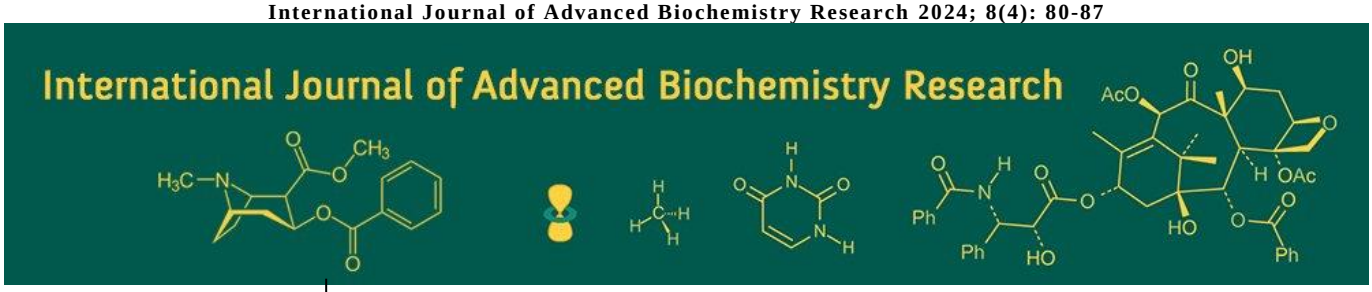

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Extensive intra-species comparative assessment of nutrient media for growth between Rhizospheric and Non-Rhizospheric *Trichoderma* Isolates

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Abstract
For their growth characteristics on seven distinct media, five *Trichoderma* isolates from non-rhizospheric regions were compared to one rhizospheric and one commercial isolate. The majority of *Trichoderma* isolates showed early sporulation and maximum mycelial development on corn meal agar and oat meal agar media, two of the seven different solid media. In terms of mycelia growth (90 mm) and early sporulation (48 hours after inoculation) on the greatest number of different solid media, non-rhizospheric isolates namely, PSV (Paddy straw) and GMV (Goat manure) performed better than commercial isolates. A rhizospheric *Trichoderma* isolate, Rkd-Cu, came in second (66 mm). Non-rhizospheric sources can also be used in search of effective bio-control agents, especially *Trichoderma* spp. for eco-friendly management against various plant pathogens, as fungi with the fastest growth rate and the earliest sporulation contribute to the virulence of bio-control agents against plant pathogens.

Keywords: Mycelial growth, non-rhizospheric, Rhizosphere sporulation period, *Trichoderma* spp.

Introduction
Trichoderma species are green-spored ascomycetes present in almost all types of tropical and temperate soils. They can regularly be found in the rhizosphere of plants and decaying plant material [9]. They are opportunistic; avirulent plant symbionts are antagonistic against many phytopathogenic fungi *viz*., *Phytophthora palmivora*, *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii*, and *Pythium* spp. [13, 6]. They are well interactive in root, soil, and foliar environments. Apart from controlling phytopathogens, *Trichoderma* spp. encourages plant growth and root development, crop productivity, resistance to abiotic stresses, and the uptake and consumption of nutrients [3]. *Trichoderma* spp. produces numerous secondary compounds, including antibacterial and antifungal antibiotics for example polyketides, terpenes and pyrones. Secondary metabolites play dynamic roles in the disease resistance mechanism, metal transport, differentiation, symbiosis and stimulating or inhibiting spore production and germination [2]. Similarly, it stimulates the induction of resistance mechanisms, much like the hypersensitive response (HR), systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants [1, 12]. *Trichoderma* spp. has been revealed to improve the growth of lettuce, tomato, and pepper plants [14]. Microbial inoculants as bio-control agents are useful and appealing alternatives to avoid the deficiencies caused by a limited reliance on chemicals [7]. *Trichoderma* sp. has evolved various mechanisms that are involved in attacking other fungi. These mechanisms consist of mycoparasitism for the production of inhibitory compounds, competition for space and nutrients, inactivation of the pathogen's enzymes, and induced resistance.
Media plays an important part in determining the effectiveness of *Trichoderma* against phytopathogens. Sharma *et al*. [11] also reported that media, pH, and temperature showed profound effects on the growth and sporulation of fungi. Similarly, the effects of different temperatures and culture media on ten strains of *Trichoderma viride* were studied by Maurya *et al*. [5]. The maximum growth of *Trichoderma* was found at 25–30 °C and the maximum weight was found in potato dextrose agar (276 mg), while the lowest was recorded in Czapek Dox broth (96 mg).

Apart from the effect of cultural studies on rhizospheric *Trichoderma* isolates, this paper describes a comparison between the cultural characteristics of rhizospheric and non-rhizospheric *Trichoderma* spp. on different cultural media which helps in the identification of the best media for mass multiplication of *Trichoderma* isolates and also understanding the different sources available for the isolation of potential *Trichoderma* isolates.

Materials and Methods

An experiment was conducted in the laboratory of the Department of Plant Pathology, College of Agriculture V. C. Farm, Mandya, under the University of Agriculture Sciences, Bangalore to evaluate the comparative cultural performance of rhizospheric and non-rhizospheric *Trichoderma* spp. on different cultural media.

