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# ***In vitro* evaluation of phytoextracts and screening of different cultivars of pigeon pea against collar and root rot disease caused by *Sclerotium rolfsii* Sacc.**

**PP Salvi, VS Pande, SV Pawar and PV Joshi**

**Abstract**

In the view of ecological management of different diseases of crops, in present era the use of chemical fungicides have been hazardous to plants, animals as well as human being also. In case of this, to avoid such a condition there were six different phytoextracts used in present investigation viz., *Sapendus tripholiarus*, *Allium sativum*, *Curcuma longa*, *Zingiber officinale*, *Callophyllum inophyllum* and *Ocimum sanctum*. Among above mentioned phytoextracts only *Sapendus tripholiarus* extract at 10 per cent concentration was most effective in inhibiting the mycelial growth and sclerotia formation. The *in vitro* screening of some cultivars of pigeon pea against *Sclerotium rolfsii* Sacc. revealed that among the varieties tested five varieties viz., BDN-711, TAT-10, ICPL-87119, T-Vishakha and UPAS-120 was found disease free from *S. rolfsii* up to 21 days.

**Keywords:** *Sclerotium rolfsii*, Pigeon pea, phytoextracts and screening

**Introduction**

Pigeo npea (*Cajanus cajan* L. Millsp) is an important pulse crop of semi-arid tropics and subtropics grown for its grain legume and as a source of fodder and fuel. It enhances the soil fertility through nitrogen fixation in the root nodules and improves the soil structure. A long duration crop of pigeon pea can fix up to 200 kg N /ha and the residual effect for next crop remains 40 kg N/ha. It finds an important place in the rain-fed farming systems in developing countries because of its ability to tolerate drought and utilise residual moisture during dry season. In the Indian subcontinent, it serves as an important dietary protein to a large section of the people especially the vegetarians and the poor. Pigeon pea represents about 5% of world legume production (Hillocks *et al.*, 2000) <sup>[6]</sup> and more than 70% is being produced in India. In India, pigeon pea is grown in an area of 4.01 million hectare with a production of 2.65million tonnes bringing an average yield of 656 kg/ha (Directorate of Economics and Statistics, 2011-12).

There is a large gap between the potential yield (2,500 kg/ha) and on farm yield and among the several factors contributing to this yield gap are the abiotic and biotic constraints. According to Nene *et al.*, (1996), pigeon pea crop is attacked by 210 pathogens of which 83 are fungi. Soil borne diseases viz; root rot incited by *M. phaseolina* (*R. bataicola*) and *Sclerotium rolfsii* this are the major constraints in reducing the yield in pigeon pea. Under favourable environmental conditions these diseases spread very quickly and develop in a heavy proportion causing huge economic losses ranging from 10-100 per cent. The pigeon pea wilt disease was first recorded by Butler (1906) <sup>[2]</sup> in India. Although the disease is more prevalent in India, East Africa and Malawi where yield losses of over 50% are common, it also occurs in Bangladesh, Grenada, Indonesia, Mauritius, Mynmar, Nepal, Nevis, Venezuela, Trinidad, and Tobago (Kannaiyan *et al.*, 1984; Reddy *et al.*, 1993; Marley and Hillocks, 1996) <sup>[9, 14, 11]</sup>. The disease is found in all pigeon pea growing areas but incidences are high in the eastern areas (Hillock and Songa, 1993) <sup>[5]</sup>. The incidence of the disease has been reported from 30-60 per cent at flowering and crop maturity stages (Kannaiyan and Nene, 1981) <sup>[8]</sup>.

In konkan region of Maharashtra pigeon pea is grown mainly on bunds of rice fields. This crop is sown during kharif in month of June-July after transplanting of rice. This crop matures in the month of November-December.

