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ANTIFUNGAL POTENTIAL OF ENDOPHYTIC *TRICHODERMA* SPP. TO ANTAGONIZE ROOT-ROTTING OF CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

The application of pesticides and organic chemicals is frequently employed to control plant diseases. However, such chemicals are difficult to degrade, their quantity build up in food chains causes increased toxicity in animals. Several strains of *Trichoderma* had a significant effect on plant diseases caused by pathogens such as *Fusarium solani, Rhizoctonia solani, F. solani* and *Macrophomina phaseolina*. The current study focused the antagonistic potential of *Trichoderma* fungi in vitro and in vivo by analysing the effect of inoculation on chickpea (*Cicer arietinum* L.) growth. In a dual culture plate assay, the *Trichoderma* strains showed significant activity against root rooting fungi, the efficient strains were taken for in-vivo studies in the pot experiment were CvR-Tr1, TaR-Tr3, AvR-Tr5, Rtl-Tr6, and AvR-Tr5+ Rtl-Tr6 demonstrated considerable biocontrol activity against *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum*, and *Fusarium solani*. These strains also had a significant impact on plant growth, increasing the weight & length of shoot and root of chickpea plants. When chickpea plants were inoculated with various strains of *Trichoderma*, significant variations in the amount of chlorophyll and carotenoid were seen when compared to control. CvR-Tr1 (0.157 mg/g), (AvR-Tr5 0.1111 mg/g) and (AvR-Tr5+RtL-Tr6 0.0900 mg/g) had the most chlorophyll. AvR-Tr5 (0.0483 mg/g), AvRTr5 + RtL-Tr6 (0.041 mg/g), and RtL - Tr6 (0.0381 mg/g) had the most carotenoid. AvR - Tr5 (5.966 µg/mL), RtL - Tr6 (5.534 µg/mL), and CvR - Tr1 (1.436 µg/mL) had the highest carbohydrate content. Endophytes inhabit an ecological niche similar to phytopathogens therefore endophyte bio-control is an efficient pest management method.

Key-words: Antagonist, *Trichoderma spp.*, Pathogenic fungi, Pestisides, biological control agent.

INTRODUCTION

Chickpea contains beneficial phytochemicals; a good source of energy, protein, fibre, vitamins, and minerals (Wood and Grusak, 2007). Among pulses, chickpea is generally preferred as legumes (Siddique *et al*., 2000). Moreover, chickpea is also considered as beneficial for health like alleviating cancer, diabetic and cardiovascular risks. Chickpea belong to the family Fabaceae. It is known from literature that in all developmental stages, chickpea can be attacked by various pathogens. During germination period and in the next development stages chickpea can be attacked by *Fusarium spp*. (Dwivedi, 1989). During vegetation period *Fusarium oxysporum* caused the most pernicious disease i.e Parasitic wilt of chickpea (Kunwar *et al*., 1989). Scale of seedlings and root rot disease of chickpea caused by *F. moniliforme* and *F. solani* (Bhatti and Kraft, 1992). Another fungus *Alternaria alternata* can also attack all aboveground parts of the plant (Raut and Somani, 1988).

Intra- or intercellular spaces of plant tissues do not show any disease symptoms due to endophytic fungi colonization. (Abedinzadeh *et al*., 2019). Endophytic fungi promote plant growth by Phyto-stimulation +, bio-control, and bio-fertilizations (Hassan, 2017; Eid *et al*., 2019) for example; Plant growth directly gets promoted by producing phytohormones like gibberellic acid and indole-3-acetc acid (IAA) by (Hashim *et al*., 2020). Moreover, endophytic fungi have greater capability of plant protection from various pathogens and, therefore, crop loss decreases because of different antibiotic compounds secretion (Murali *et al*., 2017). The endophytic distribution and colonization and inside the plants their secondary might be peril for these effects. The elaborated facts divulge potential of endophytes as biological control agent and play pivotal application in an ecological agriculture.

Trichoderma spp. is cosmopolitan fungi. They are not only involve in controlling the growth of pathogenic microorganism, but their other function include stimulating rhizospheres colonization, stimulates plant growth, root growth, and enhance plant defence responses. *Trichoderma* was first developed as a bio-control agent in the early

1930s (Weindling, 1934). *Trichoderma* products are now available as bio-pesticides, soil amendments, and plant growth enhancers (Vinale *et al*., 2008). The use of *Trichoderma* based products are worldwide and their application use in controlling of fungal soil-borne pathogens like *Rhizoctonia* and *Pythium* in nurseries, horticulture and fields. (Woo *et al*., 2014). This methodology is environment friendly and safe in abating precarious chemical pesticides effects.

The purpose behind this research was an evaluation of the antagonistic potential of *Trichoderma* species against root rotting fungi (*M. phaseolina, R. solani, F. solani and F. oxysporum*) and these species were isolated from various wild plants, to study of the biochemical properties of *Trichoderma* spp. and to examine the impacts of *Trichoderma* isolates on chickpea's (*Cicer arietinum*) growth and development in pot experiment.

