Exploration of native *Trichoderma* spp. from different eco-systems of the Canara circle, Karnataka, India

Abstract

Canara Circle of Karnataka fall under the agro-climatic zone 9 and 10, has a very unique type of coastal, plain and Ghat eco-systems, comprising mainly forest followed by horticulture and agricultureal crops. Soil borne diseases was seriously impacting quality and quantity of crop production yielding low profitability. No doubt, pesticides were effective, but have soil and environment impairments. Application of effective native *Trichoderma* sp. is the best in all angles. As this region being the organic district with diverse ecosystems, it is a treasure for exploring efficient *Trichoderma* spp. In this context, a roving survey was carried out during 2019 to in seven (7) different taluks of Canara circle (Haliyal, Mundgod, Siddapur, Sirsi, Yellapura, Ankola and Kumta). Two rhizosphere soil samples of 0.5 kg each were collected from agriculture, horticulture, and forest ecosystems of each taluk totaling 42 rhizosphere samples. Among 42the rhizosphere soils tested by serial dilution technique on Trichoderma selective medium (TSM), highest number of *Trichoderma* isolates was from forest ecosystem (11) with maximum recovery (78.57 %). Horticulture ecosystem followed next (9 isolates) with 64.28 per cent recovery and the agriculture ecosystem recorded the lowest *Trichoderma* isolates (7) with 50 per cent recovery

Keywords: Rhizosphere soils, Eco-systems, Survey, Agriculture, Horticulture. Forest, Trichoderma selective medium

Comment [u1]: ? give universal name in parenthesis

Comment [u2]: Give the name of the rhizospheres or their depth

1. INTRODUCTION

Canara circle located in agro-climatic zones 9 and 10 of Karnataka has unique diverse cropping system (agriculture, horticulture and forest eco-system). Agriculture area is very limited with groundnut, maize, sugarcane and paddy crops. Horticulture comprises areca nut based multistoried cropping system covering areca nut, coconut, black pepper, ginger, pineapple, cocoa, nutmeg, banana, cinnamon, mango and clove. The forest eco-system covering large area is one of the biodiversity hot spots, comprise all forest types (evergreen, semi evergreen to deciduous). There is a predominance of teak, bauhinia, acacia, casuarina, rosewood, matti, nandi, swamps and malabar neem. There is a serious impact on crop and timber productivity by the persistent destructive diseases specially soil borne pathogens. Exhaustive usage of chemical pesticides causing detrimental impact on soil, environment, fungicide resistance, non–target beneficial flora and fauna and human health. Biological

control with biocontrol agents (BCA's) finds an appropriate status in integrated disease management (IDM) (Singh, 2005) due to its eco-friendly benefits with high suppressive capability of soil-borne fungal pathogens (Harman *et al*., 2004). The BCA produces antifungal metabolites, cell wall-degrading enzymes, antibiotics and toxins which plays a vital role in pathogen suppression (Howell, 2003). Chandra Sekhar *et al*., 2017 also highlighted the high competitive efficiency, myco-parasitism, induced growth promotion and other host immunity boosting nature of BCA. *Trichoderma*, highly diverse soil-borne cosmopolitan filamentous saprobe is the most efficient BCA (Phylum: Ascomycota Class: Sordariomycetes, Order: Hypocreales and Family: Hypocreaceae). Diverse species of *Trichoderma* dwell on decaying wood, compost or other organic matter and most efficient ones in rhizosphere soils (Samuels, 2006). Resident (native) *Trichoderma* spp isolates have potential antagonistic performance against plant pathogens. Hence, the investigation to explore the most efficient native *Trichoderma* spp. strains across different cropping systems distributed in different eco-system of Canara circle was undertaken.

2. MATERIAL AND METHODS

The study was carried out during 2019 at Canara circle region, of Karnataka, India.

2.1 Survey and collection of rhizosphere sample

A roving survey ~~for~~ was done in agriculture, horticulture and forest eco-systems (zones 9 and 10) in the Western Ghats part of Karnataka during August 2019. Two rhizosphere soil samples (250 g each) were collected from each of Haliyal, Mundgod, Siddapur, Sirsi, Yellapura, Ankola and Kumta, the taluks representing agriculture, horticulture, and forest eco-system (42 samples). Composite of soil samples were maintained in cotton bags (Plate 1).

