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In vitro Screening for Potential Antagonistic Activity of *Trichoderma* Isolates against *Sclerotium rolfsii* Causing Collar Rot of French Bean

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ABSTRACT

French bean is an important vegetable which has high nutritional values. It has possibility to be grown round the year especially in the North eastern region of India. French bean is affected by number of diseases. Among the diseases collar rot (*Sclerotium rolfsii*) being most devastating one and cause a substantial yield loss up to 55–70%. For obtaining better insight in the antagonistic potential of native bio-control agent, 36 isolates of *Trichoderma* isolates were evaluated against *S. rolfsii* by dual culture technique. Upon *in vitro* screening of the varied isolates, the highest growth inhibition of the pathogen was recorded in isolates, T-8 (85.7 %) followed by T-20 (84.8%). Efficient mycoparasitism and microbial enzyme ability were also assessed in all *Trichoderma* isolates. All native microbial isolates showed consistent ability to produce volatile and non-volatile metabolites.

Keywords *Sclerotium rolfsii*, Antagonistic potential, Mycoparasitism, Volatile, Non-volatile metabolites.

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is one of the most important leguminous vegetable crops. It is native of South America and belongs to the Fabaceae family and started domestication in Colombia, Mexico and Peru about 8000 years ago. In 19th century the slim pods became common in France as Haricotverts, which mean slender pod and hence being referred to as "French" beans. It is widely cultivated in tropics, sub-tropics and temperate regions. In India and most of the tropical Asia, it is a major vegetable crop (Athikho *et al.* 2019) and grown both under field as well as greenhouse conditions throughout the year supplying in the fresh market as well as for processing purposes (Bhati and Kanaujia 2014) and is a popular vegetable grown under irrigated conditions almost throughout the year. Though French bean crop occupies a very important place among the vegetable crops grown in India, the average yield of this crop on farmers' fields is reasonably poor. One of the constraints for poor yield is the devastating effect of disease incidence. Number of diseases viz., leaf spot (*Alternaria alternata*), collar rot (*Sclerotium rolfsii*), anthracnose (*Colletotrichum lindemuthianum*), root rot (*Rhizoctonia solani*), fusarium root rot (*F. solani*

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Table 1. Native *Trichoderma* isolates and their collection locations.

Isolate code	Isolation from	Location	District	State
T-1	French beans rhizosphere	Agronomy field, SASRD	Chumoukedima	Nagaland
T-2	French beans rhizosphere	AICRP field, SASRD	Chumoukedima	Nagaland
T-3	French beans rhizosphere	Entomology field, SASRD	Chumoukedima	Nagaland
T-4	French beans rhizosphere	Horticulture farm (Sample-1), SASRD	Chumoukedima	Nagaland
T-5	French beans rhizosphere	Horticulture farm (Sample II), SASRD	Chumoukedima	Nagaland
T-6	French beans rhizosphere	Farmers' field (Sample-I), Kohima	Kohima	Nagaland
T-7	French beans rhizosphere	Farmers' field (Sample-II), Kohima	Kohima	Nagaland
T-8	French beans rhizosphere	Farmers' field (Sample-III), Kohima	Kohima	Nagaland
T-9	French beans rhizosphere	Farmer's field, Medziphema	Chumoukedima	Nagaland
T-10	French beans rhizosphere	Farmers' field, Mao	Senapati	Manipur
T-11	French beans rhizosphere	Kitchen garden, SASRD	Chumoukedima	Nagaland
T-12 (*T. harzianum*)	Virgin forest	DBT project site, Dziilakie	Kohima	Nagaland
T-13 (*T. virens*)	Virgin forest	DBT project site, Dziilakie	Kohima	Nagaland
T-14 (*T. asperellum*)	Virgin forest	DBT project site, Dziilakie	Kohima	Nagaland
T-15	Tomato soils	Polyhouse (Sample-I), CIH, Medziphema	Chumoukedima	Nagaland
T-16	Tomato soils	Tomato field (Sample-I), Horticulture farm, SASRD	Chumoukedima	Nagaland
T-17	Tomato soils	Farmers' field (Sample-I), Merima	Kohima	Nagaland
T-18	Tomato soils	Farmers' field (Sample-I), Tsiesma	Kohima	Nagaland
T-19	Tomato soils	Polyhouse (Sample-II), CIH, Medziphema	Chumoukedima	Nagaland
T-20	Tomato soils	Tomato field (Sample-II), Horticulture farm, SASRD	Chumoukedima	Nagaland
T-21	Rice soils	Upland rice field-V1, Agronomy farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-22	Rice soils	Upland rice field-V, Agronomy farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-23	Soils	Fallow land, Agronomy farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-24	Soils	Upland rice field-I, Agronomy farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-25	Soils	Black gram field, Agronomy farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-26	Soils	Cauliflower field, Horticulture farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-27	Soils	Fallow land, Horticulture farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-28	Soils	Upland rice field-II, Agronomy farm, SASRD, Medziphema	Chumoukedima	Nagaland