MATERIALS AND METHODS

Collection of sample for isolation of endophytic *Trichoderma* spp.

Different samples of wild plants such as *cleome viscose, Trichodesma amplexicaule, Abutilon indicum, Amaranthus viridis, Ruellia tuberosa, Sonchus oleracus, and Vernonia cinerea* were collected from the University of Karachi for the isolation of *Trichoderma*. The plant material brought to the laboratory and stored at low temperature until it was used for isolation.

IN VITRO

Isolation and identification of endophytic *Trichoderma spp*.

One g of plant material (root, leaves and stem) was rinsed with running tap water then surface sterilized with 1% calcium hypochlorite (bleach) for 3 minutes then for two to three minutes it was followed by 70% alcohol and lastly for about one minute washed with distilled water. Then, they were blend with fifty ml of water after cutting into small pieces cut so as to give the dilution of 1:50. Each sample was diluted up to 1:10 and transferred 0.1 mL suspension onto a Petri dish containing Potato Dextrose Agar with penicillin (100, 000 units / L) and streptomycin (0.2g/L) supplementation. The plates were incubated at 28°C for 5 days and fungi were identified. Barnett & Hunter (1998), Domsch *et al.,* (1980), Dugan (2006), Ellis (1971), Gilman (1957).

Isolation of root-rotting fungi from soil

Soil Dilution Technique for the Isolation of *Fusarium spp.*

A series of dilutions was prepared by using 1g of soil sample dissolved in 9 mL of 0.1 percent agar suspension. From the final dilution 0.1% agar suspension of 1 mL aliquot was poured on Petri dishes containing Pentachloronitrobenzene (PCNB) (Nash and Snyder, 1962) and the suspension was spread on the surface of agar by rotating the petridish. For 5 days plates were incubated at 28°C and after Booth and Nelson *et al.,* (1983) *Fusarium* species were identified.

Baiting Technique for the isolation of *Rhizoctonia solani*

As bait, sterilized sorghum seeds were spread on a damp soil surface. The bait was removed after 24 hours, rinsed with tap water, and transferred to a PDA. For growth and identification of *R. solani* pH was maintained at 5.5 (Wilhelm, 1955). The percentage of seeds colonized was used to estimate the population of *R. solani* in the soil.

Wet Sieving and dilution technique for the isolation of *Macrophomina phaseolina*

This method of isolating *Macrophomina phaseolina* by wet sieving was suggested by (Sheikh and Ghaffar, 1975). 20g soil sample was sieved through a 100 mesh (150- µm) screen before being placed on a 300 mesh (53- µm) screen. The residue obtained from the 53- µm screen was rinsed under running tap water for 1 minute and then transferred into a beaker containing 0.5 % Ca $(OCl)_2$ and diluted up to 100 mL to make a 1:5 dilutions. The sclerotial suspension was put on a magnetic stirrer and 1 ml aliquot was evenly spread onto the surface of plates containing PDA supplemented with penicillin (100, 000 units/L) and streptomycin 1g/L). For five days the plates were incubated at 28 °C and *Macrophomina phaseolina* was identified by the appearance of brown to black colonies.

Screening by dual culture method.

The antagonistic potential of *Trichoderma* species was identified via *In Vitro* screening by an inoculating an antagonistic 5 mm disc of agar, with the distance of 2mm from the periphery of the petri plate *Trichoderma* species were placed as an antagonist and similary it was followed by root rotting soil-borne fungi (*F. solani, F.oxysporum, R. soani and M. phaseolina*) by placing 2mm away from the petri plate's edge on the opposite end of *Trichoderma* sample. Each root rotting fungi disc as a control was similarly separately placed as on the fresh plate of PDA. All

these pairings were incubated at 28 °C and carried out in triplet. After 4 days of incubation an antagonistic activity was estimated by measurement of zone of inhibition in mm.

IN VIVO

Screen house experiment

Trichoderma culture filtrate (25 mL) is mixed with sandy loam soil. Soil was always full of saprophytic microorganisms. Transfer 1 kg of amended soil into the clay pot. Eight seeds were planted in each pot, and each pot was saturated with 25 mL of topsin (200 ppm) suspension. Only four seeds were saved one week after germination and those reported were removed. Pots without *Trichoderma* culture had poor control, while antibiotics (Topsin) had positive results. Pots were placed on a bench scale at 50% WHC (water retention) and each process was repeated four times (Keen and Raczkowski, 1921).

Growth parameters

To determine the effectiveness of *Trichoderma* and the fungicide on plant growth, plants were uprooted after six weeks of seed sowing. Shoot length (mm), shoot weight (g), root length (mm) and root weight (g) were observed.