This crop is also grown in rice fields after harvest of rice on residual moisture during the month of October and matures in the month of February-March. The farmers are using the seed material of any pigeon pea variety and therefore yield is less. It is necessary to identify the pigeon pea variety for growing on rice bunds. In konkan region of Maharashtra rice is grown on about 4.2 lakh hectare areas. This crop is grown on bunds of rice fields as the rice bunds have more residual moisture than the field, the growing of pigeon pea on rice bund increase the total cropped area and this crop grows very well and produces a good yield. The diseases on pigeon pea is one of the major constrain for lower yield of this crop. As this fungus was observed for first time in the Konkan region. Therefore it was felt necessary to carry out the basic studies against the pathogen. In view of ecological management of this disease we have used different botanicals for checking their efficacy against isolated pathogen and also screening objectives was carried out for observed resistant reaction of some cultivars.

## Materials and Methods

### Efficacy of Plant extracts against causal organism in vitro: Crude extraction

Aqueous phytoextracts were obtained as per the method adopted by Bhatti (1988) <sup>[1]</sup>. Hundred gram fresh plant materials was washed thoroughly with sterilized distilled water and ground well in 100 ml sterilized distilled water. The macerate was filtered through double layer cotton wool and centrifuged at 4000 rpm, for 5 min. The supernatant was filtered through filter paper. Extracts thus obtained was passed through filter paper and then passed through sintered glass filter to avoid bacterial contamination. This formed the standard plant extracts solution (100 %).

### Effect of plant extracts on mycelial growth of causal organism

The effect of plant extracts on mycelial growth was studied by 'Poison food technique' (PFT) (Nene and Thapliyal, 1993) <sup>[12, 14]</sup>. The principle involved in this technique is to poison the medium with toxicant and then allowing a test fungus to grow on such medium. All glassware used in the study was sterilized before their use. All the plant extracts given in Table-2 were tested at 10 per cent concentration against *Sclerotium rolfsii* using Potato Dextrose Agar medium as a basal medium. To obtain 10 per cent plant extract medium, 90 ml of molten PDA was poured in 100 ml sterilized conical flask and 10 ml of plant extract was poured in each flask with the help of sterilized pipette. Twenty ml of the medium was then poured in each sterilized Petri Plate. Then mycelial discs of 5 mm diameter were cut from seven day old culture of *Sclerotium rolfsii* with the help of sterilized cork borer and transferred aseptically to the centre of Petri Plate already poured with poisoned medium. Medium devoid of plant extract served as control. Petri Plates were incubated at room temperature ( $28 \pm 1^{\circ}\text{C}$ ) for growth of the fungus.

Three replications per treatment were maintained. The observations on colony diameter of the fungus were recorded when Petri Plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth was calculated by the following formula (Horsfall, 1956) <sup>[7]</sup>.

$$X = \frac{Y - Z}{Y} \times 100$$

Where,

X = Per cent inhibition

Y = Growth of fungus in control (mm)

Z = Growth of fungus in treatment (mm)

### In vitro screening of some cultivars against causal organism

To study *in vitro* screening of some cultivars against causal organism in pots fifteen varieties viz. BDN-T-08, AKT-88-11, BSMR-853, Phule Rajeshwari, PKV-Tara, BSMR-756, UPAS-120, Konkan Tur, BDN-711, TAT-10, ICPL-87119, ICPL-87, Vipula-1, AKTE-11-1 and T-Vishakha were selected. The seeds of test varieties were grown in pots containing sterilized soil and FYM (2:1) mixture. The soil from each pot was made sick by mixing fungal inoculum prepared by mixing 7 days old fungal mycelial growth and fully developed sclerotia in 100 ml sterilized PDA broth. Plants were watered regularly and observed for development of typical symptoms of the disease and the observations were recorded.

## Results and Discussion

In present investigation on evaluation of different phytoextracts against *Sclerotium rolfsii* it was revealed from the data presented in Table 1 that the mean colony diameter in all the plant extract treatments, except T<sub>1</sub> was statistically at par with the mean colony diameter in control. This clearly indicates that except T<sub>1</sub> (soap nut extract) all the treatments were ineffective. Soap nut extract was effective up to 72.22 per cent in inhibiting the mycelial growth. Minimum sclerotia were formed (45) in this treatment while in rest of the treatments they were within a range of 115 - 315. These results are in line with the findings of Tiwari and Singh (2004) <sup>[15]</sup>, Kolte (2007) <sup>[10]</sup> and Haralpatil and Raut (2008) <sup>[4]</sup> who reported that soap nut extract is highly effective against *S. rolfsii*.

