minute greyish or blackish microsclerotia on it (Fig. 1b) Acharya *et al*. (2021), Jayasimha *et al*. (2021) also recorded the similar kind of observations in their studies.

The stem rot symptoms in the field were noticed or observed as yellowing of the leaves, wilting, lack of vigor, complete drying of the plant and presence of the white cottony mycelium near the collar region of the plant (Fig.1c) (Gururaj *et al*., 2016) with many brown or brick red color, mustard seed like sclerotial bodies (Fig. 1d) (Ekka *et al*., 2016 and Kakade *et al*., 2017 & Manu *et al*., 2018).

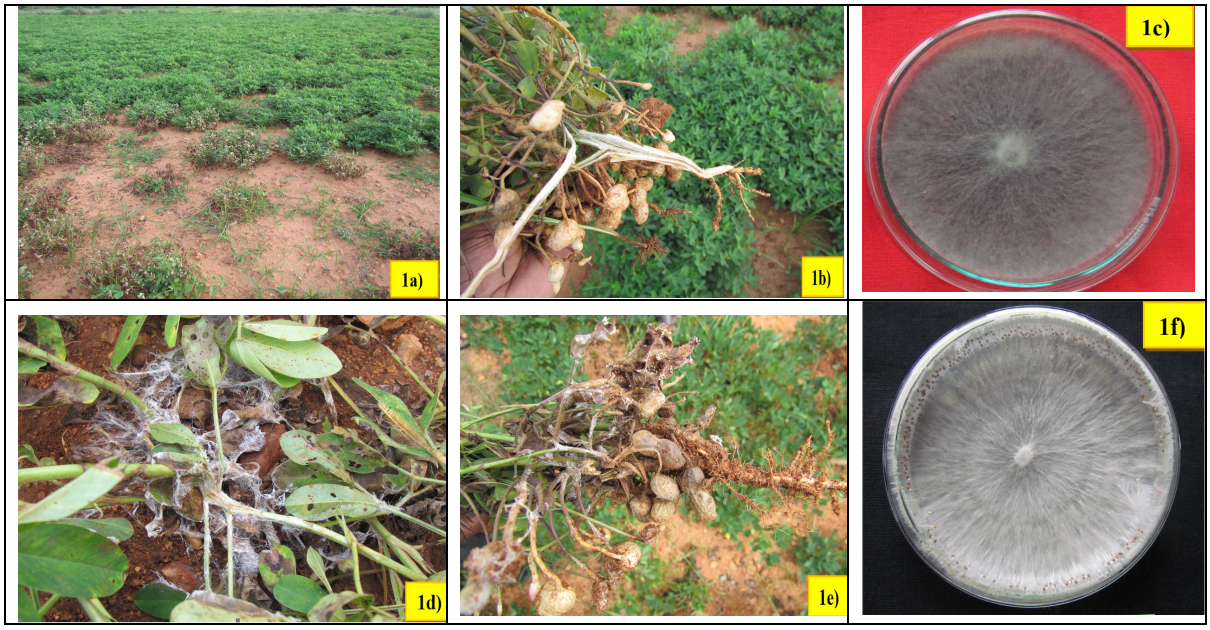


Fig. 1a. Dry root rot symptoms in the field as complete wilting and drying of the plants; **Fig. 1b.** Pinkish discoloration of mycelium with the small black microsclerotia on split opened dry root infected plant; **Fig. 1c.** Pure culture of the dry root rot pathogen Rhizoctonia bataticola with greyish mycelium; **Fig. 1d.** Stem rot infected plants in the filed as the white cottony mycelium and presence of the sclerotial bodies near the collar region; **Fig. 1e.** Presence of the white mycelium and sclerotial bodies on the stem, roots and pods near the ground; **Fig. 1f**. Pure culture of the stem rot pathogen *Sclerotium rolfsii* with white cottony mycelium and sclerotial bodies.

