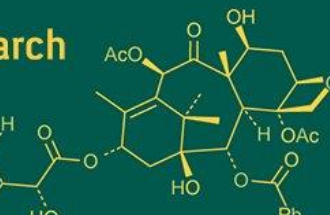
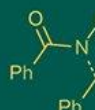
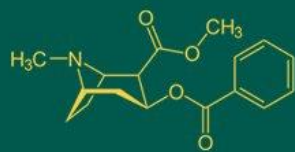


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Isolation and *In-vitro* biocontrol potential of native *Trichoderma* spp. against *Aspergillus niger* van Tieghem causing collar rot of groundnut

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Abstract

Collar rot caused by *Aspergillus niger* van Tieghem is an important soil- and seed-borne disease of groundnut (*Arachis hypogaea* L.), causing poor seed germination and seedling mortality. The present investigation was undertaken to isolate and identify the collar rot pathogen of groundnut, confirm its pathogenicity, isolate native *Trichoderma* spp. from rhizospheric soils, and evaluate their antagonistic potential against *A. niger* under *in-vitro* conditions. The study was conducted at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, during Kharif 2025. *A. niger* was isolated from infected groundnut kernels exhibiting characteristic collar rot symptoms and identified based on its cultural and morphological characteristics. Its pathogenicity was confirmed through artificial inoculation and subsequent re-isolation of the pathogen, thereby fulfilling Koch's postulates. For isolation of native *Trichoderma* spp., 52 rhizospheric soil samples were collected from different locations of middle Gujarat. Serial dilution and plating on *Trichoderma* selective medium resulted in the isolation of 25 native *Trichoderma* strains, which were purified and maintained for further evaluation. The antagonistic potential of these isolates against *A. niger* was assessed using dual culture and inverted plate techniques. Among the 25 isolates tested, Tr6 exhibited the highest inhibitory effect against *A. niger*, followed by Tr10, Tr16, Tr8 and Tr18. Based on the modified Bell's scale, these isolates were grouped as highly effective native *Trichoderma* strains. The study demonstrated considerable variation in the antagonistic ability of native *Trichoderma* isolates and identified promising strains with strong *in-vitro* inhibitory activity against *A. niger*. These efficient native isolates may serve as potential candidates for further evaluation as biological control agents for the sustainable management of collar rot of groundnut.

Keywords: *Aspergillus niger*; *Trichoderma*; dual culture; inverted plate method; volatile compounds

Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed and food legume crop cultivated predominantly in arid and semi-arid regions. It is valued for its high nutritional and economic importance, particularly as a rich source of vegetable oil and protein (Hammons, 1982; Reddy, 1976) ^[1, 2]. In India, groundnut is one of the major oilseed crops, with Gujarat being one of the leading groundnut-producing states (Anonymous, 2024; Anonymous, 2025) ^[3, 4].

Groundnut production is affected by several fungal diseases, among which collar rot caused by *Aspergillus niger* van Tieghem is an important soil- and seed-borne disease. The disease primarily affects the collar and hypocotyl regions of young seedlings and may occur in both pre- and post-emergence phases. Infected seeds may rot in the soil, while emerging seedlings develop brown lesions, followed by tissue decay, wilting, collapse and death. Under favourable conditions, collar rot can cause considerable plant mortality and yield losses (Chahal *et al.*, 1974; Ghewande *et al.*, 2002) ^[5, 6].

Aspergillus niger is widely distributed in soil and plant debris and can survive under conditions favourable for disease development. The incidence of collar rot is influenced by factors such as high soil temperature, low soil moisture, sandy soil conditions and deep sowing of seeds (Saran *et al.*, 2021) ^[7]. The pathogen may persist in the field and cause significant damage to groundnut seedlings, making effective management of the disease essential (Gajera *et al.*, 2011) ^[8].

Although fungicides such as mancozeb, tebuconazole, thiram, carbendazim and vitavax are used for the management of collar rot, their repeated and extensive use has several

limitations. These include environmental pollution, development of resistant pathogen populations, non-target effects, food and feed contamination and potential health hazards. Moreover, effective management of soil-borne pathogens through chemical application is difficult because of their persistence in the soil environment (Rakholiya *et al.*, 2012) [9]. Therefore, biological control represents a promising and sustainable alternative for the management of soil-borne diseases (Amin *et al.*, 2010; Dhotre and Vanmare, 2017) [10, 11].

