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In vitro evaluation of potential *Trichoderma* mutants against collar rot pathogen (*Sclerotium rolfsii* Sacc.) of chickpea

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Abstract
Collar rot of chickpea is an important disease which causes severe loss in chickpea production. The present study was carried out to evaluate four potential *Trichoderma* mutants against *Sclerotium rolfsii*. Among the four potential *Trichoderma* mutants tested the maximum inhibition was observed in BARC mutant (81.50%) over control which was followed by mutants M-136 (81%), M-23 (80.5%) and M-18 (79%) respectively.

Keywords: *Trichoderma*, collar rot pathogen, *Sclerotium rolfsii* Sacc., chickpea

Introduction
Chickpea (*Cicer arietinum* L.) is an important legume crop and good source of vegetable protein. Chickpea crop can be attacked by several pests and diseases. (Singh *et al*., 2015) [12]. Collar rot of chickpea is one of the economic important and most destructive disease of chickpea caused by *Sclerotium rolfsii* Sacc. It is prevalent in those areas which have high soil moisture and warm temperature with mortality rate from 10-100 per cent (Kumari and Ghatak, 2018) [6]. Seedling mortality due to attack of this pathogen is reported from 54.7 to 95 per cent and yield reduction in field condition is reported from 22 to 50 per cent (Ahsan *et al*., 2018) [1]. Due to wide host range and sclerotial formation as a resting structure by the pathogen it survives in the soil for longer period of time which makes management process quite difficult (Wavare *et al*., 2017). The antagonistic fungus *Trichoderma* spp. have capability to break the outer sclerotial shell that leads to its destruction along with several histological changes such as deformation, decay of cytoplasmic content and cell wall lysis (Rawat and Tewari, 2011) [11]. Since application of toxic chemicals for management of this disease are hazardous and have residual effect in soil. Use of biological control agents are the best alternative to these toxic chemicals. Among the bio-control agents, *Trichoderma viride* and *Gliocladium virens* were found to be most effective against *S. rolfsii. Trichoderma* spp. has been found as an effective BCA against many seed and soil borne pathogens (Eziashi *et al*., 2006) [4]. The mycelial growth inhibition of different pathogens by *Trichoderma* isolates is due to the production of diffusible compounds, lytic enzymes and water soluble metabolites. (Harman*et al*.2004; Yedidia *et al*.2003) [16]. Hence, present investigation was carried out to screen out the most compatible combinations of floral extracts, bio-control agents and fungicides to find out efficient management practices against collar rot of chickpea caused by *S. rolfsii*.

Material and methods
Potential *Trichoderma* mutants and the pathogen were grown on PDA (Potato Dextrose Agar) medium each separately and by using 7 days old cultures, 5 mm diameter disc of the potential mutants and pathogen were taken into consideration. The Petri plates (90 mm) were inoculated aseptically with *S. rolfsii* and potential mutants, by placing 5 mm diameter culture blocks at 70 mm apart from each other. Three repetitions of each treatment were kept and the petri plates with only pathogen served as control. Afterward, the plates were incubated at temperature (28±2°C) and the radial growth of the test organism and pathogen was measured after 7 days of incubation. The per cent growth inhibition (PGI) was worked out by using the formula given by Vincent (1947) [13].

$$PGI = \frac{DC\text{-}DT}{DC} \times 100$$

Where,
PGI= Per cent growth inhibition
DC = Average diameter of mycelial colony of control set
DT = Average diameter of mycelia colony of treated set

Results and discussion

In vitro antagonistic potential of different potential *Trichoderma* mutants against fungal plant pathogen *Sclerotium rolfsii* studied by following dual culture method, and growth assessed 5 days after inoculation. All the potential *Trichoderma* mutants showed varied range of antagonism against *Sclerotium rolfsii* ranging from 79 per cent to 81.50 per cent. Among potential *Trichoderma* mutants, BARC mutant showed maximum inhibiting effect on the growth of *Sclerotium rolfsii* (81.50%) over control. While remaining mutants also showed good inhibitory effect on the growth of *Sclerotium rolfsii* in order M-136 (81%), M-23 (80.5%) and M-18 (79%) respectively.