Table 1. Continued.

Isolate code	Isolation from	Location	District	State
T-29	Soils	Upland rice field-III, Agronomy farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-30	Soils	Upland rice field-IV, Agronomy farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-31	Tomato rhizosphere	Polyhouse (Sample-II), CIH, Medziphema	Chumoukedima	Nagaland
T-32	Tomato rhizosphere	Tomato field (Sample-II), Horticulture farm, SASRD, Medziphema	Chumoukedima	Nagaland
T-33	Tomato rhizosphere	Farmers' field (Sample-II), Merema	Kohima	Nagaland
T-34	Tomato rhizosphere	Farmers' field (Sample-II), Tsiesema	Kohima	Nagaland
T-35	Tomato rhizosphere	Polyhouse (Sample-III), CIH, Medziphema	Chumoukedima	Nagaland
T-36	Tomato rhizosphere	Tomato field (Sample-III), Horticulture farm, SASRD, Medziphema	Chumoukedima	Nagaland

f. sp. *phaseoli*), root knot nematode (*Meloidogyne* sp.), bacterial brown spot (*Pseudomonas syringae* pv. *syringae*), common blight (*Xanthomonas campestris* pv. *phaseoli*), halo blight (*Pseudomonas syringa*e pv. *phaseolicola*), bean yellow mosaic disease. affected on French bean (Kumar *et al.* 2018). The use of large volumes of fungicides facilitates the development of resistance in the fungi, which reduces their efficacy (Apaliya *et al.* 2017) as well as hazardous to environment and human health. Under such conditions, the most effective method is the biological control which considered both safe and eco-friendly (Dukare *et al.* 2019). The use of plant beneficial microorganisms as biological control agents (BCAs) of pests and diseases emerges as a viable alternative to the abusive use of agrochemicals (Rändler-Kleine *et al.* 2020). They are considered key players in modern crop management programs aiming to increase sustainability in agriculture (Compant *et al.* 2019).

Looking into the aforesaid realities, the use of biological agents, as biological control, promises more efficient disease control. Also, not much systematic research work has been carried out on collar rot of French bean under Nagaland condition with the following objective : To isolate the native rhizospheric microbes and test their antagonistic efficacy against *S. rolfsii* under *in-vitro* condition.

MATERIALS AND METHODS

Collection of diseased specimens

Diseased specimens of collar rot of French bean were collected from French bean field, Horticulture farm, SASRD, Nagaland University, Medziphema campus showing typical symptoms of collar rot of French bean.

Isolation and purification of the pathogen

The causal pathogen from naturally infected tissues of French bean was isolated by tissue isolation technique. The typical pathogen colonies developed within 48 hrs. *S. rolfsii* produced white cottony, compact fluffy mycelial growth on PDA medium. The pure culture of the fungus was obtained by hyphal tip isolation technique and each isolate thus obtained was coded and stored in test tube slants.

Pathogenicity test

The pathogen obtained was subjected to pathogenicity test. A pot culture experiment was conducted to test

Table 2. *In vitro* antagonistic effect of *Trichoderma* isolates on radial growth and per cent inhibition of *S. rolfsii*. *Values in parentheses are angular transformed values.