Among the biological control agents, *Trichoderma* spp. have received considerable attention because of their ability to suppress a wide range of soil-borne plant pathogens through different mechanisms, including competition, antibiosis, hyperparasitism and production of lytic enzymes and antifungal metabolites (Cook and Baker, 1983; Singh and Jadon, 2019) [12, 13]. Several *Trichoderma* species, including *T. harzianum*, *T. viride*, *T. asperellum*, *T. hamatum*, *T. virens* and *T. koningii*, have been investigated for their biocontrol potential, with *T. harzianum* being one of the most extensively studied species for biological disease management (Keswani *et al.*, 2014) [14].

The rhizosphere represents an important source of beneficial microorganisms, where plant-microbe interactions can influence plant health and development. Native *Trichoderma* isolates obtained from rhizospheric soils may therefore provide promising candidates for the biological management of soil-borne pathogens. Evaluation of their antagonistic potential against the target pathogen under controlled *in-vitro* conditions can help identify efficient isolates for further disease-management studies (Huang *et al.*, 2014) [15].

Identification of efficient native biocontrol agents and evaluation of their antagonistic potential are essential prerequisites for developing sustainable and environmentally friendly management strategies against collar rot of groundnut. However, information on the diversity and effectiveness of native *Trichoderma* isolates against *Aspergillus niger* causing collar rot under *in-vitro* conditions is limited. Therefore, the present investigation was undertaken to isolate, identify and prove the pathogenicity of *A. niger* associated with collar rot of groundnut, isolate native *Trichoderma* spp. from the rhizospheric region of higher plants, and evaluate their antagonistic potential against *A. niger* under *in-vitro* conditions.

Materials and Methods:

Isolation and identification of pathogen

Aspergillus niger was isolated from infected groundnut kernels by the agar plate method. The kernels were surface sterilized with 1% sodium hypochlorite (NaOCl) for one minute, rinsed three times with sterile distilled water and placed on Potato Dextrose Agar (PDA). Plates were incubated at 25 ± 2 °C for 4-5 days. The fungal isolates were purified by single-spore isolation and maintained on PDA slants. Identification was carried out based on cultural and morphological characteristics of the mycelium and conidia following Raper and Fennell (1965) [16].

Isolation of native *Trichoderma* spp.

Rhizospheric soil samples were collected from higher plants at a depth of 15 cm from the Anand region (Table 1) and *Trichoderma* spp. were isolated by the serial dilution

technique (Hirte, 1969) [17] on *Trichoderma* selective medium (Elad and Chet, 1983) [18]. Representative isolates were purified through single-spore isolation and preserved at 4 °C for further studies.

A total of 52 soil samples were collected, out of which 25 soil samples recovered *Trichoderma* spp.

In-vitro antagonism between native *Trichoderma* spp. and pathogen - *A. niger*

Dual culture method

The antagonistic potential of native *Trichoderma* isolates against *Aspergillus niger* was evaluated using the dual culture technique (Dennis and Webster, 1971a) [19]. A 5 mm mycelial disc of *A. niger* and each *Trichoderma* isolate were placed opposite to each other on PDA plates and incubated at 28 ± 1 °C. Three replications were maintained, with pathogen-only plates as control. Radial growth was recorded until complete growth in the control, and per cent growth inhibition was calculated according to Vincent (1947) [20].

$$PGI = \frac{DC - DT}{DC} \times 100$$

Where,

PGI = Per cent growth inhibition

DC = Growth of test pathogen (mm) in the absence of antagonistic isolate

DT = Growth of test pathogen (mm) in the presence of antagonistic isolate

Volatile compounds

The inverted plate technique was employed to evaluate the ability of native *Trichoderma* isolates to produce volatile antifungal compounds against *Aspergillus niger* (Dennis and Webster, 1971b) [21]. A 5 mm mycelial disc from a three-day-old culture of each *Trichoderma* isolate was placed at the centre of PDA plates and incubated at 28 ± 1 °C for two days, while *A. niger* was inoculated separately and incubated for 12 h. The lids were removed, and the respective plate bottoms were sealed face-to-face with Parafilm and incubated at 28 ± 1 °C. Three replications were maintained for each treatment, with pathogen-only plates as control. Per cent inhibition of mycelial growth was calculated as described earlier.

