The Pharma Innovation Journal 2022; 11(9): 2844-2848

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(9): 2826-2830

www.thepharmajournal.com
Received: 12-06-2022
Accepted: 14-07-2022

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Survey, virulence, inoculum levels and susceptible stage on the incidence of stem rot of Cluster bean (*Cyamopsis tetragonoloba* L.) caused by *Sclerotium rolfsii* Sacc

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Abstract
Stem rot of cluster bean caused by *Sclerotium rolfsii* Sacc. is a soil-borne disease which causes considerable damage to the crop and yield loss. A roving survey was carried out in different districts of Tamil Nadu *viz*., Cuddalore, Dindigul, Namakkal and Salem during the year 2020-2021.The results revealed that the maximum incidence of 18.33% was recorded in Melamoongiladi village of Cuddalore District. Pathogenicity test was carried out under pot culture by inoculating with pathogenic culture of *S. rolfsii* isolates. Among the isolates tested, the isolate (I_1) from Melamoongiladi village pertaining to Cuddalore district was found to be highly virulent in causing stem rot compared to other isolates investigated in the present study. *S. rolfsii* multiplied on sorghum grains @5 grains/plant to 1kg of soil registered the maximum incidence of 69.00% stem rot at 100 and 120 days after pathogen inoculation and it was found significantly more susceptible compared to rest of the treatments.

Keywords: Cluster bean, survey, virulence and inoculum levels

Introduction
Cluster bean (*Cyamopsis tetragonaloba* (L.) Taub) which is commonly known as guar means 'cow food' (in Hindi) belonging to the family leguminaceae. It is an annual arid and semi-arid legume crop (Singh *et al*. 2001) [19] grown as green manure, as forage crop for cattle and as a vegetable crop for human consumption. It is primarily grown for seed, animal feed, fodder, vegetable and green manuring purposes. Cluster bean is a rich source of high quality galactomannan gum and protein rich (40-50%) guar meal as animal feed. The green and tender pods of guar are cooked as favourite vegetables in many parts of the country including South India (Choudhary and Sindhu, 2015) [7]. India is the largest producer of Guar with 80% among the world production, followed by Pakistan with 10-15%. Cluster bean is a native to the Indian subcontinent. It is an erect, bushy, annual herbaceous legume up to 3 m height with trifoliate leaves up to 10 cm long and white flowers. The pods are straight, hairy, pale shiny green, up to 12 cm long and contain 5 to 12 hard seeds. The area under cluster bean production in India is 4.26 million ha with a production of 2.42 million tonnes and productivity of 567 kg/ha (Anonymous, 2020) [3]. Rajasthan is the biggest cluster bean producer state contributes about 80 per cent of the total cluster bean production in the country. In Rajasthan, area under the cluster bean crop is 35.30 lakh hectare with production of 14.04 lakh tonnes and productivity 398 kg/ha (Anonymous, 2020) [3].
The fungal infection causes variations in protein contents of plant parts. Being a multipurpose crop, there is a great demand of organic cluster bean. Sustainable cluster bean cultivation is continuously challenged by diseases that cause quantitative and qualitative losses in yield. Among the fungal diseases stem rot caused by *Sclerotium rolfsii* is a soil borne disease which causes considerable damage to the crop and yield loss was estimated up to 50-70 per cent under field condition (Ronakkumar and Sumanbhai, 2014) [15]. With this background, the present study has been undertaken with the following objectives. i) To survey and isolate the pathogen from infected samples, ii) To assess the virulence of *Sclerotium* isolates and to fix the inoculum levels on the incidence of stem rot disease.

Materials and Methods
Cluster bean seeds var. Pusa Mausami purchased from Department of Horticulture, TNAU, and Coimbatore were used in the pot culture studies.

Survey on the incidence of stem rot of cluster bean in different districts of Tamil Nadu

Surveys were carried out in important cluster bean growing tracts *viz*., Cuddalore, Dindigul, Namakkal and Salem districts of Tamil Nadu during the year 2020 to assess the severity of stem rot incidence. At each place, three fields were selected. Five plots ($1m^2$) were selected randomly in each field and 100 plants in each plot were selected randomly. Observations were recorded and per cent disease incidence was calculated using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Isolation and maintenance of pathogen

