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RESEARCH ARTICLE

In vitro evaluation of botanicals and bio control agents against *Sclerotium rolfsii* Sacc. Causing collar rot of chickpea

■ **N. Sangeeta, H. Virupaksha Prabhu and Gurupad Balol**

SUMMARY

Collar rot of chickpea is caused by *Sclerotium rolfsii* Sacc., is a devastating polyphagous soil borne fungus infecting more than 500 plant species across the world that is causing vast losses. It is more serious at seedling stage causing plant mortality ranged from 54.7 to 95%. Treating soil borne pathogens with fungicides is not reasonable due to very high costs. Environmental hazards are also involved. Therefore, integrated management of pathogens using bioagents and botanicals agents is the paramount alternative. Extracts of higher plants have demonstrated a wide range of activity against plant pathogenic organisms. The present research work was carried out to manage the pathogen and disease *in vitro* by using plant extracts by using poison food technique in which commercially available once showed maximum inhibition followed by agave extract and NSKE, whereas under *In vitro* evaluation of bioagents by dual culture technique, *Trichoderma viride* found most effective compared to other bioagents tested.

Key Words : Chickpea, *Sclerotium rolfsii*, *Trichoderma*, Collar rot, Agave, botanicals

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Plant disease outbreaks are increasing and threatening food security of the world under changing climatic scenario. Global yield losses due to crop pests and diseases on crops are large. Important diseases such as Turcicum leaf blight in maize (Hooda *et al.,* 2017), *Fusarium wilt* and dry root rot in chickpea (Talekar *et al.,* 2017 and 2021), phyllody in chickpea (Balol *et al.,*2021), leaf spots in groundnut, wilt in pigeonpea, necrosis disease in sunflower (Sundaresha *et al.*, 2012) and bud blight caused by groundnut bud necrosis virus (Balol and Patil, 2014) *etc.* are contributing

for the yield loss. Outbroken pest fall armyworm in maize (Tippannavar *et al.,* 2019) also threatening food security.

Collar rot is a devastating soil-borne disease of chickpea caused by fungus *Sclerotium rolfsii* Sacc, [teleomorph: *Athelia rolfsii* (Curzi)], causing heavy economic losses. Wherever chickpea is grown all over the world it is almost reported everywhere and caused 10 to 30 per cent yield loss annually according to severity of the disease (Nene *et al.,* 1979). Over conventional fungicides, botanicals and biological control of plant pathogens has a number of advantages, as fungicides features only a temporary effect and require repeated applications during the growing period of crop while, the biological control agents are able to establish themselves, colonize and reproduce in the ecosystem. It was reported that *Trichoderma* spp. involves wide range of key characteristic mechanisms for disease control *i.e.*, Mycoparasitism and hyphallysis, antibiosis, competition for nutrients and space and also promotion of plant growth (Swathi *et al.,* 2015). Aim of the present investigation was to identify best bioagent and botanical as an alternative method for the management of collar rot of chickpea.

MATERIAL AND METHODS

The present research work was performed at the University of Agricultural Sciences Dharwad, Karnataka.

Isolation, purification and identification of *Trichoderma* spp. and test pathogen:

Bioagents were collected from IOF Dharwad and also commercially available once from the market, total 13 bioagents were subjected to their antagonistic potential *in vitro* against *S. rolfsii* was done by using dual culture method.

Growth of antagonist and the pathogen in monoculture:

T. harzianum and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of each fungus. *B. subtilis* was maintained on nutrient agar, for maintenance of *Pseudomonas fluorescens*, King's B medium was used and selective isolates of *Trichoderma* species from soil was isolated and cultured using selective medium (Elad *et al.,*1981).

Bioagents were evaluated for their efficacy through dual culture technique. The per cent inhibition of the pathogen was calculated by using the formula given by Vincent (1947).

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

where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

In vitro evaluation of botanicals against *S. rolfsii*

Collection of plant material :

Plant based extracts are relatively cheaper, safe and non-hazardous which can be easily and successfully used against the plant pathogenic fungi. Fresh leaves of various plants were collected during December 2018 from College of Agriculture Dharwad. Leaves were thoroughly washed and air dried. The aqueous solution is prepared.

