International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(6): 1654-1656

Received: 04-09-2018
Accepted: 08-10-2018

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In vitro studies on efficacy of various *Trichoderma spps.* Against collar rot of tomato caused by *Sclerotium rolfsii* Sacc. in Manipur

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Abstract
Sclerotium rolfsii is a soil inhabitant, non-target, polyphagous, and a ubiquitous facultative parasite. Its geographic distribution, profuse mycelial growth, persistent sclerotia and large number of hosts attacked by it indicate that, economic losses are substantial every year due to infection. The present study was carried out to understand about the *in vitro* efficacy of various Trichoderma species against collar rot pathogen. Percent inhibition was observed and recorded, it was ranged from 13.02 to 63.02% among various isolates under study. *Trichoderma asperellum* had shown best results with 63.02% inhibition of pathogen. Isolates under study had been categorised according bells scale into three groups.

Keywords: *Trichoderma*, against collar, tomato, *Sclerotium rolfsii*

Introduction
India is the member of WTO. Health consciousness is one of the prime significance in the principles of WTO and WHO. The inherent hazardous effect involved conventional, chemical management couple with inclination of farmer towards organic farming and non-chemical, bio-pesticides etc. for plant protection measures are gaining importance. Biological control is inhibition of growth and infection or reproduction of one organism by other organism. (Cook, 1993) [3]. Biological control employs natural enemies of pests and pathogens to manage their population it may be by introduction of exotic species are by utilizing any form of biological control present in eco-system. Biological control of seed and seedling diseases through effective and eco-friendly is not yet very much popular on large scale. In the absence of resistance / tolerant varieties effort should be made to have bio-control measures in such a way which are eco-friendly viable in a particular set of environment.
Biological agent; Trichoderma has emerged as an alternative mean of management of soil borne diseases. (Ansari, *et al.* 2011) [1]. Trichoderma produces various volatile and non-volatile antibiotics and also inhibits by various mechanisms like hyper parasitism, enzymes (Lorito *et al.* 1993) [5] competition and iduced systemic resistance (De Mayer *et al. 1998*) [4]. Various species of Trichoderma may vary in the level of production of various chemicals and mechanism of action on pathogen, so various species, (six species) of Trichoderma available (9 isolates) were taken for evaluation against *S. rolfsii* collar rot pathogen of tomato.

Materials and Methods
Isolation of fungus from diseased samples
First brought in the lab the plant parts showing typical symptoms of wilt or collar rot suspected to be caused by *S. rolfsii w*ere collected after initial routine microscopic observations of the effected plant parts, then the part of collar or stem region showing typical symptoms of the disease was cut into small pieces. These pieces were surface sterilized with 1% sodium hypo-chloride solution for one minute. Such pieces were washed thoroughly in sterile distilled water thrice to remove traces of sodium hypo-chloride, if any and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. They were incubated at 27±1°C for three days for the growth of the fungus. Later, the mycelial tip of fungal growth was transferred to PDA slants. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions (Rangaswamy, 1972) [8]. then identification of causal organism of isolates i.e. *S. rolfsii* following standard procedures. (Narain and Mishra, 1977) [6].

The isolates were sub-cultured on PDA slants and allowed to grow at 27±1°C temperature for ten days. The cultures so obtained were stored in a refrigerator at 4°C and they were cultured periodically once in a month.

In vitro* efficacy of bio control agents on the growth of *S. rolfsii

Antagonistic test of *Trichoderma spp.* was done by dual culture technique given by Bell (1982) [2]. The experiment was conducted on PDA. A single mycelial disc of 5mm diameter of S. *rolfsii* was placed aseptically at one end of Petri plate. Mycelial disc of 5mm diameter of antagonist isolate was placed at the opposite end of the same plate. The plates were then incubated at 25 ± 1°C in B.O.D. incubator. Bio control agents are listed in table.1, isolates were procured from department of plant pathology, COA, CAU, Imphal for study.