The stem rot symptoms were collected from major cluster bean growing tracts of Tamil Nadu pertaining to districts such as Cuddalore, Dindigul, Erode, Namakkal and Salem. The infected plant materials brought back from the field were washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface sterilized in 0.5% sodium hypochlorite solution for 5 min. and rinsed in three changes of sterile distilled water. The segments were separately dried in between sheets of sterile filter paper and placed (3 pieces per plate) on fresh potato dextrose agar (PDA) medium (Ainsworth, 1961) [1] impregnated with streptomycin, and incubated for seven days at 28±2 °C. A total of seven isolates (I_1 to I_7) causing stem rot was isolated from infected plant samples collected from different tracts of Tamil Nadu. The fungal growth on 5th day, which arose through the sclerotial bodies was cut by inoculation loop and transferred aseptically to the PDA slants and allowed to grow at room (28±2 °C) temperature to obtain the pure culture of the fungus. The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub cultured periodically. The purified isolates were identified as *Sclerotium rolfsii* based on morphological and colony characteristics (Punja and Damini, 1996; Sarma *et al*. 2002; Watanabe, 2002b) [14, 16, 21].

Mass multiplication of *S. rolfsii* isolates

A total of seven isolates were multiplied in sorghum grain medium (Sennoi *et al*. 2012) [17]. The pathogen *S. rolfsii* was multiplied on sorghum grains (200 g) soaked overnight in water for pot experiment. About 100 g of soaked sorghum grains were taken in 500 ml capacity saline bottles tightly plugged. The bottles were then sterilized for 20 min. at 121 °C. After sterilization, the sorghum seeds in saline bottles were inoculated with mycelial discs taken from the advancing margins of seven days old culture of respective *S. rolfsii* at each bottle and bottles were incubated for 15 days at 28±2 °C for proper mycelial growth.

Assessing the virulence of *S. rolfsii* isolates

To know the virulence of *S. rolfsii* isolates, an experiment was conducted at Department of Plant Pathology, Faculty of Agriculture, Annamalai University during the year 2015-2016 under pot culture condition. Plants were maintained in earthen pots of 15×30 cm diameter replicated in three times and filled with sterilized soil. In each pot 3 seeds of cluster bean (var. Pusa Mausami) were sown and after thinning one plant was maintained and fertilizer dose applied as recommended. After raising all the respective the sorghum grains inoculums were added at near the collar region of the stem @ 5 grains/plant at 25 days after sowing. Inoculated plants were kept in open place for observation and the pots were irrigated as when required. Typical stem rot symptoms were observed and Per cent disease incidence was calculated using formula (Kokalis-Burelle *et al*, 1997) [9].

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Re isolation was made from such affected portion of the plant tissue and compared with that of original isolate for conformity. The highly virulent isolate (*S. rolfsii*-I_1) of pathogen obtained from infected seedling was used for subsequent studies.

Effect of different levels of inoculum of *S. rolfsii* (I_1) on the incidence of stem rot of cluster bean

To know the inoculums levels on the development of stem rot disease, an experiment was conducted at Department of Plant Pathology, Faculty of Agriculture, Annamalai University during the year 2015-2016 under pot culture condition. Five levels *viz*., 0, 1, 2, 3, 4 and 5 seeds/ plant were maintained. These levels of plants were maintained in the earthen pots of 15×30 cm diameter replicated in three times and filled with sterilized soil. In each pot 3 seeds of cluster bean (var. Pusa Mausami) were sown and after thinning one plant was maintained and fertilizer dose applied as recommended. After raising all the respective the sorghum grains inoculums were added near the collar region of the stem separately @ 1, 2, 3, 4 and 5 grains/plant at 25 days after sowing. Inoculated plants were kept in open place for observation and the pots were irrigated as when required. Stem rot severity was made at all inoculums levels at 25 days after sowing by number of plants showed typical stem rot symptoms were observed and Per cent disease incidence was calculated using formula (Kokalis-Burelle *et al.* 1997) [9].

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Identification of susceptible stage of the crop to stem rot of cluster bean

To know the susceptible stage of the crop, an experiment was conducted at Department of Plant Pathology, Faculty of Agriculture, Annamalai University during the year 2015-2016 under pot culture condition. Six stages viz., 0, 25, 50, 75, 100 and 125 days after sowing of cluster bean plants were taken for their susceptible reaction against stem rot pathogen. These stages of plants were maintained in the earthen pots of 15×30 cm diameter replicated in three times and filled with sterilized soil. In each pot 3 seeds of cluster bean (var. Pusa Mausami) were sown after thinning one plant was maintained and fertilizer dose applied as recommended. After raising all the respective the sorghum grains inoculums were added at near the collar region of the stem up to 5 grain on each plant of cluster bean on six stages. Inoculated plants were kept in open place for observation and the pots were irrigated as when required. Stem rot severity was made at 25, 50, 75, 100 and 125 days after inoculation at respective stages, number of plants showed typical symptoms due to *S. rolfsii* was observed and Per cent disease incidence was calculated using formula (Kokalis-Burelle *et al.* 1997) [9].