Sample collection

Samples were collected by random sampling method from six different sources, *viz*., soilrhizosphere of cucumber crop, manures, coirpith, paddy straw, and sawdust, from different places in Mandya, Chamarajnagar, Davangere, Hassan, and Tumkur districts of Karnataka, India.Until further use, all collected samples werestored in a polyethylene bag and labeled.

Isolation and identification of *Trichoderma* spp.

Trichoderma spp. were isolated by following the serial dilution spread plate method on *Trichoderma* selective medium. The inoculated plates were incubated at 28±2 °C for about 5-7 days and the appearance of *Trichoderma* colonies was observed. Totally, six *Trichoderma* isolates were obtained and coded as mentioned in Table 1, one commercial isolate (*T. viride*) was used for comparative studies. Further isolates were maintained on slants for future uses.

Cultural characteristics features of *Trichoderma* isolates

The cultural characteristics, *viz*., colour, mycelial form, number of concentric rings, days required to sporulate, and mycelia growth rate of seven isolates, were studied on seven solid media.. The selected media consisted of natural, synthetic, and semi-synthetic sources. The details of the media used in studies are given in Table 2.

Results and Discussion

Cultural studies of isolates of *Trichoderma* spp. on different media

Colony appearance is one of the important cultural characteristics that help in studying the colony form, pigmentation, and days required for sporulation. Apart from this, the study of fungal mycelial growth helps in the identification of the best potential bio-control agent against plant pathogens. Because the highest growth rate is one of the vital characteristics of a potential bio-control agent. So, in the present study, we studied the cultural characteristics of different *Trichoderma* isolates on seven different media. The cultural studies of seven effective isolates on seven different media revealed the variation in the colony's appearance. SMV appeared white to dark green in colour in all seven media. Similarly, GMV exhibited a greenish-yellow colour in all media except for SDA (colourless). The colony colour of PSV isolates was dark green in all media except V-8, where it appeared light yellow. In all seven media, SDkd appeared green to yellowish but transparent in V-8. Likewise, CPV appeared white to green in all media but light yellow in V-8. A dark green colour colony was observed by RKdCu on all seven media except for V-8 and SDA: yellow and colourless, respectively. Similarly, commercial isolate also appeared green in all the media but colourless in SDA.

There is no report of pigmentation of all isolates on the reverse plate of seven media, but PSV and SDKd produced pale yellow and yellowish green pigmentation in OMV and MEA media, respectively. Mycelial growth in the form of concentric rings appeared on different media. In the present study, 0–4 concentric rings were observed on different media. The highest concentric rings (4) were produced by RKdCu (PDA, V-8), and SMV (MEA). All *Trichoderma* isolates showed sporulation on different media and varied in their sporulation times (48–96 HAI (Hours After Inoculation)) except PSV and SDKd isolates, which did not show any sporulation.

The colony appearance of all the isolates varied from green to yellowish, which had no significance among the different media. Whereas in the case of the colony form, only non-rhizospheric *Trichoderma* isolates exhibited an uneven growth pattern on different solid media compared to rhizospheric *Trichoderma* isolates. The highest uneven growth patterns of isolates, *viz*., PSV, SMV, and SDKd, were observed on CMD and v-8 media, followed by SDA (SMV and SDKd). However, on PDA and OMA, only one isolate (SDKd) formed an uneven growth pattern. The results on the appearance of *Trichoderma* spp. colonies on different media are consistent with Shah et al. [10], who reported that colonies formed by *T. harzianum* and *T. viride* appeared as dark green colonies with sufficient conidiation, whereas colonies formed by *T. pseudokoningii* appeared almost whitish with little or no conidiation at the 5th DAI. However, *T. harzianum* appeared with more pigmented mycelial growth than *T. viridae*. Further, they reported that *T. pseudokoningii* produced no pigmentation at all.

However, all the isolates exhibited a maximum colony diameter of 90.00 mm on OMA at the 4th DAI, and the least colony diameter was seen on TSM (< 12.00 mm). Among all the isolates, PSV exhibited a 90.00 mm colony diameter on all seven media except TSM (33.33 mm), which was superior than the commercial isolate (90.00 mm colony growth on 5 media), followed by CPV, which showed 90.00 mm colony growth on PDA, OMA, and MEA. Savitha *et al.* [8] recorded that there was no significant difference in the growth rate among all the isolated *Trichoderma* spp. on potato dextrose agar and malt extract agar except for Th16, which was slower on synthetic nutrient agar (80.00 mm). It indicates that, among these three media, potato dextrose agar was the best medium for mycelial growth of *Trichoderma* spp. However, in our present study, we observed >85 mm radial growth on OMA, followed by >80 mm on SDA. Whereas, on PDA, apart from SDKd (71.67 mm), all six isolates showed >85 mm radial growth. However on TSM, had a lower mycelial growth (>15 mm).