Comment [u3]: Use the location name as previously mentioned in abstracts i.e canara circle

Comment [u4]: Mention the soli depth and corresponding names of the rhizosphere

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Rhizosphere soil samples collected from agricultureal eco-system were designated as soil sample for *Trichoderma* (SST). Fourteen SST (SST 1 to SST ~~2~~14) with their geo-reference from largely grown crops (groundnut, maize and sugarcane) [Table 1]. Fourteen rhizosphere soil samples (SST 15 to SST 28 isolates) with ground truth data were collected from horticultural crops (mango, chili, and black pepper) [Table 2]. Similarly, rhizosphere soil samples collected from forest ecosystem (from acacia, matti, teak, rosewood, bauhunia, and malabar neem) were designated as SST 29 to SST 42 (Table 3).

The rhizosphere soil samples were air dried at room temperature (27 ±1 ^{o}C) and sieved in 2 mm test sieve and sealed in labeled cloth bag. The bags were kept at 4 ^{o}C.

2.2 Media preparation

Potato dextrose agar (PDA) medium was use. It was prepared by standardized protocol with minor modification (Chattopadhyay, 2003). Peeled potatoes (200 g) were cut into small slices and boiled in distilled water followed by collecting the extract by filtering through muslin cloth. Dextrose (20 g) and agar agar (20 g) were dissolved in potato extract and the final volume was made up to 1000 ml with distilled water. The mixture was then autoclaved at 121 ^{o}C for 15 min.

***Trichoderma* selective media (TSM)**

TSM medium was prepared by following the set protocol with little modification (Askew and Laing, 1993). The components [0.2g $MgSO_4(7H_2O)$, 0.9g K_2HPO_4, 0.15g KCl, 1.0 g NH_4NO_3, 3.0 g D (+) glucose anhydrous, 0 15 g rose bengal and 20 g agar] were mixed with 950 ml of distilled water and autoclaved. Subsequently, 0.25 g Chloramphenicol, 0.2 g PCNB, 0.2 g Captan, and 1.6 g Metalaxyl were mixed with autoclaved distilled water (50 ml) and added to the autoclaved basal medium.

2.3 Isolation and identification of *Trichoderma* isolates

Forty-two rhizosphere soil samples collected from different eco-systems of the Canara Circle, were subjected to serial dilution technique for isolating *Trichoderma* spp. One gram of each representative SST was added separately to sterile 9 ml water blanks and labeled as 10^{-1}. The test tubes were rolled to and fro between the palms for 5 min. to assure uniform mixing. From this, 1 ml of suspension was transferred to fresh 9 ml sterile water blank and labeled as 10^{-2}. The same procedure was repeated till 10^{-5} dilution is attained (Kale *et al*., 2018). Aliquot of 0.5 ml was plated on *Trichoderma* selective media (TSM) by spread plate technique and incubated (27 ± 1^{o}C). Using hyphal tip culture technique, pure culture slants were prepared and kept at 4 ^{o}C (Chandra Sekhar *et al*., 2017). Periodical sub-culturing of each of the isolates were done on fresh PDA. Microscopic observation for presence of phialide, its shape, length, width and colony color under 40x for confirming *Trichoderma* (Tuite, 1969).

3. RESULTS AND DISCUSSION

Survey and collection of soil samples

In roving survey 42 rhizosphere soil samples were collected @ two samples per ecosystem from agriculture, horticulture, and forest ecosystems in 7 taluks of Canara circle (zones 9 and 10). This study is in agreement with earlier workers (Rangarani *et al*, 2017) who collected 27 rhizosphere soil samples from different cropping systems (groundnut, redgram, and tomato fields) in Chittoor district (Andhra Pradesh) for *Trichoderma* spp. isolation.

Rashmi *et al.* (2017) followed a similar procedure in collecting rhizosphere soil samples from groundnut crop at 5–6 cm depth from Manipur.

Isolation and identification of *Trichoderma* isolates.