Results and Discussion

A total of 52 rhizospheric soil samples collected from different locations of Anand district were processed for isolation of *Trichoderma* spp. using *Trichoderma* selective medium. Twenty-five samples yielded *Trichoderma* isolates, which were confirmed based on their cultural and morphological characteristics and purified through hyphal tip isolation

In-vitro antagonism between native *Trichoderma* spp. and pathogen - *A. niger*

Dual culture method

The antagonistic potential of 25 native *Trichoderma* isolates against *A. niger* was evaluated using the dual culture technique. Considerable variation was observed among the isolates in their ability to inhibit mycelial growth of the pathogen. Isolate Tr6 recorded the highest inhibition of mycelial growth (87.81%), restricting the colony diameter of *A. niger* to 10.96 mm, followed by Tr10 (77.44%; 20.30

mm) and Tr16 (75.22%; 22.30 mm). Isolates Tr8 and Tr18 also exhibited high inhibitory activity, with 74.84 and 72.30 per cent inhibition and corresponding pathogen colony diameters of 22.64 and 24.53 mm, respectively (Table 2).

The lowest inhibition was recorded by Tr5 (31.88%), with a pathogen colony diameter of 61.30 mm. The remaining isolates exhibited inhibition ranging from 35.22 to 72.14 per cent, indicating considerable variation in their antagonistic activity against *A. niger*. Overall, Tr6 was the most effective isolate in suppressing the mycelial growth of *A. niger* under *in-vitro* conditions (Figure 2).

The present findings are in agreement with earlier reports demonstrating the antagonistic potential of *Trichoderma* spp. against *A. niger*. Nath and Patel (2022) [22] reported maximum inhibition of 63.72 per cent by *T. viride*, while Gajera *et al.* (2011) [8] recorded 86.2 per cent inhibition by *T. virens* isolate T6. Similarly, Swathy *et al.* (2024) [23] reported 86.33 per cent inhibition by *Trichoderma* isolate TVK1 using the dual culture technique. These findings support the effectiveness of *Trichoderma* spp. as potential biocontrol agents against *A. niger*.

Volatile compounds

The antagonistic potential of 25 native *Trichoderma* isolates

against *A. niger* was evaluated using the inverted plate method. All the isolates inhibited the mycelial growth of the pathogen compared with the control (90.00 mm), with considerable variation in their activity. Isolate Tr18 recorded the highest growth inhibition (78.63%), followed by Tr6 (77.03%), Tr8 (76.41%), Tr10 (76.66%) and Tr16 (76.38%). The corresponding pathogen colony diameters were 19.23, 20.66, 21.23, 21.00 and 21.25 mm, respectively. The lowest inhibition was recorded by Tr5 (19.77%), with a maximum colony diameter of 72.20 mm. Overall, Tr18 exhibited the highest antagonistic activity against *A. niger* through volatile metabolites (Table 2) (Fig. 3).

The present findings are in agreement with earlier reports on the inhibitory effect of *Trichoderma* volatile metabolites against *Aspergillus* spp. Erfandoust *et al.* (2020) [24] reported 71.93 per cent inhibition of *A. niger* by volatile compounds of *T. atroviride*. Similarly, Stracquandano *et al.* (2020) [25] observed 45.86 and 25.56 per cent inhibition by *T. asperellum* and *T. atroviride*, respectively. Boukaew *et al.* (2024) [26] and Voloshchuk *et al.* (2024) [27] also reported significant suppression of *Aspergillus* spp. by volatile metabolites of *Trichoderma*.

Table 1: List of soil samples collected for isolation of *Trichoderma* spp. from Anand district, Gujarat