Treatment			Inhibition of *S. rolfsii* growth		
			Radial growth (cm)	Radial growth (cm) inhibited	Inhibition (%)
T_0	T_0	(Control)	7.50	0.00	0.00 (4.05)
T_1	T_1	(*S. rolfsii* + T-1)	3.33	4.16	55.55 (48.19)
T_2	T_2	(*S. rolfsii* + T-2)	3.00	4.50	60.00 (50.76)
T_3	T_3	(*S. rolfsii* + T-3)	3.03	4.36	58.22 (49.73)
T_4	T_4	(*S. rolfsii* +T-4)	2.26	5.23	69.77 (56.65)
T_5	T_5	(*S. rolfsii* +T-5)	1.33	6.16	82.22 (65.06)
T_6	T_6	(*S. rolfsii* +T-6)	2.93	4.56	60.89 (51.29)
T_7	T_7	(*S. rolfsii* +T-7)	2.93	4.56	60.88 (51.28)
T_8	T_8	(*S. rolfsii* +T-8)	1.03	6.43	85.77 (67.84)
T_9	T_9	(*S. rolfsii* +T-9)	3.66	3.83	51.11 (45.63)
T_{10}	T_{10}	(*S. rolfsii* +T-10)	2.66	4.83	64.44 (53.39)
T_{11}	T_{11}	(*S. rolfsii* +T-11)	3.36	4.13	55.11 (47.93)
T_{12}	T_{12}	(*S. rolfsii* +T-12)	2.96	4.53	60.44 (51.02)
T_{13}	T_{13}	(*S. rolfsii* +T-13)	2.03	5.46	72.88 (58.62)
T_{14}	T_{14}	(*S. rolfsii* +T-14)	3.46	4.03	53.77 (47.16)
T_{15}	T_{15}	(*S. rolfsii* +T-15)	3.36	4.13	55.11 (47.93)
T_{16}	T_{16}	(*S. rolfsii* +T-16)	2.53	4.96	66.22 (54.46)
T_{17}	T_{17}	(*S. rolfsii* +T-17)	2.56	4.93	65.77 (54.19)
T_{18}	T_{18}	(*S. rolfsii* +T-18)	2.46	5.03	67.11 (55.00)
T_{19}	T_{19}	(*S. rolfsii* +T-19)	3.76	3.73	49.77 (44.87)
T_{20}	T_{20}	(*S. rolfsii* +T-20)	1.10	6.40	84.88 (67.12)
T_{21}	T_{21}	(*S. rolfsii* +T-21)	3.76	3.73	49.77 (44.87)
T_{22}	T_{22}	(*S. rolfsii* +T-22)	4.73	2.76	36.88 (37.39)
T_{23}	T_{23}	(*S. rolfsii* +T-23)	5.40	2.10	28.00 (31.94)
T_{24}	T_{24}	(*S. rolfsii* +T-24)	4.86	2.633	35.11 (36.33)
T_{25}	T_{25}	(*S. rolfsii* +T-25)	5.10	2.40	32.00 (34.44)
T_{26}	T_{26}	(*S. rolfsii* +T-26)	4.03	3.46	46.22 (42.83)
T_{27}	T_{27}	(*S. rolfsii* +T-27)	4.26	3.23	43.11 (41.04)
T_{28}	T_{28}	(*S. rolfsii* +T-28)	4.53	2.96	39.55 (38.99)
T_{29}	T_{29}	(*S. rolfsii* +T-29)	5.86	1.63	21.78 (27.81)
T_{30}	T_{30}	(*S. rolfsii* +T-30)	4.40	3.10	41.33 (40.00)
T_{31}	T_{31}	(*S. rolfsii* +T-31)	4.86	2.63	35.11 (36.33)
T_{32}	T_{32}	(*S. rolfsii* +T-32)	6.10	1.40	18.66 (25.58)
T_{33}	T_{33}	(*S. rolfsii* +T-33)	5.36	2.13	28.44 (32.22)
T_{34}	T_{34}	(*S. rolfsii* +T-34)	4.93	2.56	34.22 (35.79)
T_{35}	T_{35}	(*S. rolfsii* +T-35)	3.66	3.83	51.11 (45.63)
T_{36}	T_{36}	(*S. rolfsii* +T-36)	3.70	3.80	50.67 (45.38)
SEm±			0.00	0.00	0.02
CV(%)			2.06	2.10	2.12
CD (p= 0.01)			0.16	0.17	2.31