Infection percentage

To determine the occurrence of fungal infection, 1 cm long pieces of tap root roots (four pieces from each plant) were surface disinfected with 1% Ca(OCl)2 solution and 70% alcohol, followed by distilled water, and then placed on potato dextrose agar. with penicillin (100,000 units/L) and streptomycin (0.2 g/L). After incubation for 5 days at 28°C, colonies of *Macrophomina phaseolina, Rhizoctonia solani and Fusarium* species were recorded.

Bio-chemical parameters

Chlorophyll and carotenoid

Chlorophyll and carotenoid were extracted using 80% aqueous acetone and estimated by method of Lichtenthaler and Wellburn (1983). One g of fresh plant sample was accurately weighted using a weight balance and homogenized thoroughly in a pestle and mortar with 5ml of 80% acetone. Homogenized sample was taken in test tube, again added 5ml of 80% acetone, shake well and centrifuged at 4500rpm for 10 minutes. The sample was centrifuged repeatedly until the supernatant became colourless. In the test tubes supernatant was transferred and volume make up was up to 10 ml with acetone 80%). For estimation about 3ml of supernatant were transferred into Cuvette and for the analysis of Chlorophyll a, Chlorophyll b and Carotenoid, took optical density of supernatant at 663, 645, 500 and 480nm with 80% reagent blank in Spectrophotometer. The amount of carotenoid and chlorophyll in the leaf sample was estimated using the formula mention below and expressed in milligram per gram fresh weight of leaf.

$$\text{Carotenoid (mg/g)} == \frac{7.6\,(A480) - 1.49\,(A510) \times V}{1000 \times W}$$

$$\text{Chlorophyll a (mg/g)} == \frac{12.7\,(A663) - 2.69\,(A645) \times V}{1000 \times W}$$

$$\text{Chlorophyll b (mg/g)} = = \frac{22.9\,(A645) - 4.68\,(A663) \times V}{1000 \times W}$$

Whereas; V= volume of total extract; W = fresh leaf sample weight

Carbohydrate

Anthrone reagent was used to estimate carbohydrate from treated and control plant samples using the Willis and Yemm method (1954). Anthrone reagent was made by dissolving 0.1g of anthrone in 500ml of 95% sulphuric acid, which was carefully weighted using a weight balance. To avoid oxidation, the anthrone reagent was kept in dark.

Using a weight scale, weigh 0.5g of treated and control fresh leaf samples, then homogenized them with 5ml of distilled water using a pestle and mortar. The homogenized material was placed in a centrifuge tube and centrifuged for 5 minutes at 500rpm. Centrifugation was repeated until the supernatant was clear. Into the new test tube the supernatant was transferred and by adding 5ml distilled water, the volume make up was 10 ml. In a separate test tube, 5ml of anthrone reagent was added into 1ml of extract for estimation. After covering the test tube with cotton, they were boiled in a water bath for around 30 min. Then in an ice cold water bath it got chilled. By using spectrophotometer an optical density was measured at 620 nm with a 3 mL anthrone reagent blank and 3 mL extract in a Cuvette.

The amount of carbohydrate was calculated in µg/mg of extract by using calibration curve and by putting values in below given formula.

$$\text{Carbohydrate (} \quad \text{g/mg)} = \frac{\text{volume of extract} \times \text{D.F} \times \text{value from calibration curve}}{\text{weight of sample}}$$

Whereas, volume = 10, D.F = 0, Weight = 500mg

ANALYSIS OF DATA

Analysis of data was determined by using ANOVA (variance, means and standard deviation), (Gomez and Gomez, 1984).

RESULTS

IN VITRO

Isolated strains of endophytic *Trichoderma*

An isolation of 8 strains of *Trichoderma spp.* was from the leaves and roots of various wild plants which were collected from the university of Karachi (Table 1; Fig 1a, 1b). Tr represents the *Trichoderma* isolate, while CvR is the short form used for *Cleome viscosa*-root, CvL is the short form used for *Cleome viscosa*-leaf, TaR is the short form used for *Trichodesma amplexicaule*-root, AiR is the short form used for *Abutilon indicum*-root, AvR is the short form used for *Amaranthus viridis*-root, RtL is the short form used for *Ruellia tuberosa*-leaf SoL is the short form used for *Sonchus oleracus*-leaf, and VcR is the short form used for *Vernonia cinerea*root.

Identification of different species of *Trichoderma*

Among the 8 isolates of Trichoderma, species level identification was determined on the basis of colony color, chlamydospore formation, characteristics of conidiophore and phialides and shape of conidia. *Trichoderma hamatum* is recognized macroscopically by the formation of greyish green pruinose postules from a cover of mostly curled sterile end of condiophore. Most specifically, branches and phialides are broad and the conidia are smooth walled, green branches and phialides are particularly broad and the conidia short cylindrical, green, smooth walled. *Trichoderma harzianum* showed subglobose to short oval conidia. Verticilate and branched conidiophore. Phialides were ampulliform and convergent. Formation of chlamydospore infrequent and producing in both terminal and intercalary. *Trichoderma koningii* showed branched conidiophore. Conidia smooth walled short cylindrical with truncate base. Phialides mostly arise singly and laterally and the whole conidiophore system is elongate. The condiophore of *Trichoderma viridi* was typically pyramidally branched i.e. short brances found close to the tip and long ones with branching repetition in the lower part. In the divergent groups of 2-4, phialides are arranged, an irregular and slender bent. Conidia almost globose and distinctly roughened.