Sr. No.	Isolates	Common name	Trees	Locations	Type of soil
1.	Tr1	Aonla	<i>Phyllanthus emblica</i> L.	AAU Campus	Alluvial and sandy loam
2.	Tr2	<i>Desi babul</i>	<i>Vachellia nilotica</i> (L.) P.J.H. Hurter & Mabb.	Navli	
3.	Tr3	Eucalyptus	<i>Eucalyptus tereticornis</i> Sm.	AAU Campus	
4.	Tr4	Subabul	<i>Leucaena leucocephala</i> (Lam.) de Wit	AAU Campus	
5.	Tr5	Wood apple	<i>Limonia acidissima</i> L.	AAU Campus	
6.	Tr6	Banyan tree	<i>Ficus benghalensis</i> L.	AAU Campus	
7.	Tr7	Vilayati <i>babul</i>	<i>Prosopis juliflora</i> (Sw.) DC.	Boriavi	
8.	Tr8	Neem	<i>Azadirachta indica</i> A. Juss.	AAU Campus	
9.	Tr9	Yellow flame tree	<i>Peltophorum pterocarpum</i> (DC.) Backer ex K. Heyne	AAU Campus	
10.	Tr10	Ashoka	<i>Polyalthia longifolia</i> (Sonn.) Thwaites	AAU Campus	
11.	Tr11	Gulmohar	<i>Delonix regia</i> (Bojer ex Hook.) Raf.	Aanand City	
12.	Tr12	Golden Shower Tree	<i>Cassia fistula</i> L.	AAU Campus	
13.	Tr13	<i>Karanj</i>	<i>Milletia pinnata</i> (L.) Panigrahi	Vallabh Vidhyanagar	
14.	Tr14	Bottle brush	<i>Callistemon citrinus</i> (Curtis) Skeels	AAU Campus	
15.	Tr15	Umbrella tree	<i>Terminalia mantaly</i> H. Perrier	AAU Campus	
16.	Tr16	<i>Peepal</i>	<i>Ficus religiosa</i> L.	AAU Campus	
17.	Tr17	Whistling Pine	<i>Casuarina equisetifolia</i> L.	AAU Campus	
18.	Tr18	<i>Jamun</i>	<i>Syzygium cumini</i> (L.) Skeels	AAU Campus	
19.	Tr19	Tamarind	<i>Tamarindus indica</i> L.	Lambhvel	
20.	Tr20	Indian almond	<i>Terminalia catappa</i> L.	AAU Campus	
21.	Tr21	Mango	<i>Mangifera indica</i> L.	AAU Campus	
22.	Tr22	Guava	<i>Psidium guajava</i> L.	AAU Campus	
23.	Tr23	Sapota	<i>Manilkara zapota</i> (L.) P. Royen	AAU Campus	
24.	Tr24	Custard apple	<i>Annona squamosa</i> L.	AAU Campus	
25.	Tr25	Flame of the forest	<i>Butea monosperma</i> (Lam.) Taub.	Jitodia	

Table 2: Evaluation of the antagonistic potential of isolated native *Trichoderma* strains against *A. niger* under *in-vitro* conditions

<i>Trichoderma</i> isolates	Dual culture technique		Inverted plate method		Mean	
	Mycelial radial growth (mm)	Growth inhibition (%)	Mycelial radial growth (mm)	Growth inhibition (%)	Mycelial radial growth (mm)	Growth inhibition (%)
Tr1	35.43	60.62	44.20	50.88	39.81	55.75
Tr2	38.08	57.68	43.76	51.37	40.92	54.52
Tr3	56.50	37.22	62.66	30.37	59.58	33.79
Tr4	35.30	60.77	51.00	43.33	43.15	52.05
Tr5	61.30	31.88	72.20	19.77	66.75	25.83
Tr6	10.96	87.81	20.66	77.03	15.81	82.42
Tr7	47.96	46.70	50.66	43.70	49.31	45.20
Tr8	22.64	74.84	21.23	76.41	21.93	75.62
Tr9	45.10	49.88	51.43	42.85	48.26	46.37

Tr10	20.30	77.44	21.00	76.66	20.65	77.04
Tr11	44.20	50.88	53.66	40.37	48.93	45.62
Tr12	49.33	45.18	63.33	29.62	56.33	37.40
Tr13	25.06	72.14	49.66	44.81	37.36	58.48
Tr14	56.73	36.96	68.33	24.07	62.53	30.51
Tr15	47.96	46.70	53.00	41.11	50.48	43.90
Tr16	22.30	75.22	21.25	76.38	21.77	75.80
Tr17	41.86	53.48	54.33	39.62	48.10	46.55
Tr18	24.53	72.30	19.23	78.63	22.08	75.45
Tr19	58.30	35.22	63.00	30.00	60.65	32.61
Tr20	44.20	50.88	60.33	32.96	52.26	41.92
Tr21	27.53	69.40	47.76	46.92	37.65	58.16
Tr22	41.30	54.11	51.66	42.59	46.48	48.35
Tr23	38.53	57.18	56.00	37.77	47.26	47.48
Tr24	51.30	43.00	61.66	31.48	56.48	37.24
Tr25	33.96	62.25	49.53	44.96	41.75	53.61
Control	90.00	-	90.00	-	90.00	-
S.E.m.±	0.50	-	1.25	-	-	-
C.D. at 5%	1.35	-	3.56	-	-	-
C.V. (%)	2.01	-	4.04	-	-	-