Among the rhizospheric and non-rhizospheric sources of *Trichoderma* isolate, GMV (non-rhizospheric) has the capacity to produce the spores earlier (48 h) on the maximum number of solid media (5), compared with rhizospheric isolate (RKdCu) and commercial isolate. On CMA, almost all the isolates showed earlier sporulation. Similarly, on CMA media, six isolates formed early

sporulation (48 HAI), followed by four on PDA and MEA. On V-8 media, only one non-rhizospheric isolate (GMV), exhibited early sporulation. Whereas, SMV and CPV isolates showed early sporulation on TSM. Similarly, on SDA, SMV and SDKd isolates produced early sporulation. So, based on the present investigation, we can conclude that OMA is the best medium for mass multiplication of non-rhizospheric *Trichoderma* isolates. Among the non-rhizospheric isolates, PSV and GMV have the highest growth rates and early sporulation compared to the rhizospheric *Trichoderma* isolate.

Table 1: List of *Trichoderma* isolates collected from rhizosphere and non rhizosphere sources from different places

Sl. No.	Source	Isolate code	Isolate name	Place of collection
1	Sheep manure	SMV	Sheep manure V C Farm	V. C. Farm
2	Goat manure	GMV	Goat manure V C Farm	V. C. Farm
3	Paddy straw	PSV	Paddy straw V C Farm	V. C. Farm
4	Saw dust	SDKd	Sawdust K M Doddi	K. M. Doddi
5	Coir pith	CPV	Coir pith V C Farm	K. M. Doddi
6	Soil rhizosphere	RKd-Cu	Rhizosphere K M Doddi- Cucumber	K. M. Doddi
7	Commercial isolate	*T. viride*	Mandya Commercial isolate	Mandya

Table 2: List of media used to study the cultural characters of effective *Trichoderma* spp.

Sl. No.	Solid media	Type of media
1	Potato dextrose agar (PDA)	Semi- Synthetic
2	Oat meal agar (OMA)	Semi- Synthetic
3	Corn meal agar (CMA)	Semi- Synthetic
4	*Trichoderma* specific medium (TSM)	Synthetic
5	V-8 juice agar (V-8)	Semi-Synthetic
6	Malt extract agar (MEA)	Non- Synthetic
7	Sabouraud's dextrose agar (SDA)	Synthetic

Table 3: Colony characteristics of seven isolates of Trichoderma spp. on seven different media

Medium	Isolates	Colony colour	Pigmentation	Colony form	Concentric rings (No.)	Time taken for sporulation (h)
PDA	SMV	White	-	Flat and even growth	0	96
	GMV	Greenish yellow	-	Flat and even growth	3	48
	PSV	Dark green	-	Flat and even growth	1	48
	SDKd	Light yellow	-	Uneven growth	1	96
	CPV	White and green	-	Flat and even growth	2	72
	RKd-Cu	Dark green	-	Even growth	4	48
	T. viride	Yellowish green	-	Even growth	3	48
OMA	SMV	White	-	Flat and even growth	0	96
	GMV	Dark green	-	Even growth	1	48
	PSV	Light green	Pale yellow	Even growth	1	72
	SDKd	Yellowish green	-	Uneven growth	1	96
	CPV	White and green	-	Flat and even growth	3	48
	RKd-Cu	Dark Green	-	Even growth	2	48
	T. viride	Light green	-	Even growth	1	72
CMA	SMV	Dark green	-	Uneven growth	0	48
	GMV	Greenish yellow	-	Even growth	2	48
	PSV	Green	-	Uneven growth	0	48
	SDKd	Dark green	-	Uneven growth	3	72
	CPV	Dark green	-	Even growth	3	48
	RKd-Cu	Dark green	-	Even growth	3	48
	T. viride	Dark green	-	Even growth	4	48
TSM	SMV	Dark green	-	Even growth	0	48
	GMV	Dark green	-	Even growth	1	72
	PSV	Green	-	Even growth	0	96
	SDKd	Yellowish green	-	Even growth	0	96
	CPV	Yellowish green	-	Even growth	0	48
	RKd-Cu	Yellowish green	-	Even growth	0	72
	T. viride	Dark green	-	Even growth	0	72
V-8	SMV	White and green	-	Uneven growth	0	72
	GMV	Greenish yellow	-	Even growth	2	48
	PSV	Light yellow	-	Uneven growth	0	-
	SDKd	White transparent	-	Uneven growth	0	-
	CPV	Light yellow	-	Flat and even growth	1	72
	RKd-Cu	Light yellow	-	Flat and even growth	4	72
	T. viride	Yellowish green	-	Even growth	1	72
MEA	SMV	Yellowish green	-	Even growth	4	72
	GMV	Dark green	-	Even growth	0	48

	PSV	Green	-	Even growth	2	72
	SDKd	Dark green	Yellowish green	Even growth	2	48
	CPV	Dark green	-	Flat and even growth	1	48
	RKd-Cu	Green	-	Even growth	3	72
	T. viride	Dark green	-	Even growth	2	48
SDA	SMV	Dark green	-	Uneven growth	0	48
	GMV	colourless	-	Flat and even growth	0	-
	PSV	Green	-	Even growth	0	96
	SDKd	Dark green	-	Uneven growth	3	48
	CPV	colourless	-	Flat and even growth	0	-
	RKd-Cu	colourless	-	Even growth	2	-
	T. viride	colourless	-	Even growth	0	-