It is apparent from the results in Table 2 that 5 varieties viz. Konkan Tur, ICPL-87, AKTE-11-1, Phule Rajeshwari and BSMR-853 exhibited collar rot symptoms, 7days after inoculation. At this stage the symptom development was not observed on any other varieties. After 9 days, the symptoms were also observed on two more varieties viz., BSMR-756 and PKV-Tara. After 12 days, 3 more varieties such as TAT-10, AKT-88-11 and BDN-T-08 were also positive to infection of *Sclerotium rolfsii*. In all 10 of the 15 varieties s were positive to the infection of the pathogen. The 5 varieties which did not exhibit collar rot symptoms up to 21 days were, BDN-711, ICPL-87119, Vipula -1, T-Vishakha, and UPAS-120. Thus it was concluded that the varieties viz., BDN-711, ICPL-87119, Vipula -1, T-Vishakha, and UPAS-120 remained free from *Sclerotium rolfsii* infection up to 21 days. In 2009 eleven sugar beet genotypes were evaluated at National Agricultural Research Centre, Islamabad, Pakistan by Farooq *et al.* (2011) <sup>[3]</sup> their resistance against root rot caused by *Sclerotium rolfsii*. out of eleven genotypes only in SD-PAK-09/07 was resistant while SD-PAK-07/071 was moderately resistance. Remaining nine genotypes were susceptible to highly susceptible to the disease. Pandav (2012) <sup>[13]</sup> screen eight varieties of gerbera against *Sclerotium rolfsii* and observed that except Goliyat all the varieties were susceptible.

**Table 1:** Efficacy of Plant extracts against mycelial growth of *Sclerotium rolfsii* Sacc.

Tr. No	Treatment	Conc. %	Plant parts used	Mean colony diameter (cm)*	Per cent inhibition over control	No. of sclerotia formed
T <sub>1</sub>	Soap nut	10	Fruits	2.5	72.22	45
T <sub>2</sub>	Garlic	10	Bulb	8.70	3.33	182
T <sub>3</sub>	Turmeric	10	Rhizome	9.00	0.0	210
T <sub>4</sub>	Ginger	10	Rhizome	8.60	4.44	115
T <sub>5</sub>	Undi	10	Seed	9.0	0.0	202
T <sub>6</sub>	Tulsi	10	Leaves	8.60	4.44	147
T <sub>7</sub>	Control	--	--	9.00	--	315
S. Em ±				0.32		
C.D at 1%				1.37		

\* Mean of three replications

**Table 2:** Reaction of pigeon pea varieties, against infection of *S. rolfsii* Sacc.

Tr. No.	Varieties	Reaction (Positive/ Negative -In days)					
		7	9	12	15	18	21
V-1	BDN-711	-	-	-	-	-	-
V-2	Konkan Tur	+	+	+	+	+	+
V-3	TAT-10	-	-	+	+	+	+
V-4	ICPL-87119	-	-	-	-	-	-
V-5	Vipula-1	-	-	-	-	-	-
V-6	ICPL-87	+	+	+	+	+	+
V-7	AKTE-11-1	+	+	+	+	+	+
V-8	T-Vishakha	-	-	-	-	-	-
V-9	BSMR-756	-	+	+	+	+	+
V-10	Phule Rajeshwari	+	+	+	+	+	+
V-11	PKV- Tara	-	+	+	+	+	+
V-12	AKT-88-11	-	-	+	+	+	+
V-13	UPAS-120	-	-	-	-	-	-
V-14	BSMR-853	+	+	+	+	+	+
V-15	BDN-T-08	-	-	+	+	+	+

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