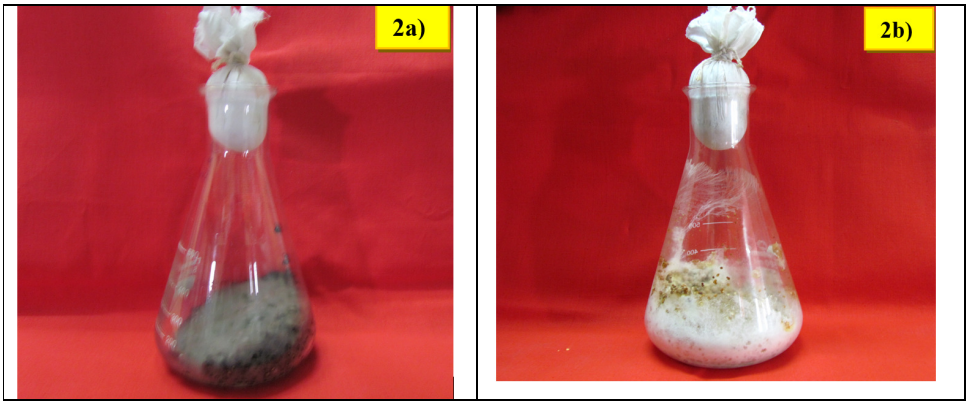
Fig. 1. Typical symptoms of dry root rot and stem rot diseases of groundnut and pure cultures of the pathogens *Rhizoctonia bataticola* and *Sclerotium rolfsii.*

Isolation and purification of the pathogen isolates

A total of 44 No's of *Rhizoctonia bataticola* and 40 No's of the *Sclerotium rolfsii* isolates were isolated from the field collected samples from various locations in Rayalaseema region covering the Anantapur, Chittoor, Y.S.R. Kadapa, Kurnool and SPSR Nellore districts. After the initial isolation and purification, the isolates were identified based on the morphological characters.

Mass multiplication of pathogens *Rhizoctonia bataticola* and *Sclerotium rolfsii*

After the initial isolation and purification, the isolates were mass multiplied on the sorghum grains by inoculation and incubated the conical flasks at 28 + 2° C, for about 15-20 days. After incubation the greyish black mycelial mat will be observed on the *Rhizoctonia bataticola* flasks (Fig. 2a). Whereas cottony white mycelium was spreaded in the sorghum grains in case of the *Sclerotium rolfsii* (Fig 2b). This mass multiplied pathogen culture were used for the pathogenicity studies.



a. Mass multiplication of the *Rhizoctonia bataticola*; **b.** Mass multiplication of the *Sclerotium rolfsii*

Pathogenic variability of *Rhizoctonia bataticola* isolates.

Fig. 2. Mass multiplication of dry root rot and stem rot pathogens.

The pathogenic variability for the *Rhizoctonia bataticola* isolates was studied by following the sick soil inoculation method in glasshouse and the percent disease incidence was recorded (Table 1) and categorized the *Rhizoctonia bataticola* isolates into various categories (Table.2). Germination of groundnut seed ranged from 73.33 % to 100 % among different isolates and the least germination of seed recorded by the isolate of GNRb-2 (73.33%). Similar kind of results was also noticed by the earlier workers (Om Gupta *et al*., 2012, Rajamohan and Balabaskar, 2012 & Rani, 2014, Pandey *et al*., 2021).

Based on their virulence nature on the susceptible host *i.e*., groundnut cv.Kadiri-6. The isolates recorded the different range of the percent disease incidence. The maximum percent disease incidence was recorded as 89.39 % by the GNRb-2 isolate. Whereas in the isolates GNRb-5, GNRb-17, GNRb-29 recorded the lowest disease incidence of 13.33 respectively. The isolates were further grouped into the non-pathogenic, weakly pathogenic, moderately pathogenic, strongly pathogenic and aggressively or highly pathogenic (Fig 3.) based on the percent disease incidence on the susceptible cultivar (Table.2) (Khan *et al*., 2013 & Kumari and Ghatak, 2018).

Table 1: Percent disease incidence of the *Rhizoctonia bataticola* isolates.