Isolation of *Trichoderma* spp. from 42 rhizosphere soil samples of three different ecosystems was done on *Trichoderma* specific medium (TSM) by following serial dilution plate technique. A similar kind of procedure was also followed by other workers to explore *Trichoderma* spp. (Elad and Chet, 1983; Dhingra and Sinclair, 1995; Shukla *et al.,* 2016 and Chandra Sekhar *et al.,* 2017). The rhizosphere soils from the vanilla crop collected from vanilla growing areas of Kottayam were explored for *Trichoderma* spp. (Johnson and Curl 1972). Such studies in Martin's Rose Bengal Streptomycin agar medium and malt extract agar medium were done and succeeded (Sandheep and Jisha, 2014; Cyriac *et al.,* 2021; Hinterdobler *et al.,* 2021 and Alwadai *et al.,* 2022).

From 42 rhizosphere soil samples 27 isolates of *Trichoderma* were identified based on microscopic morphological traits (phialide shape, length, width, and colony color). The study is in accordance to Gams and Bissett (2002). *Trichoderma* isolates were identified using the keys proposed by taxonomists (Rifai, 1969; Barnett and Hunter, 1972 and Gomez-Mendez *et al.,* 2020). A similar type of study was done in South Africa by Plessis *et al.* (2018), who explored 14 *Trichoderma* sp. (*T. afroharzianum, T. asperelloides, T. asperellum, T. atrobrunneum, T. atroviride, T. camerunense, T. gamsii, T. hamatum, T. koningii, T. koningiopsis, T. saturnisporum, T. spirale, T. virens,* and *T. viride*). Hewavitharana *et al.* (2018) have identified 12 *Trichoderma* spp. to genus level on a morphology basis. The highest number of *Trichoderma* spp. isolated were from forest eco-system (11 No.) with 78.57 per cent followed by horticulture eco-system (9 No.) with 64.28 per cent recovery and the least from agriculture eco-system (7 No.) with 50.00 per cent recovery respectively [Table 1, 2, and 3; Plate 2, 3 and 4]. The findings are in line with Liu *et al.* (2020), who isolated 181 *Trichoderma* strains (T1-T181) from 16 samples collected from the *Syringa oblata* rhizosphere in 8 districts of Harbin City, China. Likewise, ninety *Trichoderma* isolates were recorded in soil samples collected from different locations of Taif city (Gherbawy *et al.,* 2014). Maximum numbers of *Trichoderma* spp. recovered from the forest ecosystem can be attributed to undisturbed forest soil environment and enriched organic sources for the saprophytic survival of *Trichoderma* sp. (Zhou *et al.,* 2020).

4. CONCLUSION

The Canara circle, which is located in Karnataka's agro-climatic zones 9 and 10, is an incredible source for exploring *Trichoderma*. Totally 27 *Trichoderma* isolates were recovered

from 42 rhizosphere soil samples of three different ecosystems of the Canara circle. Highest number of *Trichoderma* isolates was recovered from forest eco-system (11No.) followed by the horticulture ecosystem (9 No.) and agriculture ecosystem (7 No.). These native *Trichoderma* isolates can be exploited to combat different plant diseases using an appropriate bio-formulation.

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Table 1. Status of *Trichoderma* in the rhizosphere soil samples of forest eco-system

Sl. No.	Taluk	Name of locality	Agro climatic Zone.	Geo-reference details		Crop	Soil sample code	*Trichoderma* occurrence
				Latitude (N°)	Longitude (E°)			
1	Haliyal	Nirlagi	9	15° 18' 20.63''	74° 43' 07.50''	Bauhinia	SST-29	+
2		Banadur	9	15° 21' 02.70''	74° 53' 39.70''	Teak	SST-30	-
3	Mundgod	Wadagatta	9	15° 02' 30.01''	75° 04' 15.43''	Rosewood	SST-31	+
4		Borangudde	9	14° 50' 13.92''	75° 02' 12.84''	Teak	SST-32	+
5	Siddapur	Aigod	9	14° 23' 29.90''	74° 56' 29.97''	Matti	SST-33	+
6		Tumbargod	9	14° 25' 39.36''	74° 52' 49.8''	Malbar neem	SST-34	+
7	Sirsi	Gudnapura	9	14° 32' 38.04''	74° 58' 40.44''	Malbar neem	SST-35	+
8		Sahasralingha	9	14° 43' 07.32''	74° 48' 39.96''	Matti	SST-36	+
9	Yellapura	Tarehalli	9	14° 28' 30.72''	74° 29' 32.28''	Malbar neem	SST-37	+
10		Shistmudgi	9	14° 35' 04.2''	74° 24' 52.56''	Teak	SST-38	-
11	Ankola	Bogadde	10	14° 35' 35.87''	74° 23' 48.00''	Matti	SST-39	+
12		Hebbul	10	14° 40' 57.99''	74° 29' 17.42''	Malbar neem	SST-40	+
13	Kumta	Anthravalli	10	14° 28' 51.19''	74° 28' 37.74''	Matti	SST-41	+
14		Bidrageri (Gokarna)	10	14° 26' 17.36''	74° 25' 33.81''	Acacia	SST-42	-
Total No. (Per cent)								11 (78.57 %)