the pathogenicity. For this purpose, the pathogen was inoculated on susceptible French bean cv Anupama plants. After 48 hrs, the observation on disease development was recorded where lesions appeared near the collar region subsequently followed with the withering and yellowing of leaf. Then after 96 hrs, full wilting of the plant was observed. The pathogen was re-isolated from the same infected tissue on a PDA plate and when compared they were observed to be akin with the original pathogen. No symptoms were observed on un-inoculated plants. The pathogen was then undergoing for further characterization studies.

Collection and isolation of native antagonists from rhizospheric soil

Soil samples were taken from the rhizosphere of healthy French bean plants from different places of

Table 3. *In vitro* effect of volatile and non-volatile metabolites production of *Trichoderma* isolates on mycelial growth and per cent inhibition of *S. rolfsii*.

	Treatment	Volatile production			Non-volatile production		
		Diameter growth (cm)	Diameter growth (cm) inhibited	Inhibition (%)	Diameter growth (cm)	Diameter growth (cm) inhibited	Inhibition (%)
T_0	T_0 (Control)	9.00	0.00	0.00 (4.05)	9.00	0.00	0.00 (4.05)
T_1	T_1 (*S. rolfsii* + T-1)	1.33	7.66	85.18 (67.36)	0.00	9.00	100.00 (85.94)
T_2	T_2 (*S. rolfsii* + T-2)	2.10	6.90	76.67 (61.11)	2.36	6.63	73.70 (59.16)
T_3	T_3 (*S. rolfsii* + T-3)	6.63	2.36	26.30 (30.85)	2.80	6.20	68.89 (56.09)
T_4	T_4 (*S. rolfsii* +T-4)	2.63	6.36	70.74 (57.25)	3.06	5.93	65.93 (54.28)
T_5	T_5 (*S. rolfsii* +T-5)	6.30	2.70	30.00 (33.21)	5.63	3.36	37.41 (37.70)
T_6	T_6 (*S. rolfsii* +T-6)	3.66	5.33	59.26 (50.33)	0.00	9.00	100.00 (42.87)
T_7	T_7 (*S. rolfsii* +T-7)	5.90	3.100	34.44 (35.93)	1.93	7.06	78.52 (62.40)
T_8	T_8 (*S. rolfsii* +T-8)	0.90	8.10	90.00 (71.58)	0.00	9.00	100.00 (85.94)
T_9	T_9 (*S. rolfsii* +T-9)	1.10	7.90	87.78 (69.57)	0.73	8.26	91.85 (73.42)
T_{10}	T_{10} (*S. rolfsii* +T-10)	8.03	0.96	10.74 (19.12)	0.00	9.00	100.00 (85.94)
T_{11}	T_{11} (*S. rolfsii* +T-11)	2.73	6.26	69.63 (56.55)	0.80	8.20	91.11 (72.65)
T_{12}	T_{12} (*S. rolfsii* +T-12)	1.23	7.70	85.56 (67.66)	0.96	8.03	89.26 (70.87)
T_{13}	T_{13} (*S. rolfsii* +T-13)	2.76	6.23	69.26 (56.33)	0.00	9.00	100.00 (85.94)
T_{14}	T_{14} (*S. rolfsii* +T-14)	4.33	4.66	51.85 (46.06)	0.00	9.00	100.00 (85.94)
T_{15}	T_{15} (*S. rolfsii* +T-15)	1.03	7.96	88.52 (70.21)	0.00	9.00	100.00 (85.94)
T_{16}	T_{16} (*S. rolfsii* +T-16)	1.13	7.86	87.41 (69.22)	0.00	9.00	100.00 (85.94)
T_{17}	T_{17} (*S. rolfsii* +T-17)	1.10	7.90	87.78 (69.55)	2.63	6.36	70.74 (57.25)