The antagonistic fungus *Trichoderma* spp. have capability to break the outer sclerotial shell that leads to its destruction along with several histological changes such as deformation, decay of cytoplasmic content and cell wall lysis (Rawat and Tewari, 2011) [11]. The present study revealed that different *Trichoderma* mutants have capacities as biological weapons in inhibiting the pathogens. This might be due to the production of secondary metabolites and antibiotics production, which diffused into the PDA and air filed spores which showed detrimental effect towards growth of *S. rolfsii* as well as due to higher competitive ability of potential *Trichoderma* mutants. Overall BARC mutant was found to be more efficient against *S. rolfsii* which indicates that it can be exploited as potential candidates for development of bio-pesticides.

Above findings are in agreement with the observations made by Bhuiyan *et al*., (2012) [3] reported that *T. harzianum* isolate Th-18 showed the highest (83.09%) reduction of the radial growth against *S. rolfsii* . This might be due to the production of secondary metabolites and antibiotics production, which diffused into the PDA and air filed spores which showed detrimental effect towards growth of *S. rolfsii* as well as due to higher competitive ability of potential *Trichoderma* mutants. The antagonistic fungus *Trichoderma* spp.

Bandyopodhyay *et al*. (2003) [2] reported that *Trichoderma* strains inhibited the growth of *Sclerotium rolfsii* and *Rhizoctonia solani* by 76.6% and 73.3% respectively. Yadub and Shahzad (2005) [15] reported that *T. harzianum* and *T. longibrachiatum* restricted the growth of *S. rolfsii* under *in vitro* condition by coiling around mycelium of *S. rolfsii* which leads in lysis of hyphae. Several workers reported that *Trichoderma viride* as an important antagonist inhibiting the growth of *Sclerotium rolfsii* (Kolte and Raut., 2007 ; Mandhare and Suryawanshi., 2008) [8, 9]. Prajapati *et al*. (2015) [10] observed that among different species of *Trichoderma* tested against *S. rolfsii* through dual culture technique, *T. asperellum* showed strong antagonistic effect in terms of mycelia growth inhibition *i.e.* 61.48, 75.00 and 73.33 per cent at 4, 6 and 8 days of incubation, respectively.

Table 1: *In vitro* evaluation of potential *Trichoderma* mutants against *Sclerotium rolfsii* (Dual Culture technique, Dennis and Webster, 1971)

Treatment	Treatment Name	Per cent Inhibition
T_1	**BARC mutant**	**81.5**
T_2	M-18	79
T_3	M-23	80.5
T_4	M-136	81
T_5	Control	-