Test against root rotting fungi dual plate culture methods

In a dual plate culture method, the impact of eight *Trichoderma* strains against 4 root rotting fungi (*Macrophomina phaselina, Fusarium oxysporum, Fusarium solani and Rhizoctonia solani*) was investigated (Table 2). The highest suppression in *Macrophomina phaseolina* was achieved by making zones of inhibition for AvR-Tr5 (11.6mm), RtL-Tr6 (11.3mm), TaR-Tr3 (11.0mm), and CvR-Tr1 (8.6mm). The most effective inhibitors of Fusarium oxysporum were AvR-Tr5 (9.3mm), TaR-Tr3 (7.6mm), CvR-Tr1 (6.6mm), and RtL-Tr6 (5.0mm). CvR-Tr1 (10.0mm) and TaR-Tr3 (9.0mm) had the best results against *Fusarium solani*. In case of *Rhizoctonia solani* lysis was seen in CvR-Tr1, CvL-Tr2, and VcR-Tr8.

IN VIVO

Endophytic *Trichoderma* effect as soil drench on plant growth parameters and in controlling root infecting fungi on chickpea in screen house

This experiment was performed to observe the growth of chickpea i.e. weight of shoot and roor, length of shoot and root and root rotting fungi infection percentage was determined and amount of chlorophyll, carotenoid and carbohydrates after 45 days of *Trichoderma* inoculation. Maximum shoot length was observed in TaR-Tr3 (55.31 cm), CvR-Tr1 (54.91 cm), AvR-Tr5 (47.27 cm) and RtL-Tr6 (44.96 cm). Maximum root length was shown by TaR-Tr3 (26.88 cm), AvR-Tr5 (26.23 cm), AvR-Tr5+RtL-Tr6 (25.69 cm) and CvR-Tr1 (24.22 cm). Maximum shoot weight was observed in AvR-Tr5 (5.04 g), CvR-TR1 (4.45 g), RtL-Tr6 (4.43 g) and TaR-Tr3 (4.42 g).Whereas, maximum root weight was shown by RtL-Tr6 (1.57 g), AvR-Tr5+RtL-Tr6 (1.53 g), CvR-Tr1 (1.51 g), TaR-Tr3 (1.49 g), AvR-Tr5 (1.46 g) (Table 3). The following strains reduced root rotting fungus (Table 4). AvR-Tr5 (4.15%), AvR-Tr5+RtLTr6 (4.16%) and TaRTr3 (10.4%) had the least amount of *Fusarium solani* infection. Negative control

and CvR-TR1 had the lowest percentage of *Fusarium oxysporum* (2.082%). In the positive control and RtL-Tr6, *Macrophomina phaseolina* infection was minimal (2.082%). There was no evidence of *Rhizoctonia solani* infection.

Table 1. *Trichoderma* species isolated from wild plants, location, and locality.

S.no	Strain	Name of Plant	Part of Isolation	Name of Species	Locality
1	CvR-Tr1	*Cleome viscosa*	Root	*Trichoderma viridi*	Department of Zoology
2	CvL-Tr2	*Cleome viscosa*	Leaf	*Trichoderma harzianum*	Department of Zoology
3	TaR-Tr3	*Trichodesma amplexicaule*	Root	*Trichoderma viridi*	Department of Chemistry
4	AiR-Tr4	*Abutilon indicum*	Root	*Trichoderma hamatum*	Department of Physiology
5	AvR-Tr5	*Amaranthus viridis*	Root	*Trichoderma hamatum*	Department of Botany
6	RtL-Tr6	*Ruellia tuberosa*	Leaf	*Trichoderma koningii*	Department of Islamic learning
7	SoL-Tr7	*Sonchus oleracus*	Leaf	*Trichoderma harzianum*	Department of Islamic learning
8	VcR-Tr8	*Vernonia cinerea*	Root	*Trichoderma viridi*	Department of Islamic learning

Tr represents the *Trichoderma* isolate, while CvR is the short form used for *Cleome viscosa*-root, CvL is the short form used for *Cleome viscosa*-leaf, TaR is the short form used for *Trichodesma amplexicaule*-root, AiR is the short form used for *Abutilon indicum*-root, AvR is the short form used for *Amaranthus viridis*-root, RtL is the short form used for *Ruellia tuberosa*-leaf SoL is the short form used for *Sonchus oleracus*-leaf, and VcR is the short form used for *Vernonia cinerea*-root.

Table 2. *In -vitro* inhibition of *Fusarium oxysporum, Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani* by strains of T*richoderma* spp.