T_{18}	T_{18} (*S. rolfsii* +T-18)	1.10	7.90	87.78 (69.55)	0.00	9.00	100.00 (85.94)
T_{19}	T_{19} (*S. rolfsii* +T-19)	8.30	0.70	9.26 (17.67)	1.33	7.66	85.18 (67.36)
T_{20}	T_{20} (*S. rolfsii* +T-20)	0.93	8.06	89.63 (71.21)	0.00	9.00	100.00 (85.94)
T_{21}	T_{21} (*S. rolfsii* +T-21)	1.56	7.43	82.59 (65.34)	4.13	4.86	54.07 (47.33)
T_{22}	T_{22} (*S. rolfsii* +T-22)	1.70	7.30	81.11 (64.23)	2.90	6.100	67.78 (55.41)
T_{23}	T_{23} (*S. rolfsii* +T-23)	2.03	6.966	77.41 (61.62)	3.10	5.90	65.56 (54.06)
T_{24}	T_{24} (*S. rolfsii* +T-24)	2.43	6.56	72.96 (58.67)	2.53	6.46	71.85 (57.96)
T_{25}	T_{25} (*S. rolfsii* +T-25)	6.73	2.26	25.18 (30.12)	2.23	6.76	75.07 (60.05)
T_{26}	T_{26} (*S. rolfsii* +T-26)	8.10	0.90	10.00 (18.37)	3.00	6.00	66.67 (54.73)
T_{27}	T_{27} (*S. rolfsii* +T-27)	4.70	4.30	47.78 (43.72)	3.16	5.83	64.81 (53.62)
T_{28}	T_{28} (*S. rolfsii* +T-28)	3.06	5.93	65.93 (54.28)	2.93	6.06	67.41 (55.18)
T_{29}	T_{29} (*S. rolfsii* +T-29)	2.83	6.16	68.52 (55.87)	3.40	5.60	62.22 (52.07)
T_{30}	T_{30} (*S. rolfsii* +T-30)	2.76	6.23	69.26 (56.32)	3.90	5.10	56.67 (48.84)
T_{31}	T_{31} (*S. rolfsii* +T-31)	5.00	4.00	44.44 (41.80)	4.06	4.93	54.81 (47.76)
T_{32}	T_{32} (*S. rolfsii* +T-32)	2.40	6.60	73.33 (58.91)	3.23	5.76	64.07 (53.17)
T_{33}	T_{33} (*S. rolfsii* +T-33)	4.76	4.23	47.04 (43.30)	2.90	6.10	67.78 (55.41)
T_{34}	T_{34} (*S. rolfsii* +T-34)	3.76	5.23	58.15 (49.69)	3.10	5.96	65.55 (54.06)
T_{35}	T_{35} (*S. rolfsii* +T-35)	3.03	5.96	66.30 (54.51)	3.50	5.50	61.11 (51.41)
T_{36}	T_{36} (*S. rolfsii* +T-36)	2.20	6.80	75.56 (60.37)	1.23	7.76	86.30 (68.28)
SEm±		0.00	0.00	0.02	0.00	0.00	0.02
CV(%)		2.31	1.43	1.39	3.14	1.05	1.02
CD (p= 0.01)		0.17	0.17	1.55	0.14	0.15	1.65

Nagaland (Table 1).

For the isolation of *Trichoderma* the soil samples were serially diluted (10^{-4}) and plated on *Trichoderma* selective medium. Totally 36 isolates were subjected to preliminary screening against *S. rolfsii*, to test their bio-control ability. The isolates were designated serially as T-1 to T-36.

In vitro screening for potential antagonistic effect of *Trichoderma* isolates on radial growth of the pathogen

The antagonistic effect of *Trichoderma* isolates was

Table 4. Qualitative assay of enzymes production and hyphal interaction by native *Trichoderma* isolates. Whereas: + = low production of pectolytic and amylase, ++ = medium production of pectolytic and amylase, +++ = strong production of pectolytic and amylase, - = no production of pectolytic and amylase; @ = absence of coiling and * = presence of coiling.