The per cent inhibition of mycelial growth over control which was calculated by using the formula given by Vincent (1927) given below:

Percent inhibition = (C-T)/C x100

Where C= linear growth of the fungus in control, T=linear growth of the fungus in treatment

Bell's scale with slight modification

Class I: The antagonist completely overgrew the pathogen (100 % overgrowth)

Class II: The antagonist overgrew at least 2/3rd of the pathogen surface (75% over growth)

Class III: The antagonist colonized on half of the growth of the pathogen surface (50% over growth)

Class IV: The pathogen and the antagonist locked at the point of contact

Class V: The pathogen overgrew the my coparasite.

Class VI: The pathogen and antagonist form inhibition zone

Results and Discussion

Various available species of *Trichoderma* were evaluated against *S. rolfsii* by dual culture technique and percent inhibition was recorded (Table 2, plate 1, graph 1). Among the 9 isolates evaluated *T. asperellum* (KU933476) had shown the best result with 63.02% inhibition followed by *T. harzianum* (KU933471) with 59.37% inhibition. *T. atroviride* (KU933472) had shown least inhibition with 13.02%. *T. harzianum* (KU904458), *T. ovalisporum* (KU904456), *T. hypocrea* (KX0113223), *T.koningiopsis* (KU904460), *T. asperellum* (KU933475) and *T. harzianum* (KU933468) had shown 51.56, 45.31, 43.75, 43.75, 42.18, and 27.61 percent inhibition respectively. Among the 9 isolates evaluated *T. asperellum* (KU933476) had shown the best result with 63.02% inhibition followed by *Trichoderma harzianum* (KU933471) with 59.37% inhibition. *T. atroviride* (KU933472) had shown least inhibition with 13.02%. Similar findings were observed by (Ramzan *et al.,* 2016), who reported that *Trichoderma* has better inhibitory effects on growth of *S. rolfsii.* Most of the species *of Trichoderma generally* produces various metabiolites like β- xylosidase, α-glycosidase, chymotrypsin, N-acetyl β-glucosaminidase, viridian, glioviridin, β-glucosidase, cellobiohydrolase, trypsin, and chymoelastase-like proteases etc., they also have various mechanism like myco parasitism, rapid substrate colonization, competition for various sources of energy like carbon, nitrogen and also for free space, which may be responsible for the biocontrol activity of various *Trichoderma species* against *S. rolfsii.*

Among the 9 isolates evaluated *T. asperellum* (KU933475), *T. hypocrea* (KX0113223), *T. harzianum* (KU933468), *T. ovalisporum* (KU904456), *T. atroviride* (KU933472) were under class IV, i.e. above Trichoderma species had stopped the growth of pathogen by competing for space and nutrients. Whereas *T. harzianum* (KU904458), *T. harzianum* (KU933471) colonized on half of the growth of the pathogen surface (50% over growth i.e. class III) these mode of inhibition may be due to hyperparasitic nature of *T. harzianum.*

T. koningiopsis (KU904460) and pathogen formed inhibition zone between the points of contact, this may be due to some antibiotics produced by *T.koningiopsis* (KU904460) which comes under class VI of bells scale.

Conclusion

As *S. rolfsii* is a soil inhabitant and its sclerotia persist in and germinate on availability of host various species of Trichoderma can effectively inhibit the growth of mycelium and crop management can be done effectively in an eco-friendly manner. Therefore it may be concluded that some Trichoderma species may have positive impact for reduction of *S. rolfsii* inoculum in field condition.

Table 1: List of bio control agents under study

Sl. no.	Biocontrol agent	Accession number
1	*T. asperellum*	(KU933475)
2	*T. koningiopsis*	(KU904460)
3	*T. hypocrea*	(KX0113223)
4	*T. harzianum*	(KU933468)
5	*T. harzianum*	KU904458
6	*T. ovalisporum*	KU904456)
7	*T. harzianum*	(KU933471)
8	*T. atroviride*	(KU933472)
9	*T. asperellum*	(KU933476)