S.no	Fungal strains	*F. oxysporum* (mm)	*F. solani* (mm)	*M. phaseolina* (mm)	*R. solani* (mm)
1	CvR-Tr1	6.6	10.0	8.6	**
2	CvL-Tr2	**	**	1.6	**
3	TaR-Tr3	7.6	9.0	11.0	*
4	AiR-Tr4	**	4.3	4.6	*
5	AvR-Tr5	9.3	**	11.6	*
6	RtL-Tr6	5.0	3.6	11.3	*
7	SoL-Tr7	*	*	3.0	*
8	VcR-Tr8	**	*	1.6	**

KEY: *- no zone of inhibition; **- *Trichoderma* spp. grew over the test fungus.

Table 3. Effect of endophytic *Trichoderma* spp. and Topsin-M on the growth of chickpea.

S.no	Treatments	Root length (cm)	Root weight (g)	Shoot length (cm)	Shoot weight (g)
1	Control	16.06 ±0.41	0.56 ±0.22	35.30 ±0.66	3.08 ±0.52
2	Topsin-M	23.92 ±0.05	0.56 ±0.07	43.31 ±0.85	3.60 ±0.04
3	CvR-Tr1	24.22 ±0.47	1.51 ±0.10	54.91 ±0.15	4.45 ±0.37
4	TaR-Tr3	26.88 ±0.52	1.49 ±0.22	55.31 ±0.83	4.42 ±0.81
5	AvR-Tr5	26.23 ±0.09	1.46 ±0.16	47.27 ±0.97	5.04 ±0.47
6	RtL-Tr6	22.83 ±0.58	1.57 ±0.04	44.96 ±0.20	4.43 ±0.03
7	AvR-Tr5 + RtL-Tr6	25.69 ±0.41	1.53 ±0.14	45.25 ±0.51	4.03 ±0.57

The values show the mean ± standard deviation.

Table 4. Effect of endophytic *Trichoderma* and topsin as soil drench on the infection of *Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani* of chickpea roots.

S.no	Treatments	*F. solani* (%)	*F. oxysporum* (%)	*M. phaseolina* (%)	*R. solani* (%)
1	Control	27.05 ±1.04	12.08 ±0.16	4.15 ±0.32	-
2	Topsin-M	19.15 ±0.45	-	2.08 ±0.16	-
3	CvR-Tr1	18.73 ±0.78	2.08 ±0.16	-	-
4	TaR-Tr3	10.48 ±0.34	-	-	-
5	AvR-Tr5	4.15 ±0.31	-	-	-
6	RtL-Tr6	18.71 ±0.48	4.16 ±0.80	2.08 ±0.19	-
7	AvR-Tr5+ RtL-Tr6	4.16 ±0.80	4.15 ±0.31	-	-

The values show the mean ± standard deviation.

Table 5. Amount of carotenoid, chlorophyll and carbohydrate in plant sample.

Treatments	Carotenoids (mg/g)	Chlorophyll- a (mg/g)	Chlorophyll- b (mg/g)	Total chlorophyll (mg/g)	Carbohydrate (µg/mL)
Control	0.00326 ±0.00015	0.0134 ±0.0017	0.038 ±0.00532	0.0514 ±0.0055	2.433 ±0.0577
Topsin-M	0.0041 ±0.0009	0.0382 ±0.007	0.0395 ±0.0175	0.0778 ±0.0164	3.5333 ±0.115
CvR-Tr1	0.0059 ±0.00284	0.0693 ±0.0117	0.0877 ±0.0106	0.157 ±0.0211	5.233 ±0.0577
TaR-Tr3	0.0128 ±0.0024	0.013 ±0.0007	0.0378 ±0.0063	0.0508 ±0.0056	3.436 ±0.2113
AvR-Tr5	0.0483 ±0.0162	0.0383 ±0.0054	0.0727 ±0.0096	0.1111 ±0.0066	5.966 ±0.1527
RtL-Tr6	0.0381±0.0107	0.0277 ±0.0087	0.0484±0.01734	0.0761 ±0.0261	5.534 ±0.1154
AvR-Tr5+ RtL-Tr6	0.049 ±0.0093	0.053 ±0.0067	0.0369 ±0.0082	0.0900±0.0147	4.5667 ±0.057

The values show the mean ± standard deviation.