Table 4: Colony diameter of seven isolates of *Trichoderma* spp. on seven different media

Sl. No.	Medium/Treatments	Mean colony diameter in mm						
		SMV	GMV	PSV	SDKd	CPV	RKd-Cu	*T. viride*
1	Potato dextrose agar	90.00	89.00	90.00	71.67	90.00	90.00	90.00
2	Oat meal agar	90.00	89.67	90.00	90.00	90.00	90.00	90.00
3	Corn meal agar	61.67	90.00	90.00	61.67	88.00	78.33	80.00
4	*Trichoderma* specific medium	15.00	89.67	33.33	31.67	16.67	12.00	13.00
5	V-8 juice agar	90.00	89.00	90.00	85.00	87.33	66.67	90.00
6	Malt extract agar	71.67	90.00	90.00	90.00	90.00	90.00	90.00
7	Sabouraud's dextrose agar	80.00	87.33	90.00	88.33	80.00	90.00	90.00
S.Em±		0.89	0.89	0.79	1.67	1.67	1.02	0.38
CD@1%		3.75	3.75	3.31	7.02	7.02	4.28	1.59

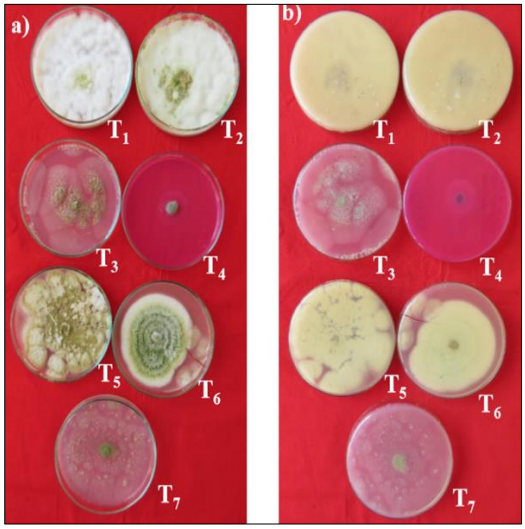

Fig 1: a) Colony appearance b) Pigmentation of SMV isolate on different media

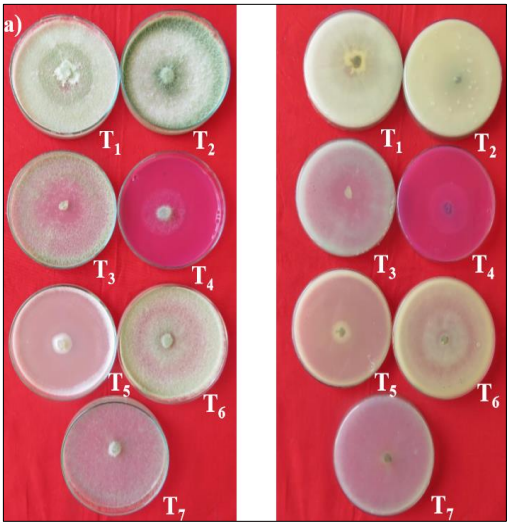

Fig 3: a) Colony appearance b) Pigmentation of PSV isolate on different media

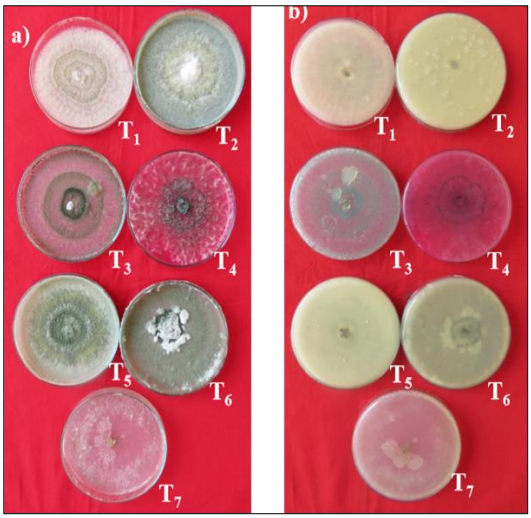

Fig 2: a) Colony appearance b) Pigmentation of GMV isolate on different media

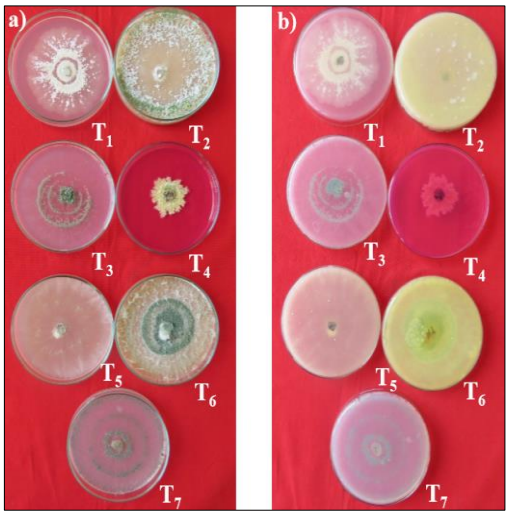

Fig 4: a) Colony appearance growth b) Pigmentation of SDKd isolate on different media