Preparation of cold aqueous extract :

Twenty grams of corresponding plant material were cut in to small pieces and macerated using pestle and mortar in 50 ml of distilled water. The contents were filtered through a clean double layered muslin cloth. This volume was made upto 100 ml to obtain 20 per cent concentration. Further, it was diluted with distilled water to get 10 and 15 per cent concentrations. These extracts were again centrifuged for 5 min at 3,000 rpm to get a clear plant extract. This supernatant extract of different concentrations was used for evaluation.

Different concentrations from stock solution were taken and mixed with sterilized molten potato dextrose agar medium. This medium was shaken thoroughly for uniform mixing of the extract. Twenty ml of medium was poured into each of the 90 mm sterilized Petriplates. Each plate was inoculated with 5 mm mycelial discs which were taken from the periphery of fungal culture and incubate at $27 \pm 1\ ^0C$ till the growth of colony touched the periphery in the control plate. The disc was placed upside down in the center of the Petriplate, so that the mycelium comes in direct contact with the poisoned medium with the requisite plant extract at required concentration.Three replications were maintained for each treatment. Suitable control plates were maintained where in culture discs are inoculated into the center of potato dextrose agar plates without plant extracts.

The botanicals total of nine were tested with different concentration *viz*., 5%, 7.50% and 10% which were evaluated by using poisoned food technique and per cent

inhibition over control was calculated by using (Vincent, 1947) formula.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Effects of bioagents against *Sclerotium rolfsii* Sacc.:

A total of thirteen isolates were tested and among them three were of *Trichoderma harzianum* isolates, two were of *Trichoderma viride* isolates and four were Bacillus species, *Pseudomonas fluorescence* each. The per cent inhibition of different bioagents on the growth of *Sclerotium rolfsii* is illustrated in Table 1 and Fig 1a. Among the *Trichoderm harzianum* isolates, IOF Dharwad isolate found effective (62.79%) followed by Raichur isolate (61.67%), commercially available Trikowin of *T. viride* found effective (60.18%), among the *Pseudomonas fluorescence* isolates maximum inhibition observed in case of IOF isolate (34.71%). Least inhibition was observed in case of *Bacillus subtilis* about (22.16%).Many other workers have also discovered similar results. Kulkarni (2007), found maximum inhibition by *T. harzianum* (59.81 %) and the least inhibition of mycelial growth were observed in *Bacillus subtilis* (10.74 %).

Effects of botanicals against *Sclerotium rolfsii* Sacc.:

The effect of different concentrations of the botanicals on the growth of *Sclerotium rolfsii* is illustrated in Table 2 Fig 1.b and 1.c. All the commercially available botanicals significantly suppressed the growth of *S. rolfsi* at all concentration (5, 7.5, 10 %) except *Neem* Ashirvad (45.80%). There was maximum inhibition observed in

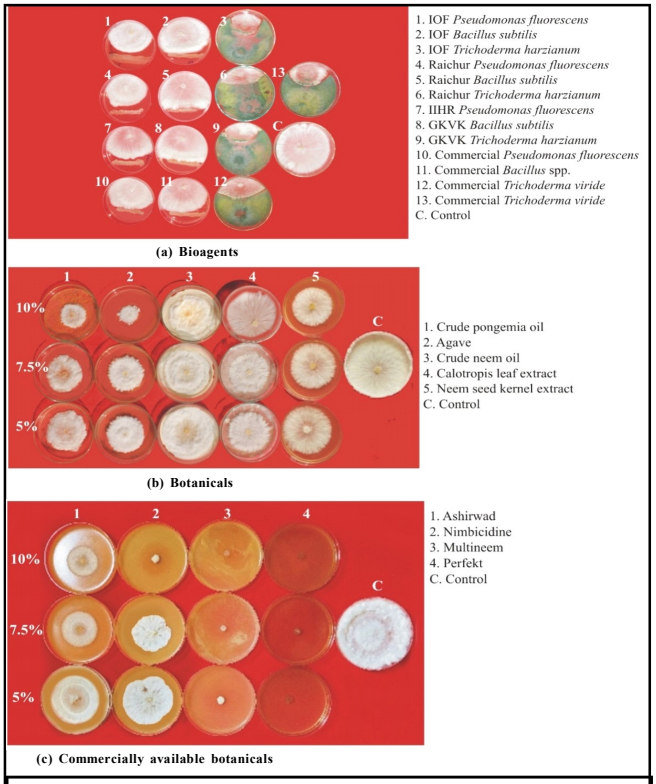

Fig. 1: *In vitro* evaluation of botanicals and bio control agents against *Sclerotium rolfsii* Sacc. Causing collar rot of chickpea