Sr. No.	Name of the isolate	Germination* (%)	Percent disease incidence* (PDI)
1.	GNRb-1	93.33 (75.21)	71.43 (57.74)
2.	GNRb-2	73.33 (58.90)	89.39 (78.55)
3.	GNRb-3	93.33 (77.69)	64.29 (53.28)
4.	GNRb-4	86.67 (68.63)	30.77 (33.63)
5.	GNRb-5	100.00 (90.00)	13.33 (21.40)
6.	GNRb-6	86.67 (68.63)	61.54 (51.69)
7.	GNRb-7	100.00 (90.00)	40.00 (39.21)
8.	GNRb-8	86.67 (68.63)	53.85 (47.29)
9.	GNRb-9	100.00 (90.00)	66.67 (54.76)
10.	GNRb-10	100.00 (90.00)	40.00 (39.21)
11.	GNRb-11	100.00 (90.00)	53.33 (46.90)
12.	GNRb-12	86.67 (68.64)	30.77 (33.58)
13.	GNRb-13	93.33 (81.14)	28.57 (32.18)
14.	GNRb-14	86.67 (68.83)	38.46 (38.27)
15.	GNRb-15	86.67 (68.64)	28.57 (32.18)
16.	GNRb-16	93.33 (77.69)	35.71 (36.63)
17.	GNRb-17	100.00 (90.00)	13.33 (21.40)
18.	GNRb-18	93.33 (77.69)	57.14 (49.08)
19.	GNRb-19	86.67 (68.83)	53.85 (47.20)
20.	GNRb-20	100.00 (90.00)	33.33 (35.25)
21.	GNRb-21	93.33 (77.69)	21.43 (27.54)
22.	GNRb-22	93.33 (77.69)	35.71 (36.63)
23.	GNRb-23	100.00 (90.00)	53.33 (46.90)
24.	GNRb-24	86.67 (68.64)	57.14 (49.12)
25.	GNRb-25	93.33 (77.69)	50.21 (45.09)
26.	GNRb-26	96.67 (81.37)	57.14 (49.08)
27.	GNRb-27	93.33 (77.69)	28.57 (32.29)
28.	GNRb-28	86.67 (68.64)	35.71 (36.69)
29.	GNRb-29	100.00 (90.00)	13.33 (21.40)
30.	GNRb-30	100.00 (90.00)	33.33 (35.25)
31.	GNRb-31	93.33 (75.21)	57.14 (49.08)
32.	GNRb-32	100.00 (90.00)	33.33 (35.25)
33.	GNRb-33	100.00 (90.00)	53.33 (46.90)
34.	GNRb-34	100.00 (90.00)	26.67 (31.07)
35.	GNRb-35	100.00 (90.00)	26.67 (31.07)
36.	GNRb-36	86.67 (68.64)	38.46 (38.31)
37.	GNRb-37	93.33 (77.69)	14.29 (22.18)
38.	GNRb-38	100.00 ((90.00)	53.33 (46.90)
39.	GNRb-39	86.67 (68.83)	64.50 (53.43)
40.	GNRb-40	80.00 (63.41)	75.00 (59.97)
41.	GNRb-41	93.33 (81.14)	28.57 (32.27)
42.	GNRb-42	100.00 (90.00)	46.67 (43.07)
43.	GNRb-43	100.00 (90.00)	53.33 (46.89)
44.	GNRb-44	100.00 (90.00)	42.86 (40.90)
45.	Control	100 (90.00)	0.00 (0.00)
C.D.		9.73	6.38
SE(m)		3.45	2.26
C.V.		7.49	9.57

-Mean of three replications -Figures in parenthesis are angular transformed values

Table 2: Categorization of the *Rhizoctonia bataticola* isolates based on the incidence of Dry root rot.

Sr. No.	Category	Number of isolates	Name of the isolates
1.	Non- pathogenic (0%)	Nil	-
2.	Weakly pathogenic (1-20%)	4	GNRb-5, GNRb-17, GNRb-29, GNRb-37
3.	Moderately pathogenic (20-50%)	21	GNRb-4, GNRb-7, GNRb-10, GNRb-12, GNRb-13, GNRb-14, GNRb-15, GNRb-16, GNRb-20, GNRb-21, GNRb-22, GNRb-25, GNRb-27, GNRb-28, GNRb-30, GNRb-32, GNRb-34, GNRb-35, GNRb-36, GNRb-41, GNRb-42
4.	Strongly pathogenic (51-70%)	15	GNRb-3, GNRb-6, GNRb-8, GNRb-9, GNRb-11, GNRb-18, GNRb-19, GNRb-23, GNRb-24, GNRb-26, GNRb-31, GNRb-33, GNRb-38, GNRb-39, GNRb-43
5.	Highly pathogenic (> 71%)	3	GNRb-1, GNRb-2, GNRb-40