*Percent inhibition is calculated 96 hours of incubation

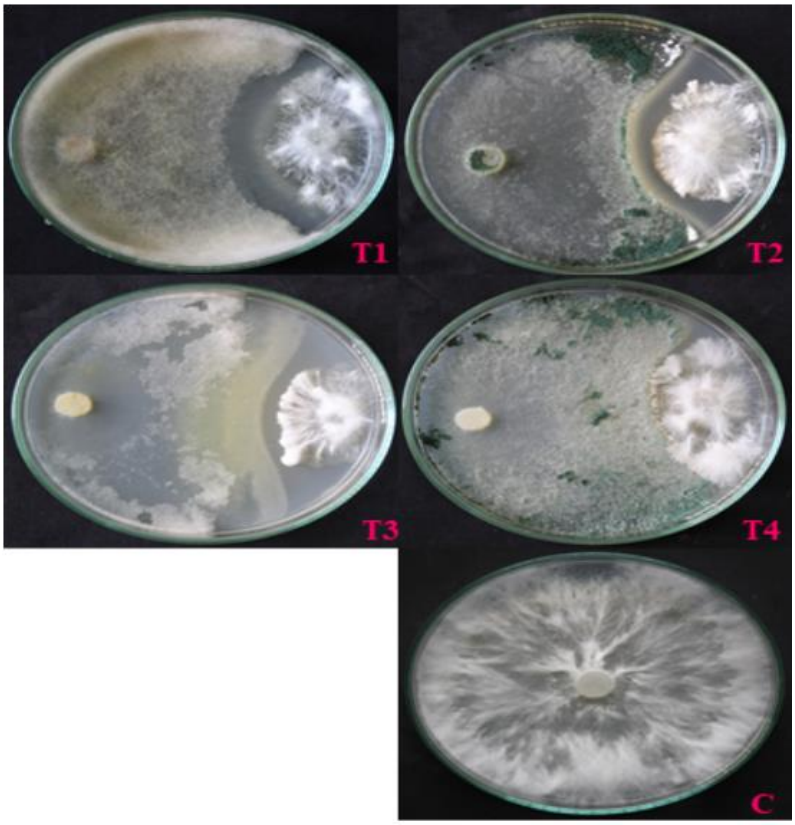

Fig 1: *In vitro* effect of antagonists against *S. rolfsii*

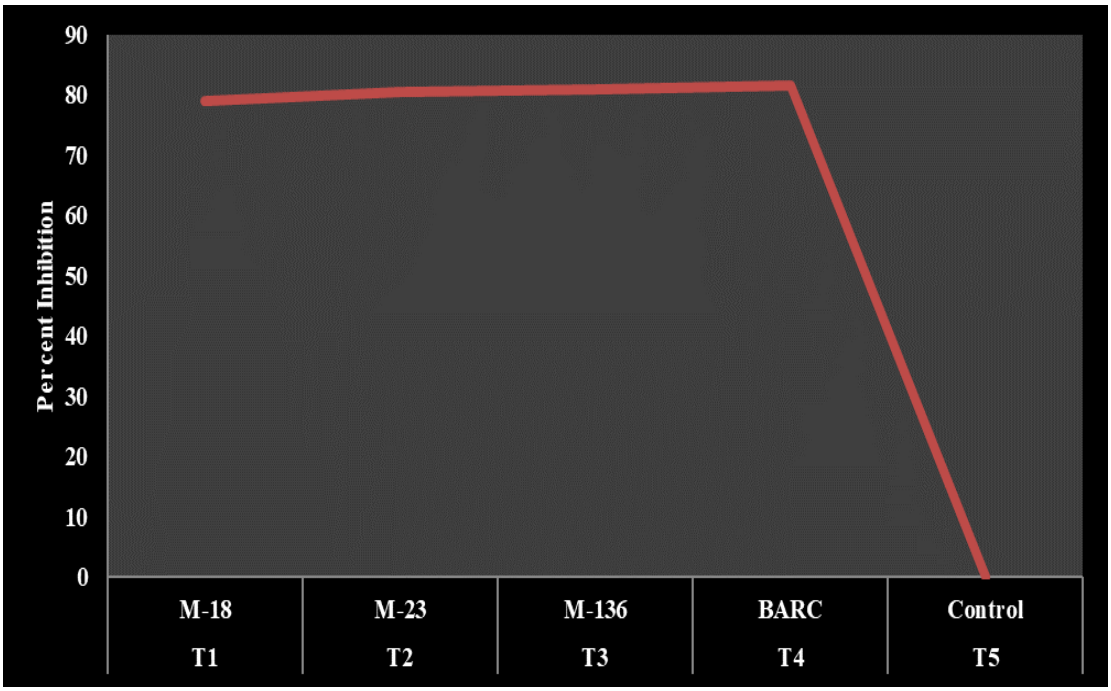

Fig 2: *In vitro* effect of antagonists against *S. rolfsii*

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