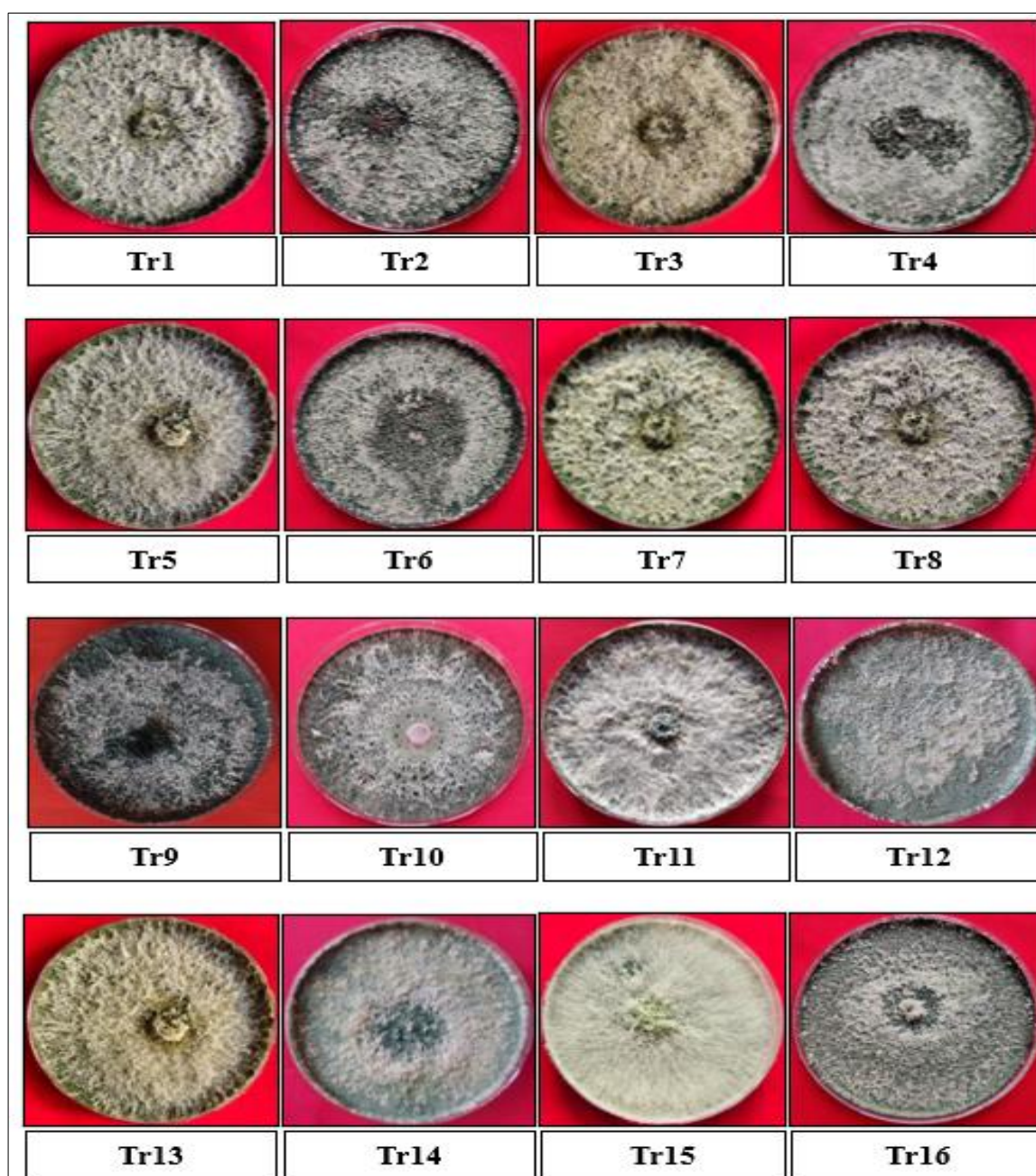


Fig 1: *Trichoderma* strains isolated from rhizospheric soil of higher plants