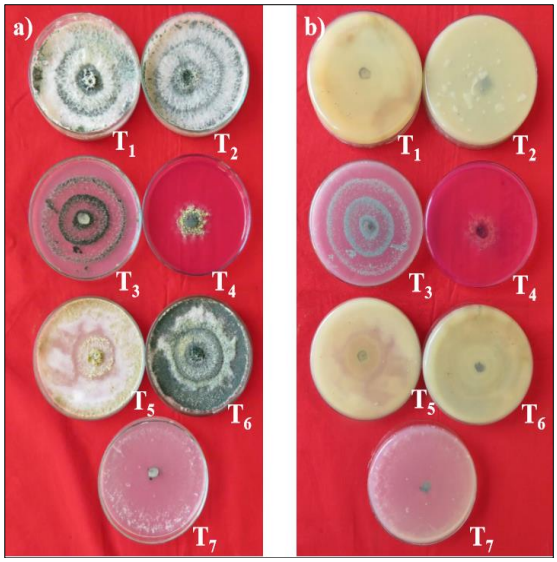

Fig 5: a) Colony appearance b) Pigmentation of CPV isolate on different media

Fig 6: a) Colony appearance b) Pigmentation of Kd-Cu isolate on different media

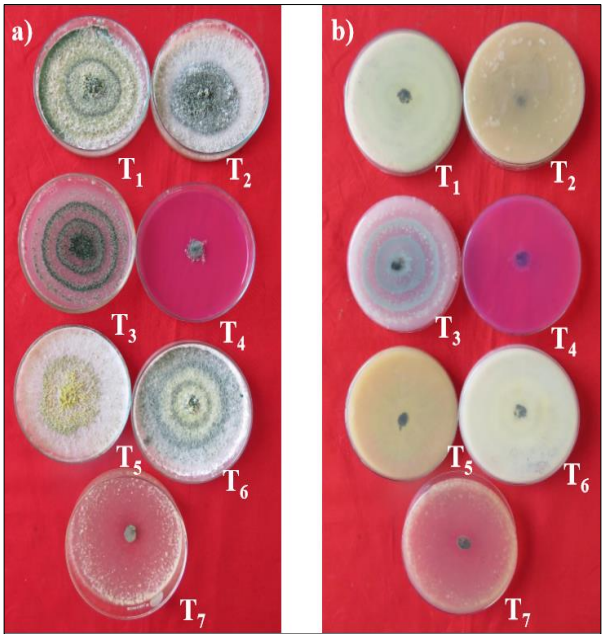

Fig 7: a) Mycelial growth of commercial isolate (*T. viride*)b) Pigmentation on different media