BCAs	Pectolytic production	Amylase production	Hyphal interaction
Control	-	-	@
T-1	-	++	*
T-2	+++	+++	*
T-3	++	++	*
T-4	++	+++	*
T-5	++	++	*
T-6	++	+	*
T-7	-	+++	*
T-8	+++	+++	*
T-9	++	+	*
T-10	+++	++	*
T-11	+++	+++	*
T-12	++	+++	*
T-13	-	+++	*
T-14	++	+++	*
T-15	-	+	*
T-16	+++	+	*
T-17	+++	++	*
T-18	++	+	*
T-19	++	++	*
T-20	+++	+++	*
T-21	+	++	*
T-22	++	+	*
T-23	++	+	*
T-24	+	+	*
T-25	+	++	*
T-26	+	+	*
T-27	++	++	*
T-28	+	+	*
T-29	+	+	*
T-30	++	+	*
T-31	++	+	*
T-32	++	+	*
T-33	+	+	*
T-34	+	+	*
T-35	++	+	*
T-36	++	++	*

evaluated against *S. rolfsii* by dual culture technique given by Sivakumar *et al.* (2000) with slight modification. A 10 mm diameter mycelial disc of *S. rolfsii* (7 days old) was placed on one side of a Petri plate (90 mm diameter) containing PDA medium (20 ml). Simultaneously, 10 mm diameter disc of *Trichoderma* isolates (5 days old) were placed on another side 60 mm away from the pathogen leaving 10 mm from both periphery on the dual plates, whereas sterile PDA disc was placed in the control plates and incubated at 25±2°C. The radial growth of the pathogen was measured after fully grown of control plate.

Linear mycelial growth was recorded from the center of the disc towards periphery of the Petri plate after the control plate was completely covered by mycelia growth of the test pathogen.

Per cent inhibition of the growth of pathogen by BCAs over control was calculated as per the formula given by Vincent (1927).

$$\text{Per cent radial growth inhibition : PI} = \frac{c-r}{c} \times 100$$

Where C = Radial growth of *S. rolfsii* (cm) in control plate

T = Radial growth of *S. rolfsii* (cm) in dual plate

PI = Per cent inhibition

The experiment was conducted in a Complete Randomized Design (CRD) and three replications were maintained for each treatment. Data were analyzed statistically.

In vitro characterization of bio-control features

To investigate the bio-control mechanism, the efficient rhizospheric isolates were tested for the production of volatile production, non-volatile diffusible antibiotic, pectolytic production, amylase production and mycoparasitism assay.

Screening of volatile metabolites production

The effects of volatile metabolites of BCAs were assessed following Dennis and Webster (1971) technique. The pathogen *S. rolfsii* was inoculated (10 mm diameter disc) at the center of a Petri plate containing PDA medium (20 ml). After 3 hrs of incubation at 25±2°C, the Petri plates were inverted on the actively grown five days old culture of *Trichoderma* and sealed with parafilms under aseptic condition and incubated at 25±2°C. Diameter mycelial growth was measured

when control plate was completely covered by growth of the test pathogen and inhibition of the growth of pathogen by volatile metabolites over control was calculated as per the formula given by Vincent (1927).

Screening of non-volatile production assay

For testing non-volatile test of *Trichoderma* and *Pseudomonas* isolates was followed with the protocol given by You *et al.* (2016). The isolates of bacterial and fungal antagonists were inoculated in 100 ml sterile nutrient broth and potato dextrose broth in 250 ml conical flask. Inoculated flasks were then incubated at 25±2^{0}C for 15 days. Supernatant of the liquid culture was prepared by filtering through a 0.22-µm filter, then mixed to un-solidified PDA (40^{0}C) at a ratio of 10% (v/v). Control plate was maintained without amending the culture filtrate of *Trichoderma* isolates. Then, a mycelial block (10 mm) of *S. rolfsii* was inoculated on poured media plates, and the plates were kept for six days at 25±2^{0}C. Colony diameter of mycelial growth was measured when control plate was completely covered and calculated as per the formula given by Vincent (1927).