Table 2: *In vitro* efficacy of bio control agents on the growth of *S. rolfsii*

Sl. no.	Biocontrol agent	Duration point of contact(days)	Bell's scale	Inhibition (%)
1	*T. asperellum* (KU933475)	2	Class iv	42.18 (6.53)
2	*T.koningiopsis* (KU904460)	2	Class vi	43.75 (6.65)
3	*T. hypocrea* (KX0113223)	2	Class iv	43.75 (6.65)
4	*T. harzianum* (KU933468)	3	Class iv	27.61 (5.30)
5	*T. harzianum* (KU904458)	3	Class III	51.56 (7.21)
6	*T. ovalisporum* (KU904456)	3	Class iv	45.31 (6.76)
7	*T. harzianum* (KU933471)	2	Class III	59.37 (7.73)
8	*T. atroviride* (KU933472)	3	Class iv	13.02 (3.67)
9	*T. asperellum* (KU933476)	2	Class III	63.02 (7.96)
	SE(d)			0.055
	$CD_{(P=0.05)}$			0.116

*Mean of three replications

**Figures in parenthesis are square root transformed values

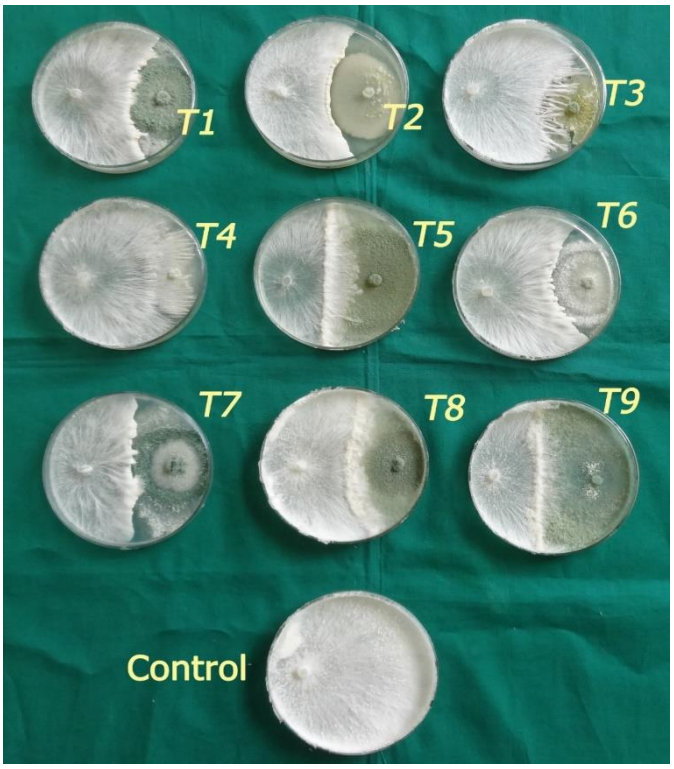
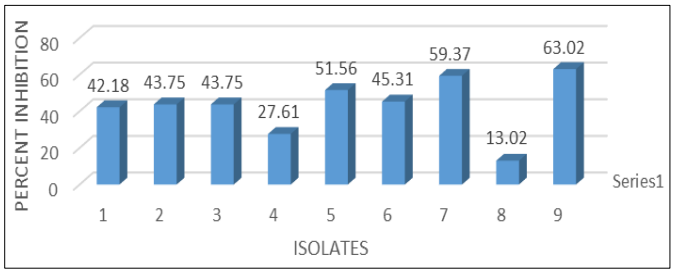


Plate 1: *In vitro* efficacy of bio-control agents on the growth of *S. rolfsii.*

C. Control, 1. *T. asperellum* (KU933475), 2. *T. koningiopsis* (KU904460),
3. *T. hypocrea* (KX0113223), 4. *T. harzianum* (KU933468),
5. *T. harzianum* (KU904458), 6. *T. ovalisporum* (KU904456),
7. *T. harzianum* (KU933471), 8. *T. atroviride* (KU933472)
9. *T. asperellum* (KU933476),



Graph 1: Percent inhibition of pathogen's mycelial growth by *Trichoderma spp.*

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