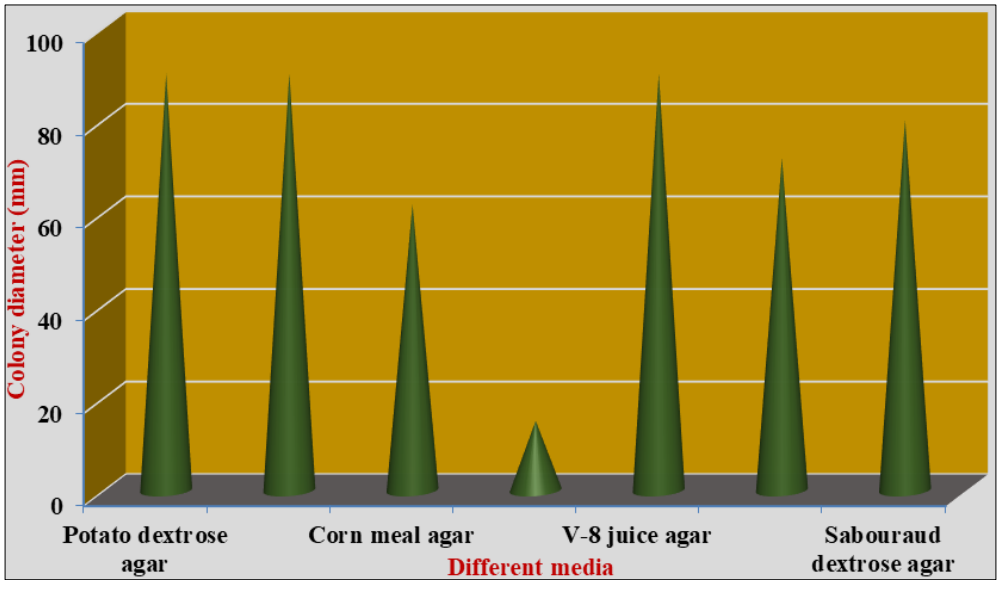

Fig 8: Mycelial growth of SMVisolate on different media

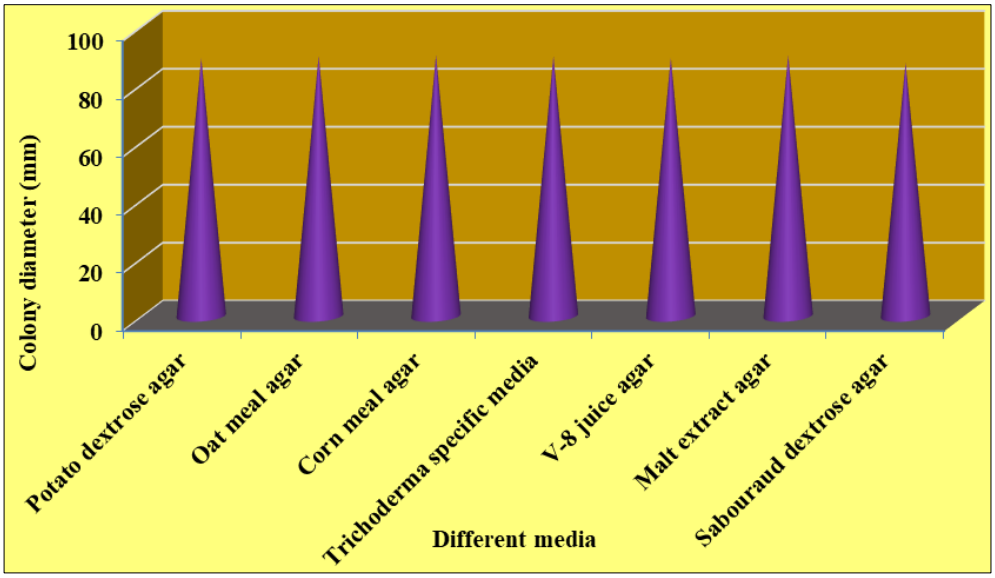

Fig 9: Mycelial growth of GMV isolate on different media

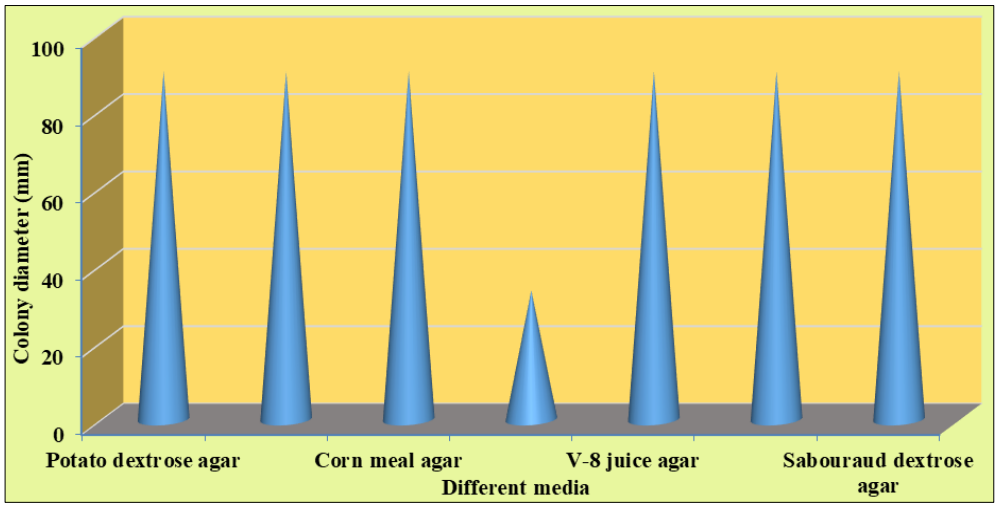

Fig 10: Mycelial growth of PSV isolate on different media

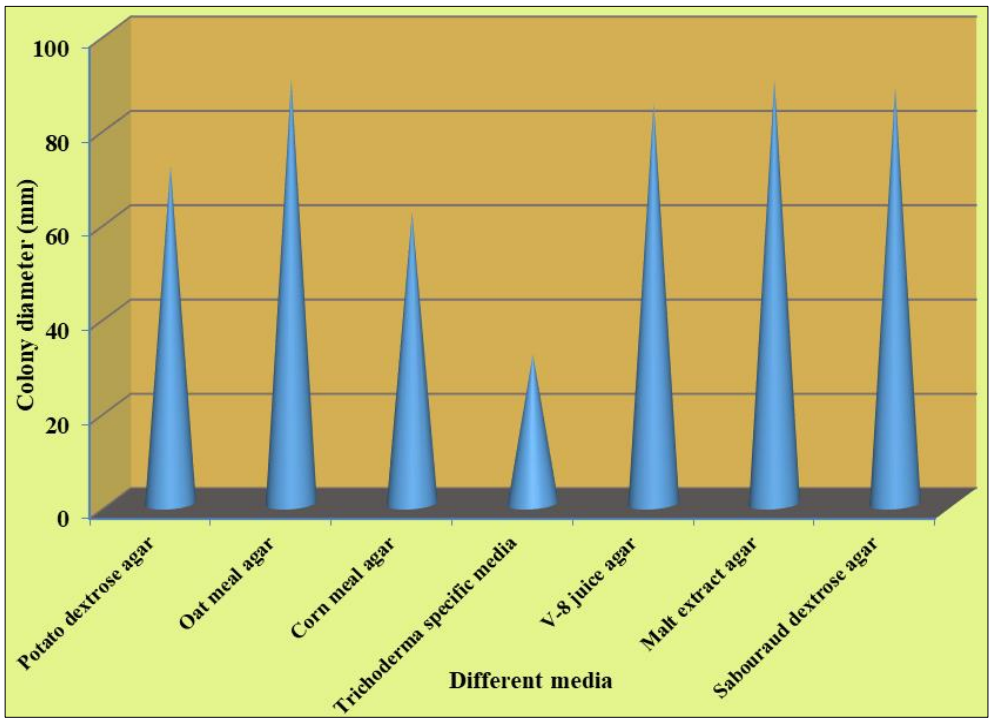

Fig 11: Mycelial growth of SDKdisolate on different medi

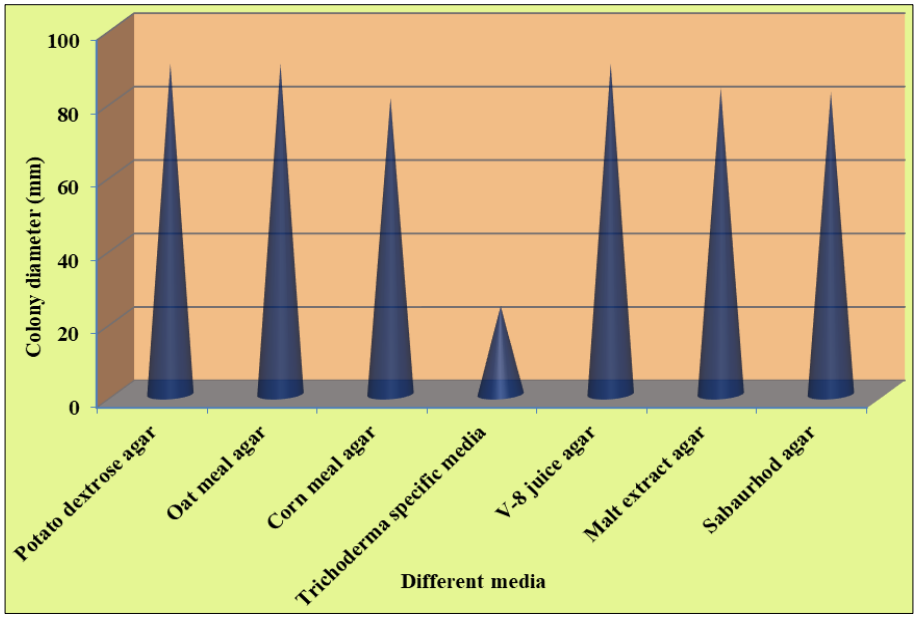

Fig 12: Mycelial growth of CP Visolate on different media

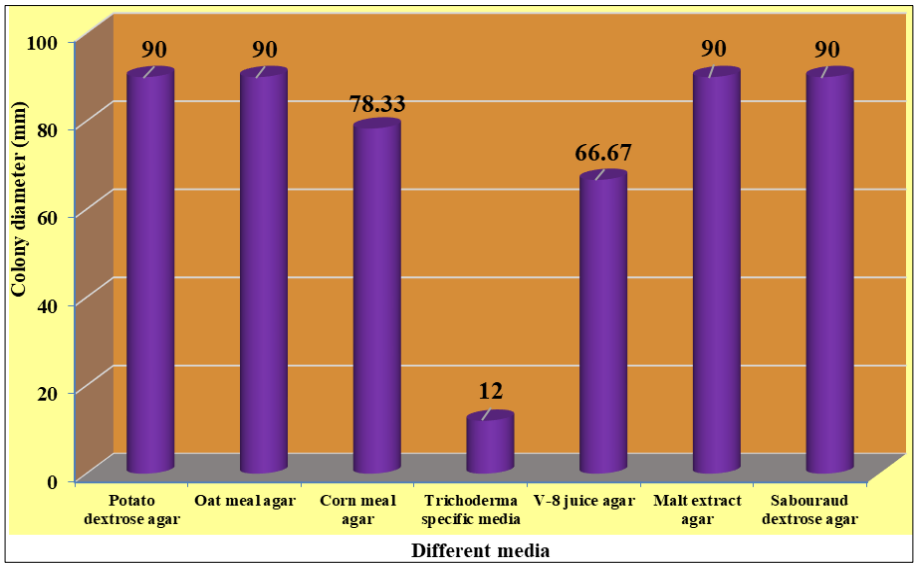

Fig 13: Mycelial growth of RKd-Cu isolate on different media

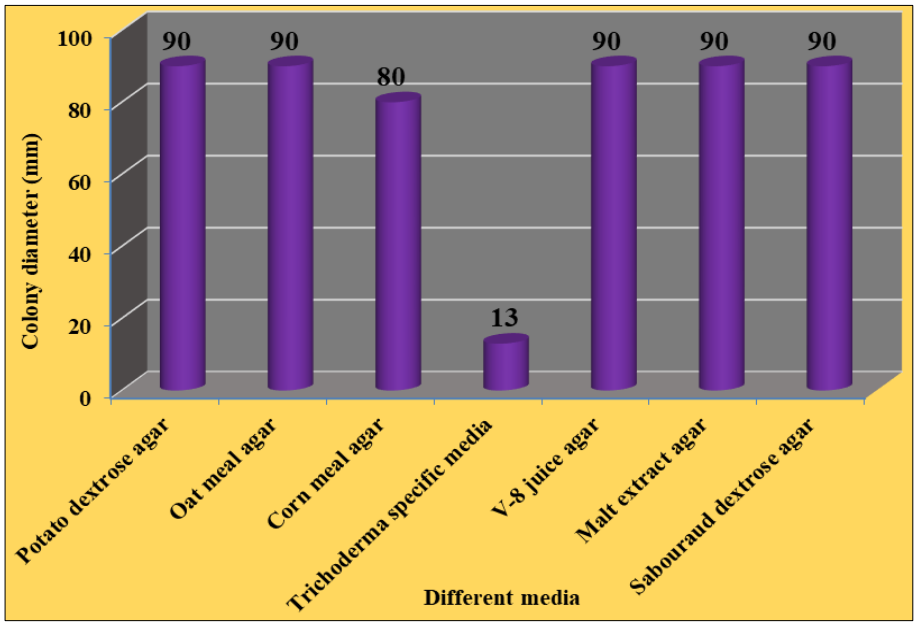

Fig 14: Mycelial growth of commercial isolate (*T. viride*) isolate on different media

Conclusion

The highest mycelia growth and early sporulation are the important characteristics of a good bio-control agent to increase the virulence against plant pathogens. In the present study, *Trichoderma* isolates from non rhizospheric source showed the highest growth rate (PSV) and early sporulation (GMV) on a more number of test media than rhizospheric and commercial isolate thus proving to be superior to them. So, apart from rhizospheric sources, we can use the non-rhizospheric sources, *viz*., goat manure, sheep manure, coir pith, sawdust, and paddy straw, for the isolation of good bio-control agents against different plant pathogens.