Qualitative assay of microbial enzymes production

Qualitative screening of pectinolytic enzyme producing isolates

Pectinolytic activity of *Trichoderma* isolates were carried out on solid medium. The medium was aseptically poured to Petri dishes and inoculated with a 10 mm disc from 5-day old *Trichoderma* isolates separately. After 24-48 hrs growth, plates were flooded with gram's iodine solution (2 g KI and 1g I2 crystals dissolved in 100 ml of water). A clear zone around the colony indicated the pectinolytic activity (Kaur *et al.* 1998).

Qualitative screening for amylase producing isolates

Amylase production test (Hankin and Anagnostakis 1975) was assessed by growing the *Trichoderma* isolates on Starch Agar Medium (Starch 20.00g, Beef extract 3.00 g, Peptone 5.00g, Agar 16.00 g and Distilled water 1000 ml). The medium was aseptically transferred to Petri dishes and inoculated with a 6mm agar disc cut from 5-day old fungal culture of each strain separately and incubated at 25 ± 2°C in darkness for 3 to 5 days. The plates were then flooded with 1% iodine in 2% potassium iodide. The clear zone formed surrounding the colony was considered positive for amylase production.

Mycoparasitism activity of native Trichoderma isolates

This method consisted of inoculating the antagonist in the same Petri plate containing PDA medium (20 ml) culture having three sterile cover slips in the center of the plate for check interaction between the hyphae. Mycelial disc (10 mm) of each isolate of *Trichoderma* and pathogen were inoculated as opposite poles in the dual plate and incubated at a temperature of 25±2°C for 10 days in the absence of light.

RESULTS AND DISCUSSION

In vitro screening for potential antagonistic effect of Trichoderma isolates on radial growth of the pathogen

Altogether 36 promising isolates of *Trichoderma* were evaluated for their inhibitory action on the radial growth of *S. rolfsii* by adopting dual culture technique (Sivakumar *et al.* 2000) and the data obtained are presented in Table 2. The isolates of fungal antagonists screened and exhibited varied level of bio-control traits against the virulent *S. rolfsii* and showed significantly superior over control. It was found that the growth of the pathogen in dual culture plates progressed until they came in contact with the leading edges of the antagonist. Among the different treatments, least radial mycelial growth of the pathogen was recorded in T_8 (1.06 cm) which is statistically at par with T_{20} (1.10 cm) followed by T_5 (1.33 cm) and T_{13} (2.03 cm), respectively.

The per cent inhibition over control was calculated and it was observed that highest inhibition per cent was recorded in T_8 (85.77 %) which is statistically at par with T_{20} (84.88%) followed by T_5 (82.22 %), T_{13} (72.88%) and the least antagonistic effect was

observed in T_{32} (20.00%) at 5 days after incubation at 25±2°C.

In the present investigation, the probable reasons of high inhibitory activity of the *Trichoderma* spp. against *S. rolfsii* in dual cultures may be due to the fact that *Trichoderma* spp. produce extracellular cell-wall degrading enzymes like chitinase, β-1, 3 glucanase, protease, cellulase and lectin, competition, mycoparasitic activity like coiling of mycelium which help them in colonising the host and inhibit soil-borne fungi. Sharma *et al.* (2020) evaluated four potential *Trichoderma* mutants against *S. 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. Similar trend of present studies was observed by earlier worker Bhuiyan *et al.* (2012).

In vitro screening of volatile metabolites production

The effects of volatile metabolites of selected twenty *Trichoderma* isolates were assessed against *S. rolfsii* by following the technique given by Dennis and Webster (1971) and the data thus obtained are presented in Table 3. All isolates assessed against *S. rolfsii* were significantly superior over control treatment. Among the different treatments, least mycelial growth of the pathogen was recorded in T_8 (0.90 cm) which was found to be statistically at par with T_{20} (0.93 cm) and T_{15} (1.03 cm). This was followed by T_9, T_{17} and T_{18} with 1.10 cm, respectively.