Pathogenic variability among *Sclerotium rolfsii* isolates

The pathogenic variability for the *Sclerotium rolfsii* isolates was using sick soil inoculation method in glasshouse condition. The isolates showed the various degrees of the percent disease incidence. The maximum percent disease incidence was recorded as 95.83 % in the GNSr-25 isolate. Whereas the lowest disease incidence with 13.33 was recorded in GNSr-24. The percent disease incidence was recorded (Table 3 & Fig. 4) and categorized the *Sclerotium rolfsii* isolates into various categories (Table 4) based on incidence of stem rot on the susceptible host *i.e*., Kadiri-6 groundnut cultivar. The isolates were grouped into the non-pathogenic, weakly pathogenic (WP), moderately pathogenic (MP), strongly pathogenic (SP) and aggressively or highly pathogenic (HP) based the percent disease incidence on the susceptible cultivar (Table. 4) (Mahato and Biswas, 2017 & Kumari and Ghatak, 2018). Similar kind of results was also noticed by the earlier workers (Sivakumar *et al*., 2016, Naresh *et al*., 2017, Sekhar *et al*., 2017, Hawaladar *et al*., 2021). The grouping of isolates into various groups will help to understand the virulence pattern of the pathogens. Further these virulent isolates can be used for the screening of groundnut cultivars for dry root rot and stem rot diseases and to identify the resistance source and it ultimately used in the breeding programmes for development of resistance varieties etc.

Table 3: Percent disease incidence of the *Sclerotium rolfsii* isolates.

Sr. No.	Name of the isolate	Percent disease incidence* (PDI)
1.	GNSr-1	75.00 (59.97)
2.	GNSr-2	46.15 (42.80)
3.	GNSr-3	61.54 (51.67)
4.	GNSr-4	60.00 (50.74)
5.	GNSr-5	61.54 (51.69)
6.	GNSr-6	45.45 (42.36)
7.	GNSr-7	38.46 (38.31)
8.	GNSr-8	35.71 (36.69)
9.	GNSr-9	81.82 (64.72)
10.	GNSr-10	33.33 (35.25)
11.	GNSr-11	46.15 (42.80)
12.	GNSr-12	35.71 (36.67)
13.	GNSr-13	58.33 (49.79)
14.	GNSr-14	75.00 (59.97)
15.	GNSr-15	46.15 (42.80)
16.	GNSr-16	38.46 (38.31)
17.	GNSr-17	59.17 (50.28)
18.	GNSr-18	61.54 (51.64)
19.	GNSr-19	61.54 (51.64)
20.	GNSr-20	61.54 (51.64)
21.	GNSr-21	53.85 (47.18)
22.	GNSr-22	76.92 (61.31)
23.	GNSr-23	33.33 (35.23)
24.	GNSr-24	13.33 (21.40)
25.	GNSr-25	95.83 (72.73)
26.	GNSr-26	58.33 (49.79)
27.	GNSr-27	61.54 (51.64)
28.	GNSr-28	50.00 (44.98)
29.	GNSr-29	42.86 (40.90)

30.	GNSr-30	46.15 (42.80)
31.	GNSr-31	26.67 (31.05)
32.	GNSr-32	57.14 (49.08)
33.	GNSr-33	50.00 (44.98)
34.	GNSr-34	58.33 (49.80)
35.	GNSr-35	35.71 (36.66)
36.	GNSr-36	66.67 (54.75)
37.	GNSr-37	76.92 (61.31)
38.	GNSr-38	21.43 (27.54)
39.	GNSr-39	19.23 (25.95)
40.	GNSr-40	35.71 (36.66)
41.	Control	0.00 (0.00)
C.D.		2.45
SE(m)		0.87
C.V.		3.28

Table 4: Categorization of the *Sclerotium rolfsii* isolates based on incidence of Stem rot.