+ = Present, - = Absent

Table 2. Status of *Trichoderma* in the rhizosphere soil samples of horticulture eco-system

Sl. No.	Taluk	Name of locality	Agro climatic Zone.	Geo-reference details		Crop	Soil sample Code	*Trichoderma* occurrence
				Latitude (N°)	Longitude (E°)			
1	Haliyal	Mavina Koppa	9	15° 21' 03.32''	74° 49' 48.43''	Mango	SST-15	-
2		Kesarolli	9	15° 18' 17.67''	74° 43' 52.67''	Mango	SST-16	+
3	Mundgod	Wadagatta	9	15° 02' 39.58''	75° 04' 01.89''	Chilli	SST-17	-
4		Naginkeri	9	14° 44' 36.24''	74° 59' 27.06''	Black pepper	SST-18	-
5	Siddapur	Mankod	9	14° 22' 21.00''	74° 55' 18.12''	Black pepper	SST-19	+
6		Nidagad	9	14° 24' 55.08''	74° 53' 8.88''	Black pepper	SST-20	+
7	Sirsi	Ajjarane	9	14° 32' 52.98''	74° 59' 26.16''	Black pepper	SST-21	+
8		Hulagol	9	14° 42' 48.96''	74° 48' 59.4''	Black pepper	SST-22	-
9	Yellapura	Chavathi	9	14° 46' 11.28''	74° 48' 46.8''	Black pepper	SST-23	+
10		Tallikere	9	14° 34' 57.72''	74° 24' 51.12''	Black pepper	SST-24	+
11	Ankola	Mogta	10	14° 35' 41.40''	74° 23' 51.65''	Black pepper	SST-25	+
12		Gundabala	10	14° 40' 58.49''	74°29' 17.97''	Black pepper	SST-26	+
13	Kumta	Anthravalli	10	14° 28' 06.00''	74° 27' 31.79''	Black pepper	SST-27	-
14		Hiregutti	10	14° 33' 52.24''	74° 23' 14.78''	Black pepper	SST-28	+
Total No. (Per cent)								09 (64.28 %)

+ = Present, - = Absent

Table 3. Status of *Trichoderma* in the rhizosphere soil samples of agriculture eco-system

Sl. No.	Taluk	Name of locality	Agro climatic Zone	Geo-reference details		Crop	Soil sample Code	*Trichoderma* occurrence
				Latitude (N°)	Longitude (E°)			
1	Haliyal	Kyarawada	9	15° 20' 48.79''	74° 48' 04.50''	Sugarcane	SST-1	+
2		Kesarolli	9	15° 18' 09.47''	74° 44 22.00''	Sugarcane	SST-2	+
3	Mundgod	Wadagatta	9	15° 02' 40.21''	75° 04'01.25''	Maize	SST-3	+
4		Haraganalli	9	14° 43' 43.68''	75° 00' 55.80''	Maize	SST-4	+
5	Siddapur	Aigod	9	14° 23' 29.76''	74° 56' 29.97''	Groundnut	SST-5	+
6		Nidagad	9	14° 24' 50.76''	74° 53' 07.80''	Maize	SST-6	+
7	Sirsi	Andagi	9	14° 37' 00.12''	75° 00' 50.76''	Maize	SST-7	-
8		Sonda	9	14° 44' 28.68''	74° 48' 14.76''	Sugarcane	SST-8	-
9	Yellapura	Manchikeri	9	14° 51' 29.01''	74° 49' 04.47''	Maize	SST-9	+
10		Tallikere	9	14° 35' 00.24''	74° 24' 51.84''	Sugarcane	SST-10	-
11	Ankola	Moralli	10	14° 36' 34.50''	74° 24' 52.08''	Sugarcane	SST-11	-
12		Hillur	10	14° 37' 30.97''	74° 25' 27.89''	Groundnut	SST-12	-
13	Kumta	Anthravalli	10	14° 28' 26.13''	74° 27' 44.02''	Sugarcane	SST-13	-
14		Kumta	10	14° 25' 54.09''	74° 25' 20.57''	Groundnut	SST-14	-
Total No. (Per cent)								07 (50.00 %)