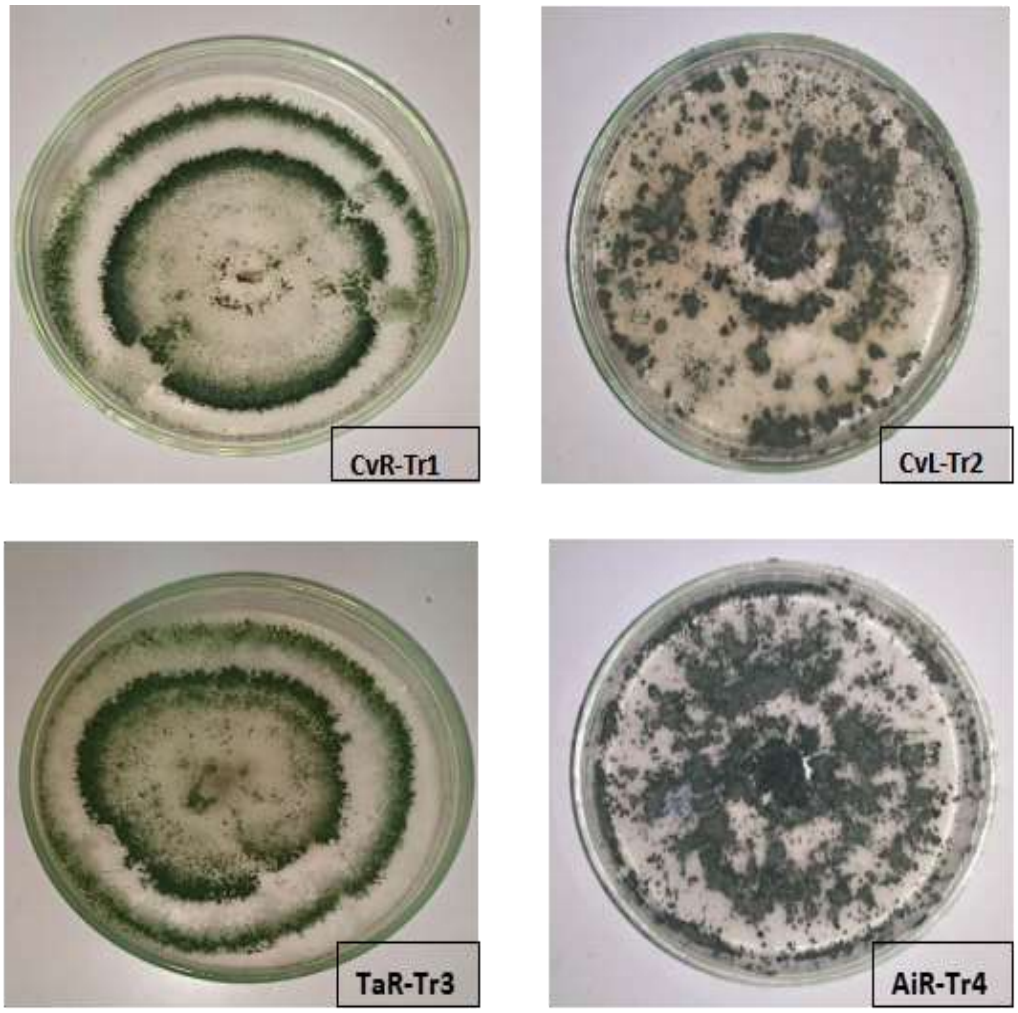

Fig. 1a. Isolated strains of endophytic *Trichoderma* spp. from various wild plants.
Tr represents the *Trichoderma* isolate, while **CvR** is the short form used for *Cleome viscosa*-root, **CvL** is the short form used for *Cleome viscosa*-leaf, **TaR** is the short form used for *Trichodesma amplexicaule*-root, **AiR** is the short form used for *Abutilon indicum*-root, **AvR** is the short form used for *Amaranthus viridis*-root, **RtL** is the short form used for *Ruellia tuberosa*-leaf **SoL** is the short form used for *Sonchus oleracus*-leaf, and **VcR** is the short form used for *Vernonia cinerea*-root.

Total Chlorophyll content

The amount of chlorophyll in chickpea plants treated with various strains of *Trichoderma* changed significantly when compared to control. AvR-Tr5 (0.111 mg/g), AvR-Tr5 + RtL-Tr6 (0.090 mg/g), and RtL-Tr6 (0.076 mg/g) had the highest levels of chlorophyll (Table 5).

Amount of Carotenoids

There was a significant rise in the amount of carotenoids. AvR-Tr5 (0.048mg/g), AvRTr5 + RtLTr6 (0.049 mg/g), and RtL-Tr6 (0.038mg/g) had the most carotenoid (Table 5).

Amount of Carbohydrate

There was a significant rise in carbohydrate content. AvR-Tr5 (5.966 µg/mL), RtL-Tr6 (5.534 µg/m), CvR-Tr1 (5.233 µg/mL), and AvR-Tr5 + RtL-Tr6 (4.566 µg/mL) had the highest carbohydrate content (Table 5).