Sr. No.	Category	Number of isolates	Name of the isolates
1.	Non- pathogenic (0%)	Nil	-
2.	Weakly pathogenic (1-25%)	3	GNSr-24, GNSr-38, GNSr-39
3.	Moderately pathogenic (25-50%)	18	GNSr-2, GNSr-6, GNSr-7, GNSr-8, GNSr-10, GNSr-11, GNSr-12, GNSr-15, GNSr-16, GNSr-23, GNSr-28, GNSr-29, GNSr-30, GNSr-31, GNSr-33, GNSr-35, GNSr-38, GNSr-40
4.	Strongly pathogenic (51-75%)	16	GNSr-1, GNSr-3, GNSr-4, GNSr-5, GNSr-13, GNSr-14, GNSr-17, GNSr-18, GNSr-19, GNSr-20, GNSr-21, GNSr-27, GNSr-32, GNSr-34, GNSr-36
5.	Aggressively/ Highly pathogenic (> 75%)	4	GNSr-9, GNSr-22, GNSr-25, GNSr-37

Fig. 3. Categorization of the *Rhizoctonia bataticola* isolates based on their virulence as a) non- pathogenic b) Weakly pathogenic c) Moderately pathogenic d) Strongly pathogenic e) Highly pathogenic.

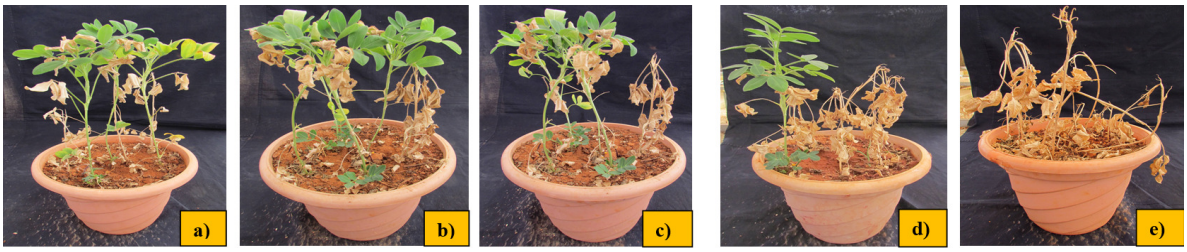


Fig. 4. Categorization of the *Sclerotium rolfsii* isolates based on their virulence as a) non-pathogenic b) Weakly pathogenic c) Moderately pathogenic d) Strongly pathogenic e) Highly pathogenic.

CONCLUSIONS

The plants showing the typical symptoms of the dry root rot and stem rot were collected from the major groundnut growing regions of the Andhra Pradesh *viz.*, Anantapuramu, Chittoor, YSR Kadapa, Kurnool and SPSR Nellore districts. A total of 44 No's of *Rhizoctonia bataticola* and 40 No's of the *Sclerotium rolfsii* isolates were isolated from the field collected samples and were tested for their pathogenic variability. The *R. bataticola* and *S. rolfsii* isolate(s) *viz.*, GNRb-2 and GNSr-25 isolates showed the maximum percent disease incidence of 89.39 and 95.83 respectively. The isolates *viz.*, GNRb-5, GNRb-17, GNRb-29 of *R. bataticola* and GNSr-24 of *S. rolfsii* were recorded the lowest disease incidence with 13.33 and 13.33 respectively, when compared with other isolates tested. Further the isolates were categorized into the non-pathogenic, weekly pathogenic (WP), moderately

pathogenic (MP, strongly pathogenic (SP) and highly/ aggressively pathogenic (HP/AP) based on their percent disease incidence (PDI) on the susceptible host (K-6 groundnut cultivar). Among the isolates tested for *R. bataticola* the non-pathogenic were found as zero or nil, whereas WP were found as 4 and 13, MP as 21 and 18, SP as 15 and 16 and HP as 3 and 4 in *Rhizoctonia bataticola* and *Sclerotium rolfsii* respectively. The categorization of the pathogen isolates will help to understand the virulence pattern of the pathogens. Further it was helpful to study the major factors responsible for their variation in the pathogenicity at the genomic level.

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