+ = Present, - = Absent

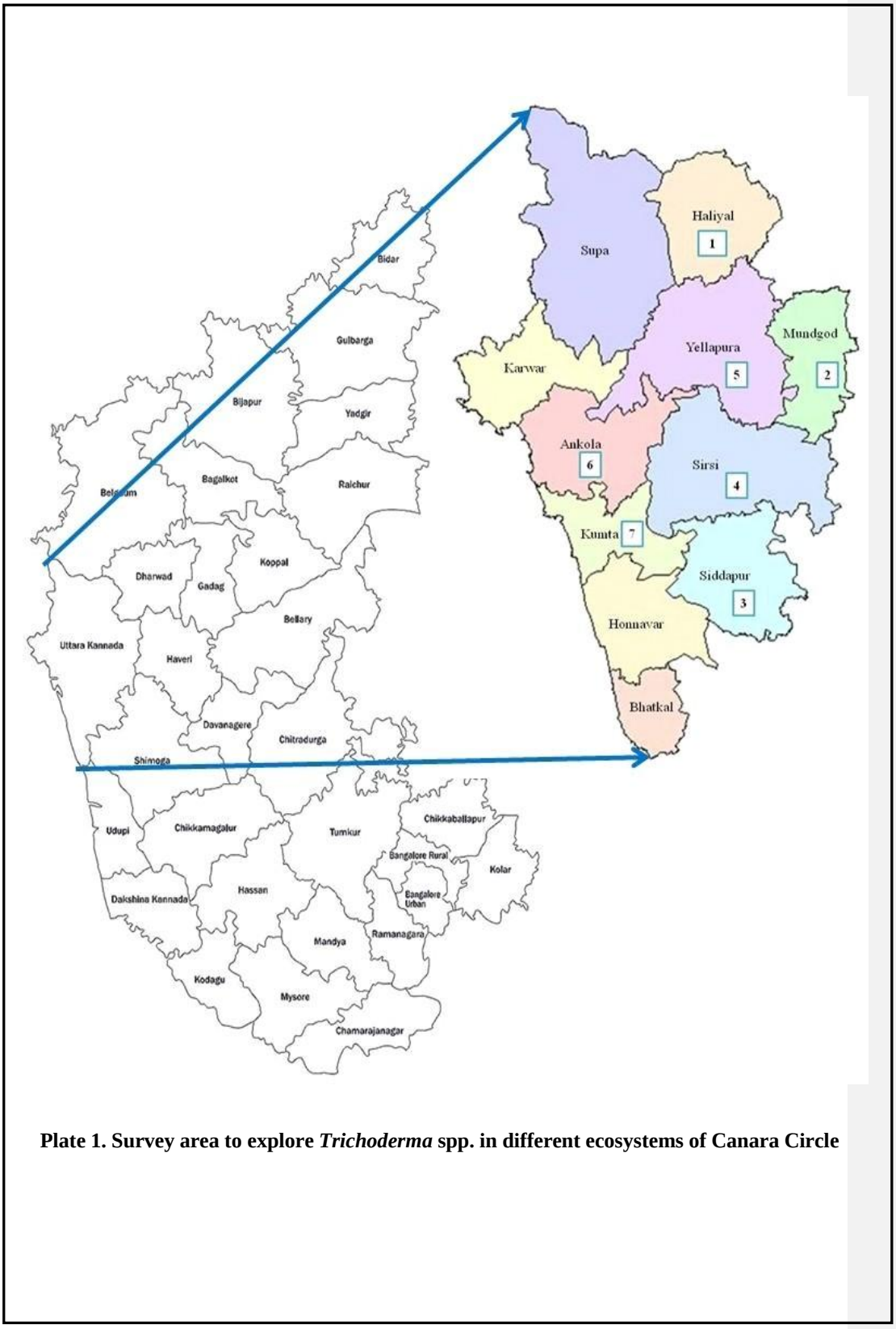

Plate 1. Survey area to explore *Trichoderma* spp. in different ecosystems of Canara Circle