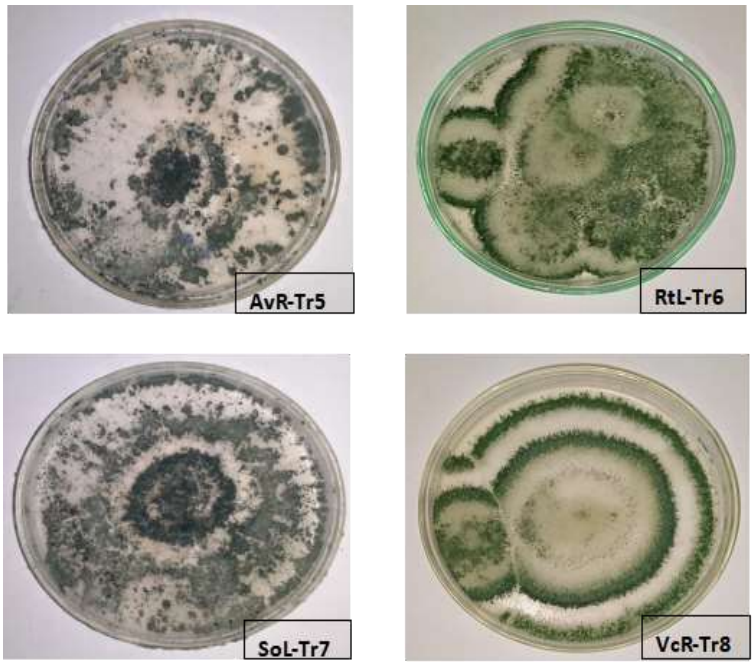

Fig. 1b. Isolated strains of endophytic *Trichoderma* spp. from various wild plants.

Figure 1a, 1b- **Tr** represents the *Trichoderma* isolate, while **CvR** is the short form used for *Cleome viscosa*-root, **CvL** is the short form used for *Cleome viscosa*-leaf, **TaR** is the short form used for *Trichodesma amplexicaule*-root, **AiR** is the short form used for *Abutilon indicum*-root, **AvR** is the short form used for *Amaranthus viridis*-root, **RtL** is the short form used for *Ruellia tuberosa*-leaf **SoL** is the short form used for *Sonchus oleracus*-leaf, and **VcR** is the short form used for *Vernonia cinerea*-root.

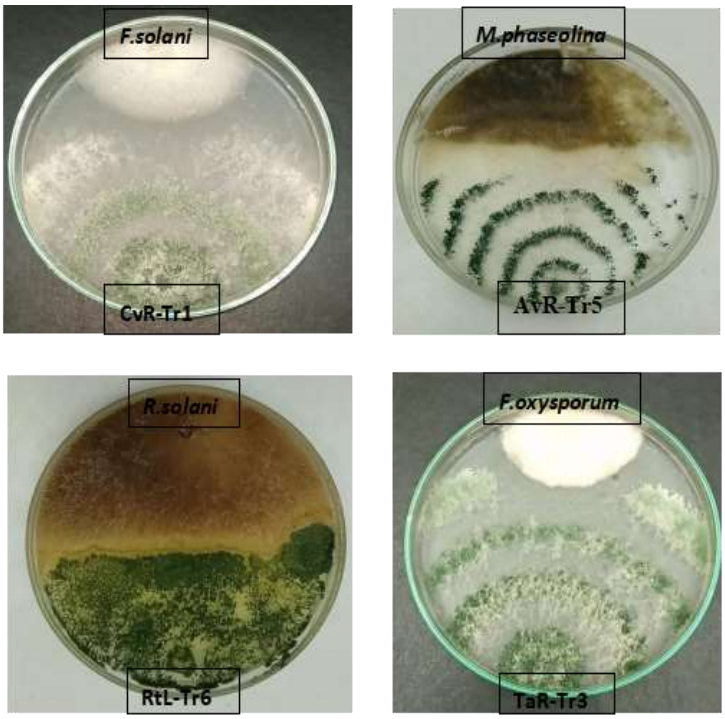

Fig. 2. Inhibition of root rooting fungi by endophytic *Trichoderma* in dual culture plate method.

Fig. 3. Effect of endophytic *Trichoderma* strain CvR-Tr1 on the growth of chickpea compared to negative control (control), and positive control (Topsin-M).

DISCUSSION

Traditional approaches to plant disease management and yield enhancement, such as chemical pesticides, herbicides, or fertilizers, are not ecologically friendly since they contain aromatic groups or methylation and ethylated compounds that have serious environmental implications. To overcome these concerns, scientists are looking into using biocontrol agents (BCA) for disease control, either alone or in combination with other chemicals, for ecologically friendly and long-term disease control (Khan *et al.,* 2022; Shafique *et al.,* 2015a; Shafique *et al.,* 2015b). In this study, eight endophytic *Trichoderma* strains were isolated from the roots and leaves of eight different wild plants and the effects of them on root-infecting fungi was observed in-vitro and in-vivo. *Trichoderma* is known as the most widely used fungal biological control agent, and known as effective in an against of pathogenic fungi. *Trichoderma spp*. is a versatile biocontrol agent that has been used for a long time to manage plant pathogenic fungus.