The per cent inhibition over control was calculated and it was observed that T_8 with 90% were found to be most promising in production of volatile compounds by *Trichoderma* isolates against *S. rolfsii* which was statistically at par withT_{20} (89 %).

In vitro screening of non-volatile metabolites production

Non-volatile (culture filtrates) production of 36 potential isolates of *Trichoderma* spp. tested for their inhibitory effect on mycelial growth of *S. rolfsii* are presented in Table 3. All isolates assessed against *S. rolfsii* were significantly superior over control treatment. Among the different treatments, least mycelial growth of the pathogen was recorded in T_1, T_6, T_8, T_{13}, T_{14}, T_{15}, T_{16}, T_{18} and T_{20} (0.00 cm).

The per cent inhibition over control was calculated and it was observed that T_1, T_6, T_8, T_{13}, T_{14}, T_{15}, T_{16}, T_{18} and T_{20} were found total inhibition 100% of the growth of *S. rolfsii*.

The results of the present investigation on the production of non-volatile metabolites by both *Trichoderma* isolates have definite influence on the high degree of inhibition on the growth of *S. rolfsii.* This might be due to the presence of several antifungal proteins in the culture filtrate of *Trichoderma* spp. which limited the mycelial growth under *in vitro* condition.

The results are in conformity with the reports of earlier workers ; Muthukumar *et al.* (2010), Chanutsa *et al.* (2014) and Kumar *et al.* (2018).

Qualitative screening of pectolytic enzyme production

Trichoderma spp. were screened for secretion of pectolytic enzyme. The results of qualitative assay of pectolytic enzyme production by the fungal isolates are depicted in Table 4. The results revealed that *Trichoderma* isolates, T-2, T-8, T-9, T-10, T-11, T-16, T-17 and T-20 showed strong production of pectolytic enzymes.

Similar work done by Maria *et al.* (2001) confirms the present investigation. They reported that *T. harzianum*, *T. viride* and *T. koningii* produced extracellular pectinases. Further Thi *et al.* (2020) reported that 15 rhizobacterial isolates were subjected to pectolytic activity test and found out that 5 isolates could not produce pectinase while the other 10 strains could produce a positive amount of pectolytic enzyme. The present results are similar with earlier work done by Qualhato *et al.* (2013), Yannam *et al.* (2014), Tepe and Dursun (2014) and Tsegaye *et al* (2019).

Qualitative screening of amylase production

Trichoderma spp. were screened for secretion of am-

ylase enzyme. The isolates were showing in varying range of amylase production (Table 4).

The results revealed that *Trichoderma* isolates T-2, T-4, T-7, T-8, T-11, T-12, T-13, T-14, T-17 and T-20 showed strong production of amylase.

Tsegaye *et al.* (2019) reported that 95 isolates of rhizobacteria exhibited positive results for amylase production. The present results are also similar with earlier work done by Malleswari *et al.* (2013), Verma and Shahi (2015) and Thi *et al.* (2020).

Mycoparasitism activity of native *Trichoderma* isolates

All 36 isolates of *Trichoderma* under the study were tested for mycoparasitism activity against *S. rolfsii.* The presence or absence of coiling was observed under compound microscope (Table 4). All, 36 isolates showed the presence of coiling as hyphal interactions between *Trichoderma* isolates and *S. rolfsii*.

In the present investigation characteristic envelopment and coiling around of the hyphae by all isolates of *Trichoderma* spp. was observed. The hyphae of *Trichoderma* spp. were also observed to grow in close proximity to the hyphae of *S. rolfsii* before coagulation and disintegration occurred.

Similar work done by Saravanakumar (2002) confirms the present findings. They observed the zone of interaction between *Trichoderma* spp. and *S. rolfsii* which revealed the hyperparasitic activity of antagonist on the test pathogen. This may result in disorganization or digestion of protoplasm contents or directly penetrates the hyphae of *S. rolfsii*. The results of mycoparasitic behaviors of *Trichoderma* spp. followed almost the same pattern against *S. rolfsii* was compared with the earlier workers Coley-Smith and Cooke (1971), Elad *et al.* (1980) and Elad and Chet (1983).

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