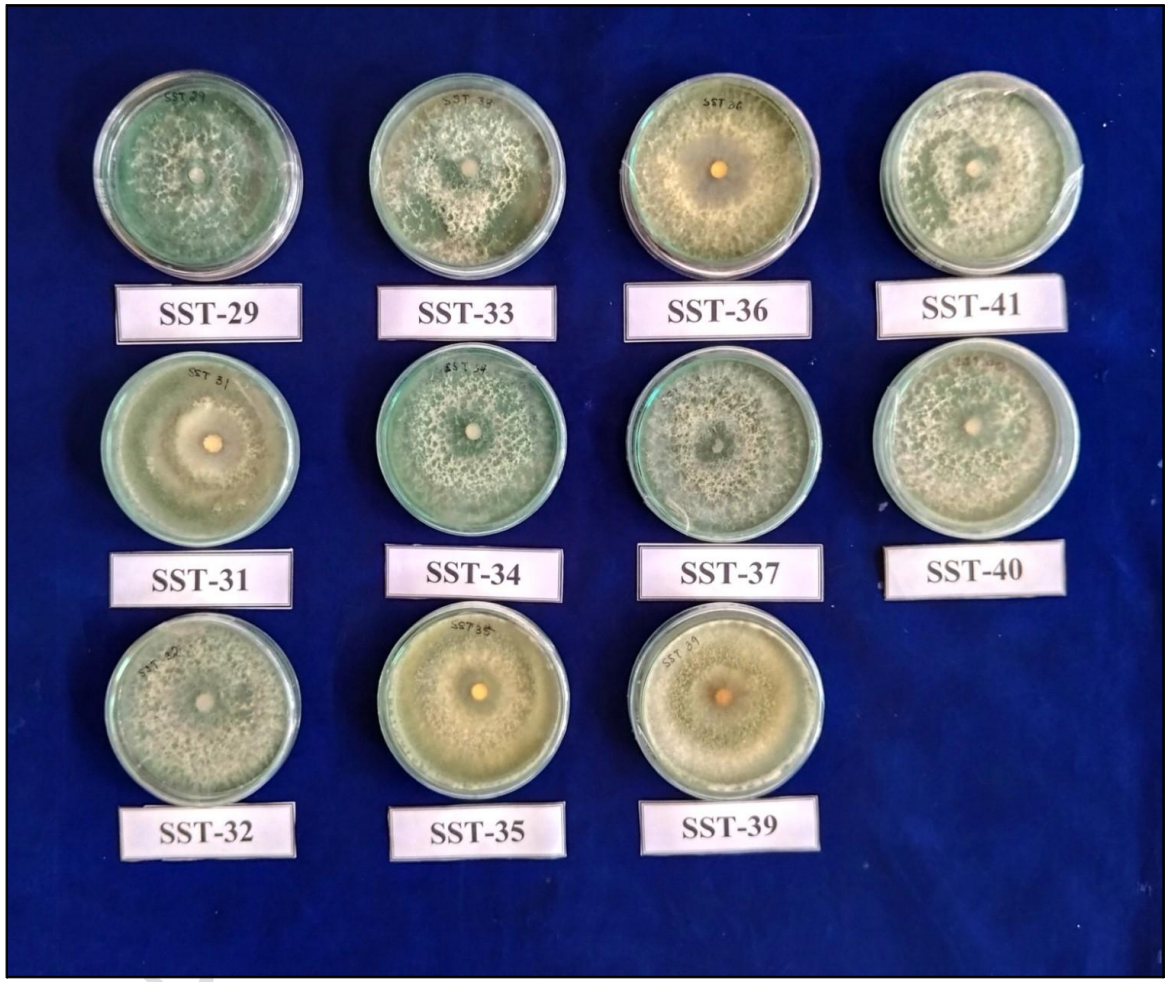

Plate 2. ***Trichoderma*** **isolates explored from forest ecosystem on potato dextrose agar medium**