In this study, endophytic fungus *Trichoderma* strains were found to be eminent in abating the root rotting fungi infection on chickpeas (*Cicer aritinum* L). *Trichoderma harzianum* is one of the most recognized bio-control agents against numerous soil-borne phytopathogenic fungi in current years (Samuels *et al.,* 1998). There are multiple bio-control strategies have been proposed. They comprised space or nutritional competition, the antibiotic synthesis, the phytopathogenic fungal enzymes inactivation and parsitism (Vinale *et al.,* 2006). An investigation was made in vitro using dual plate culture method to observe the effet of 8 strains of *Trichoderma* against 4 root rotting fungi (*F.solani, F. oxysporum, R. solani & M. phaseolina*). Both, in vitro an din vivo applications were involved in observing the species of *Trichoderm*a (Kexiang *et al.,* 2002). In dual culture investigations against *Macrophomina phaseolina*, showed effective results. A number of studies have shown that several fungi, particularly *Trichoderma spp*., can efficiently control diseases caused by *Macrophomina phaseolina* (Aly *et al*., 2007). Trichoderma played a pivotal potential as bio-control agent in an against of *Rhizoctonia solani.* Isolates of *Trichoderma* AvR-Tr5, CvR-Tr6, RtL-Tr6, and TaR-Tr3 parasitize *Rhizoctonia solani* hyphae, sclerotia, and other structures. Biological management of *Rhizoctonia solani* on potato or other host crops is an alternate technique that may give effective and long-term control (Brewer and Larkin, 2005).

According to the results of an *in vitro* experiment, eight distinct strains of *Trichoderma* species were screened against root rotting fungi and shown to have variable degrees of radial colony growth inhibition. To determine their in vitro bio-control activity, the zone of inhibition at the contact point in between the pathogen and an antagonist, also the colony diameter werew used to determine (Anand and Reddy, 2009). On an agar media, the antagonist and

pathogen interaction, zone of inhibition occurrence could be colloquially recognized as an output of the production of antibiotics, competition for space and nutrition and cell wall degrading enzymes (Upadhyay and Rai, 1987; El-Katatny, 2001). The strains AvR-Tr5, RtL-Tr6, and TaR-Tr3 showed the greatest suppression against mycelial growth of *Macrophomina phaseolina*, whereas other studies have found that *Trichoderma spp*. isolates are excellent biocontrol agents against *Macrophomina phaseolina* (Ehteshamul-haque *et al.,* 1990; Ehteshamul-haque and Ghaffar, 1992).

Thirty three Pots treated with endophytic fungi *Trichoderma* reveal a prominenet growth in length and weight of root and shoot in comparison to control and Topsin which aren't treated. The species of *Trichoderma* are potentially recognized at utilization of nutrients and have prominent reproductive potential in an against of *F. solani, F.oxysporum, R. slani and M. phaseolina,* allowing them to survive in harsh environments and making them competitive in *in vivo* studies. *Trichoderma* has to wide range of phytopathogenic fungi due to the presence of a broad range of secodary metabolites and lytic enzymes (cell wall degrading enzymes) e.g. gliovitin, gliotoxin, vinidin, vinidiol and few more (Vinale *et al.,* 2008). The mycroparasitism and antibiosis are the major bio-control mechanism of *Trichoderma* species, with pathogen (Howel, 2003). The amount of chlorophyll and carotenoid was measured, and both showed an increase. In this study chlorophyll levels were higher in CvR-Tr1, AvR-Tr5, and AvR-Tr5+RtLTr6. AvR-Tr5, AvR-Tr5+RtL-Tr6, and RtL-Tr6 all enhanced the quantity of carotenoid in the plant. Chlorophyll is an essential component of plant and plays an important role in photosynthesis. Plants cannot perform photosynthesis without a proper amount of chlorophyll. Chlorophyll has been shown to have a pivotal application in an ATP synthesis and for essential constituents preservation (Kumar *et al.,* 2012). AvR-Tr5 + RtL-Tr6, RtL-Tr6, and TaR-Tr3 all had a high carbohydrate content. In this investigation, various *Trichoderma* strains showed significant antagonistic potential in an against of root rotting fungi both in vitro and in vivo activity. Although some species of Trichoderma have been recognized as plant pathogens, their capacity to antagonize and parasitize other fungi has made them useful biocontrol agents against a variety of plant infections (Elias *et al.,* 2016). The species *Trichoderma* have also been recognized as Plant growth inducers and inducers of plant defence mechanisms (Saba *et al.,* 2012). The roots are colonized by *Trichoderma* and delivers advantages to plants, because the roots are colonized by effective strains completely till their growth.

Trichoderma is widely used as fungal biological control agent and have potential antagonistic properties in an against of plant pathogenic fungi. Few *Trichoderma* spp. Isolates are included in the proposed study significantly inhibited several pathogens and increased the amount of total chlorophyll and carbohydrates as compared to the controlled plants. So it is suggested that the biocontrol agents like *Trichoderma* should be used instead of chemical fertilizers as they don't have environmental constrains.

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