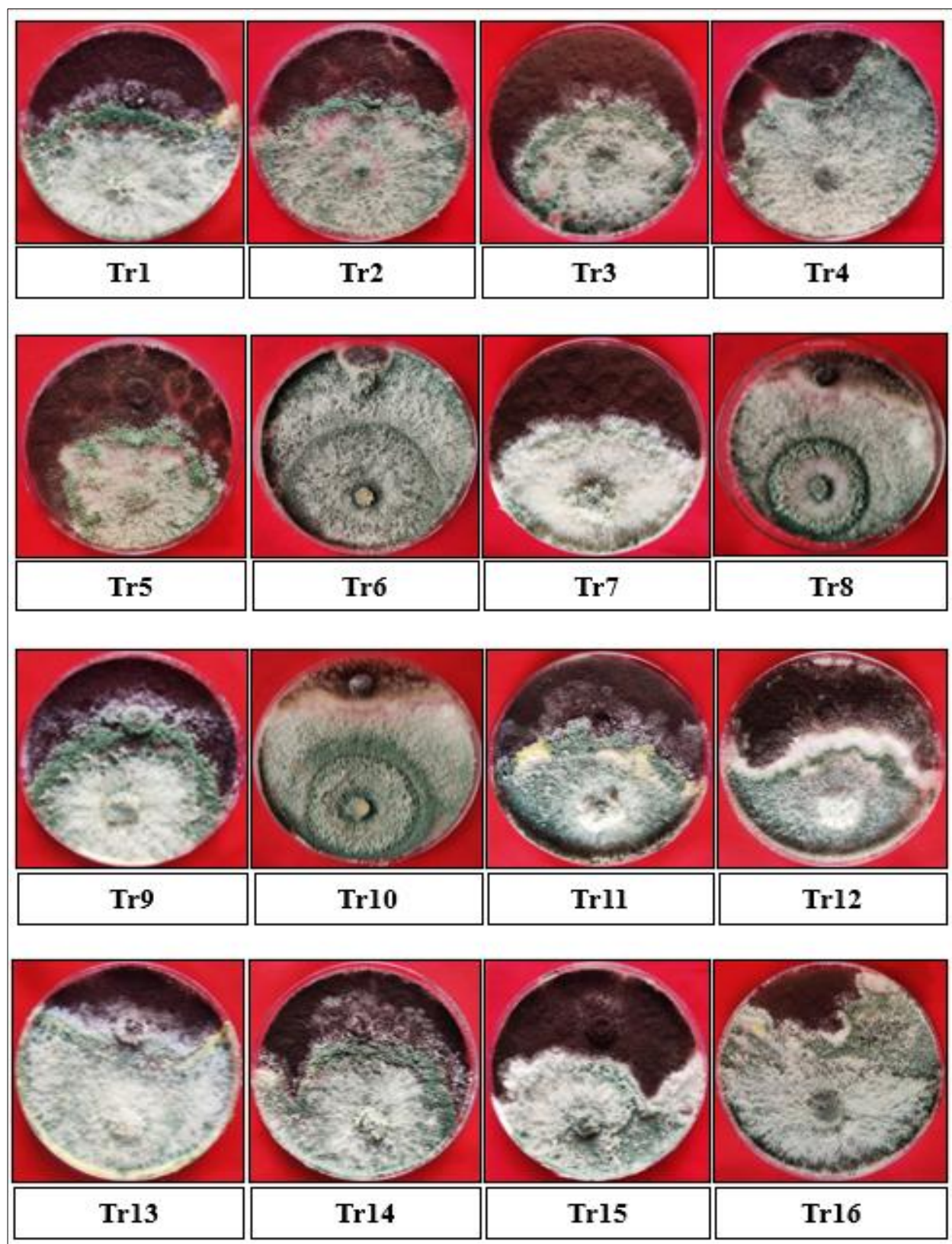


Fig 2: Evaluation of native *Trichoderma* strains against *A. niger* by using dual culture method