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Sl. No.	Treatments	Stage	Days after emergence
1.	T_1	1st	Zero stage
2.	T_2	2nd	25 days old crop
3.	T_3	3rd	50 days old crop
4.	T_4	4th	75 days old crop
5.	T_5	5th	100 days old
6.	T_6	6th	125 days old crop

Results and Discussion

Survey of stem rot of cluster bean in Tamil Nadu: A roving survey was carried out in different districts of Tamil Nadu *viz*., Cuddalore, Dindigul, Namakkal and Salem during the year 2020-2021. The data on survey revealed that the incidence of stem rot from different districts of Tamil Nadu ranged from 18.33 to 7.33%. The maximum incidence of 18.33% was recorded in Melamoongiladi village of Cuddalore District. This was followed by Kavarapattu village of Cuddalore District (15.00 per cent). Whereas, least disease incidence of 7.33% was recorded in Koothanatham village of Namakkal district (Table 1). The incidence of stem rot of cluster bean may vary from locality to locality, because of varied agro climatological situations, cropping patterns and also cultural practices followed. Even, it could also be attributed to the existence of variability (or) pathogenic diversity present in the fungus. This is in agreement with Anahosur and Kulkarni (1997) [2] who reported variable incidence of *S. rolfsii* at different localities of Belgaum and Dharwad districts. Latha and Rajeswari (2019) [10] reported that a field survey was conducted during December 2015 to March 2016 in jasmine growing areas revealed that the Jasmine *Sclerotium* wilt disease incidence ranged 123 from 5.27 to 17.00 per cent. The disease was maximum in Sathyamangalam (Pavuthampalayam) (17.00 per cent) followed by Dhandapalayam (15.65 per cent) and Sultanpet (14.66 per cent).The highest incidence of stem rot was observed in Melamoongiladi village may be due to continuous cropping and presence of pathogen over long period, because continuous cultivation of cluster bean crop over the season and years will build up inoculum level to such an extent as observed by various workers (Ronakkumar and Sumanbhai, 2014; Suneeta *et al.* 2016) [15, 20].

Table 1: Survey on the incidence of stem rot of cluster bean in Tamil Nadu

Sl. No.	Name of the district	Name of the Village	Disease incidence (%)
1.	Cuddalore	Melamoongiladi	18.33 a (25.34)
2.	Cuddalore	Kavarapattu	15.00 b (22.78)
3.	Dindigul	Ottanchatram	14.66 b (22.51)
4.	Dindigul	Chatranpatti	11.33 c (19.66)
5.	Namakkal	Vattur	8.00 d (19.42)
6.	Namakkal	Koothanatham	7.33 d (15.70)
7.	Salem	Kalipatti	13.66 b (21.69)

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (P= 0.05)

Pathogenicity of *Sclerotium rolfsii* isolates on cluster bean

Pathogenicity test was carried out under pot culture by inoculating with pathogenic culture of *S. rolfsii* isolates. Among the isolates tested, the isolate (I_1) from Melamoongiladi village pertaining to Cuddalore district was found to be highly virulent in causing stem rot compared to other isolates investigated in the present study. This was followed by isolate (I_2) from Kavarapattu village pertaining to Cuddalore district (Table 2). As early as 1988, Siddaramaiah and Chandrappa [18] proved the pathogenicity of *S. rolfsii* on cardamom in pot culture studies by inoculating 25 days old sclerotial cultures which was grown on sand corn meal medium and observed the symptoms a week after inoculation. Mahato and Biswas (2017) [11] tried pathogenicity test on tomato seedlings using soil infestation method. One month old healthy tomato seedling (var- Punjab Chuhara) was transplanted singly in each pots and after few days when seedling get well established, the inoculation of different isolates were incorporated in collar region at the rate of 20 g/kg soil in five selected pots separately. Pots without inoculation were served as control for each case. The inoculated plants were observed daily. As soon as the disease symptoms were evident, the pathogen was again re-isolated to confirm the infectivity of the isolated pathogen. The plants showing typical wilting symptoms were recorded subsequently. Praveen Kumar (2009) [13] and Bhuiyan *et al.* (2012) [5] observed variation in the virulence of the isolates of *S. rolfsii* causing collar rot of potato and soybean. Present study also corroborate with the findings of above researchers explaining that the isolate of *S. rolfsii* (I_1) is highly virulent in causing stem rot disease. The above results lend support to the present findings.