Table 1 : *In vitro* evaluation of bioagents against *Sclerotium rolfsii*

Sr. No.	Bioagents	Source/isolate	Per cent inhibition
1.	*Trichoderma harzianum*	Institute of organic farming, Dharwad	62.79 (52.41)*
2.	*Trichoderma harzianum*	Biocontrol lab, Raichur	61.67 (51.75)
3.	*Trichoderma harzianum*	Biocontrol lab, GKVK	61.11 (51.42)
4.	*Trichoderma viride*	Commercial multiplex	59.26 (50.34)
5.	*Trichoderma viride*	Commercial Trikowin	60.18 (50.88)
6.	*Bacillus subtilis*	Institute of organic farming, Dharwad	20.20 (26.71)
7.	*Bacillus subtilis subtilis*	Biocontrol lab, Raichur	16.16 (23.7)
8.	*Bacillus subtilis subtilis*	Biocontrol lab, GKVK	14.12 (22.07)
9.	*Bacillus* spp.	Commercial Sutlex	22.16 (28.08)
10.	*Pseudomonas fluorescence*	Institute of organic farming, Dharwad	34.71 (36.10)
11.	*Pseudomonas fluorescence*	Biocontrol lab, Raichur	34.51 (35.98)
12.	*Pseudomonas fluorescence* Strain IIHR -PF-52	Commercial sumonax	31.96 (34.43)
13.	*Pseudomonas fluorescens*	Commercial multiplex	28.82 (32.47)
	S.E.±		0.80
	C.D. (P=0.01)		3.16

*Arcsine transformed values

Table 2 : *In vitro* evaluation of botanicals against *Sclerotium rolfsii*

Sr. No.	Extracts	Per cent mycelial inhibition Conc. of botanicals			Mean
		5 %	7.50 %	10 %	
1.	*Neem* seed kernel extract	38.52 (38.36)*	39.63 (39.02)	47.41 (43.51)	41.85 (40.30)
2.	Crude *Neem* oil	6.85 (15.17)	30.00 (33.21)	37.78 (37.93)	24.88 (28.77)
3.	Crude Pongamia oil	21.48 (27.61)	33.33 (35.26)	39.63 (39.02)	31.48 (33.96)
4.	Agave	38.33 (38.25)	50.00 (45.00)	68.89 (56.10)	52.41 (46.45)
5.	Calotropis leaf extract	5.93 (14.09)	16.30 (23.81)	20.37 (26.83)	14.20 (21.58)
6.	Nimbicidine (Azadirachtin 0.03 %)	35.56 (36.60)	53.70 (47.12)	100.00 (90.00)	63.09 (57.91)
7.	*Neem* Ashirvad (Herbal extract of *Neem* seed kernel)	32.59 (34.81)	47.04 (43.30)	57.78 (49.47)	45.80 (42.53)
8.	Multineem (Azadirachtin)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
9.	Perfekt (Herbal mixture)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	Mean	42.14 (42.77)	52.22 (49.64)	63.54 (58.10)	
		S.E.+		C. D.(P=0.01)	
	Botanicals	0.36		1.42	
	Concentration	0.22		0.82	
	Botanicals x concentration	0.65		2.45	

* Arcsine transformed values

case of Agave (68.89 %) followed by NSKE (47.41%) at 10 per cent concentration and least inhibition in case of calotropis leaf extract (mean of 14.20 %) as compared to control in which 100% growth was seen. Similar results were obtained by Sab (2013), the least per cent inhibition was observed about 14.20 per cent for calotropis leaf extract. Commercially available botanicals showed cent per cent inhibition.

Conclusion:

In vitro evaluation of bioagents, botanicals and fungicides and their consortia against *Sclerotium rolfsii* was resulted that, among the different bioagents, *Trichoderma harzianum* showed highest inhibition (62.79 %), in botanicals tested Agave (at 10 %) recorded highest inhibition (68.89 %) and commercially available ones showed cent per cent inhibition (Multineemore and perfekt).

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