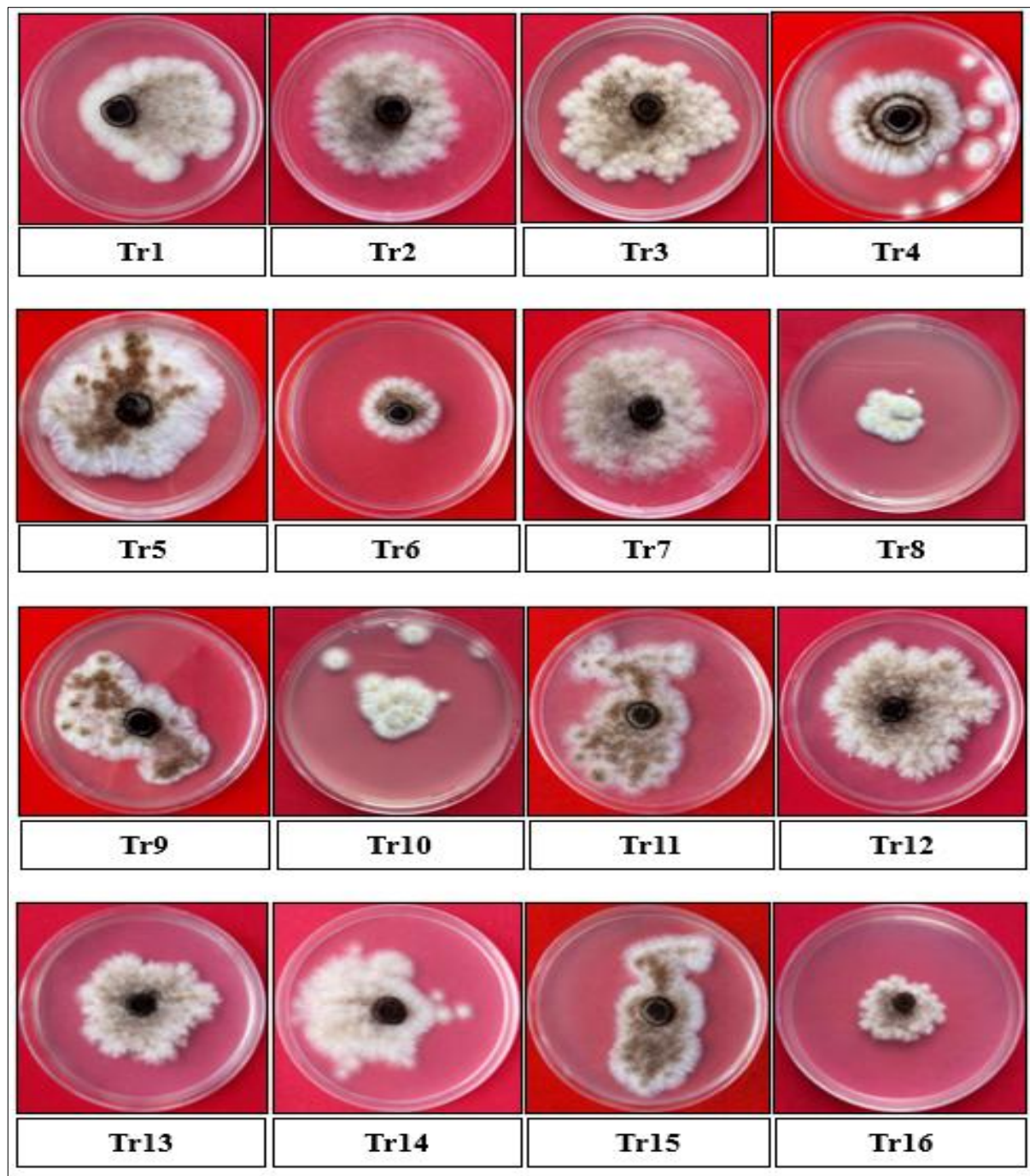


Fig 3: Evaluation of native *Trichoderma* strains against *A. niger* by using inverted plate method

Conclusion

Collar rot-affected groundnut seedlings exhibited characteristic collar rotting, wilting and eventual death. *Aspergillus niger* van Tieghem was successfully isolated from infected groundnut kernels and identified based on its cultural and morphological characteristics.

A total of 25 native *Trichoderma* isolates were recovered from rhizospheric soil samples collected from different locations around Anand. Their antagonistic potential against *A. niger* was evaluated using dual culture and inverted plate techniques. Considerable variation was observed among the isolates, with Tr6 showing the highest antagonistic activity, followed by Tr10, Tr16, Tr8 and Tr18.

Thus, the study identified promising native *Trichoderma* isolates with strong *in-vitro* antagonistic potential against *A. niger*, particularly Tr6, as a potential biocontrol agent for the management of collar rot of groundnut.

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