Plate 3. ***Trichoderma*** **isolates explored from horticulture ecosystem on potato dextrose agar medium**

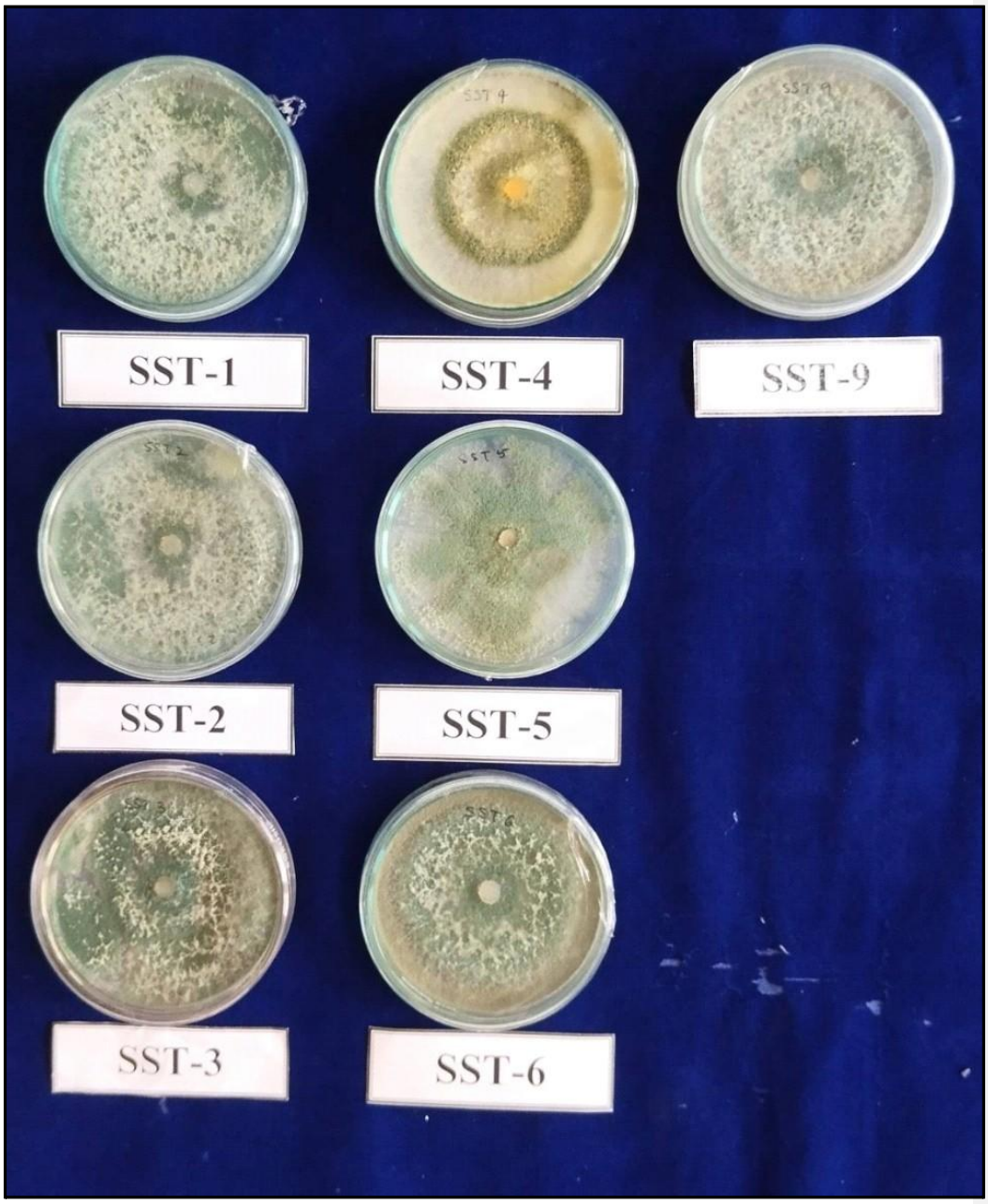

Plate 4. ***Trichoderma*** **isolates explored from agriculture ecosystem on potato dextrose agar medium**