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References

1. Benitez T, Rincon AM, Limon MC Codon AC. Bio-control mechanisms of *Trichoderma* strains. Int. J Microbiol. 2004;**7**(4):249-260.
2. Demain AL, Fang A. The natural functions of secondary metabolites. Adv. Biochem Eng. Biotechnol. 2000;69:1-39.
3. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species opportunistic, Avirulent plant symbionts. Nat Rev Microbiol. 2004;2(1):43-56.
4. Lieckfeldt E, Samuels GJ, Nirenberg HI, Petrini O. A morphological and molecular perspective of *Trichoderma viride*: is it one or two species? Appl Environ Microbiol. 1999;65(6):2418-2428.
5. Maurya MK, Srivastava M, Singh A, Pandey S, Ratan V. Effect of different temperature and culture media on the mycelia growth of *Trichoderma viride* isolates. Int. J Curr Microbiol Appl. Sci. 2017;6(2):266-269.
6. Nago BH, Vu DN, Tran DQ. Analyze antagonist effects of *Trichoderma* spp. for controlling southern stem rot caused by *Sclerotium rolfsii* on peanut. Plant Prot. 2006;1:12-14.
7. Nakkeeran S, Krishnamoorthy AS, Ramamoorthy V, Renukadevi P. Microbial inoculants in plant disease control. J Ecobiol. 2002;14(2):83-94.
8. Savitha MJ, Sriram S. Morphological and molecular identification of *Trichoderma* isolates with biocontrol potential against *Phytophthora* blight in red pepper. Pest Manage Horti Ecsyst. 2015;21(2):194-202.
9. Schuster A, Schmoll M. Biology and biotechnology of *Trichoderma*. Appl Microbiol Biotech. 2010;87:787-799.
10. Shah S, Nasreen S, Sheikh PA. Cultural and morphological characterization of *Trichoderma* spp. associated with green mold disease of *Pleurotus* spp. in Kashmir. Res J Microbiol. 2012;**7**(2):139-144.
11. Sharma RL, Singh BP, Thakur MP. Thapak SK. Effect of media, temperature, pH and light on the growth and sporulation of *Fusarium oxysporum* f. sp. *lini*. Ann Plant Prot Sci. 2005;13:172-174.
12. Tjamos EC, Papavizas GC, Cook RJ. Biological control of plant diseases. Progress and challenges for the future. Plenum Press. 1992; New York.
13. Tran TT. Antagonistic effectiveness of *Trichoderma* against plant fungal pathogens. Plant Prot. 1998;4:35-38.
14. Vinale F, Sivasithamparam K, Ghisalberti FL, Marra R, Woo SL, Lorito M. *Trichoderma*–plant–pathogen interactions. Soil Biol Biochem. 2008;40(1):1-10.