Table 2: Pathogenicity of *S. rolfsii* isolates on cluster bean

Sl. No.	Isolate number	Disease incidence (%)
1.	I_1	68.66 a (55.95)
2.	I_2	64.33 b (53.32)
3.	I_3	56.00 c (48.44)
4.	I_4	46.00 e (42.70)
5.	I_5	42.33 f (40.58)
6.	I_6	40.00 g (39.23)
7.	I_7	51.66 d (45.95)

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p= 0.05)

Effect of different inoculum levels on the stem rot of cluster bean: Among the inoculum levels tested, *S. rolfsii* multiplied on sorghum grains @ 5 grains/plant to 1kg of soil registered the maximum incidence of 69.00% stem rot which was followed by inoculum load of *S. rolfsii* multiplied on sorghum grains @ 4 grains/plant (Table 3). Among the inoculum levels tested, *S. rolfsii* multiplied on sorghum grains @ 4 grains/plant to 1kg of soil registered the maximum foot rot incidence of 73.92 per cent which was followed by inoculum load of *S. rolfsii* multiplied on sorghum grains @ 3 grains/plant (Dhivya, 2020) [8] . Unal *et al*. (2019) [21] showed that the fungal inoculums grown on wheat bran and applied to the soil (@4g/kg soil) showed maximum disease incidence of *S. rolfsii* on turfgrass. Higher inoculum density resulted in highest disease incidence, which may be due to the fact that higher inoculum always ensures the certainty of the infection. Mahato and Biswas (2017) [11] reported that isolates were incorporated in collar region at the rate of 20 g/kg soil (multiplied on wheat grains) recorded the maximum incidence of collar rot of tomato. Similar results have been reported by Chitrampalam *et al.* (2010) [6] and Sennoi *et al.* (2012) [17]. These earlier reports corroborates with the present observations.

Table 3: Effect of different inoculum levels on the incidence of stem rot of cluster bean

Sl. No.	Inoculum level (Number of grains/plant)	Disease incidence (%)
1.	0	0.00 f (0.00)
2.	1	12.33 e (20.55)
3.	2	21.66 d (27.73)
4.	3	40.00 c (39.23)
5.	4	55.33 b (48.05)
6.	5	69.00 a (56.16)

Mean of three replications
Values in each column followed by the same letter are not significantly different according to the DMRT method (p= 0.05)

Identification of susceptible stage of the crop: In the present study, it was observed that *S. rolfsii* can infect all the stages of the cluster bean crop, when inoculum was added to all the stages. But the susceptible stage of the crop can be identified based on the stage at which the crop shows the maximum symptoms of stem rot caused by *S. rolfsii*. It was found that, higher per cent of wilting of 90.66 % was recorded in plants at 100 and 120 days after pathogen inoculation and it was found significantly more susceptible compared to rest of the treatments (Table 4). Dhivya (2020) [8] reported that Identification of susceptible stage of the crop to foot rot of brinjal revealed that 50 and 70 days old seedlings were highly susceptible to foot rot disease. Bekriwala *et al*. (2016) [4] found that there was no difference in disease severity percentage among the different stage of plant. Forty five days old plant had maximum 79.04% ground nut stem rot disease followed by 30 and 15 days old plants with 74.45% and 69.36% disease severity, respectively. Muthukumar and Venkatesh (2013) [12] reported that moreover, susceptibility or resistance of plants to collar rot disease is often influenced by their age. The mortality rate was increased with increased in age up to (5 to 10) but it was decreased beyond 15 days, in peppermint. Susceptibility of the plant is decided based on the type of pathogen and duration of the crop. The above results lend support to the present findings.

Table 4: Identification of susceptible stage of the crop

Sl. No.	Treatments	Disease incidence (%)
1.	T_1-Zero stage	0.00 d (0.00)
2.	T_2-25 days old crop	0.00 d (0.00)
3.	T_3-50 days old crop	42.66 c (40.77)
4.	T_4-75 days old crop	65.33 b (53.92)
5.	T_5-100 days old	90.66 a (72.20)
6.	T_6-125 days old crop	90.66 a (72.20)

Mean of three replications
Values in each column followed by the same letter are not significantly different according to the DMRT method (p= 0.05)

Conclusion

During *in vivo* studies the isolate (I_1) from Melamoongiladi village pertaining to Cuddalore district was found to be highly virulent in causing stem rot disease. *S. rolfsii* multiplied on sorghum grains @ 5 grains/plant to 1 kg of soil registered the maximum collar rot incidence of at 100 and 120 days after pathogen inoculation

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