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Evaluation of *Trichoderma* isolates for their Antagonistic Potential against *Fusarium oxysporum* f. sp. *ciceri in-vitro*

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ABSTRACT

Twenty four sympatric isolates of *Trichoderma* from six different chickpea growing mandals of Prakasam district, Andhra Pradesh and one isolate from Bapatla were assessed for their antagonistic efficacy against Bapatla isolate of *Fusarium oxysporum* f. sp. *ciceri (Foc)*, chickpea wilt causing soil borne plant pathogen. Variation was observed among *Trichoderma* isolates tested in their antagonistic potential. Screening of *Trichoderma* isolates was done based on the radial growth of interacting test fungi, overgrowth, zone of inhibition, pigmentation of *Foc* and sporulation of *Trichoderma*. Five potential isolates were identified, *i. e*., T 19001, T 19007, T 19012, T 19020, T 19023 which were fast in growth and overgrowing the *Foc*. T 19001, T 19007 and T 19012 isolates were found to overgrow and sporulate on *Foc*. Media pigmentation in *Foc* changed from light colour in monocultured *Foc* plates to dark in dual cultured plates in interactions involving T 19020 and T 19023 isolates, however, without sporulation on the test pathogen.

Keywords: *Antagonistic potential, Fusarium oxysporum f. sp. ciceri, inhibition zone, Trichoderma.*

Chickpea (*Cicer arietinum* L.) is one of the major legume crops widely grown in the Indian sub continent and in other Asian countries. Indian subcontinent accounts for 90% of the total world chickpea production (Juan *et al*., 2000). In India, it is grown in 10.56 million ha with a production of 11.37 million t and productivity is 1078 kg /ha (Indiastat, 2018-19). It is a cheap source of protein compared to animal protein. Predominately, it is consumed as dhal or variety of snacks and condiments (Duke, 1981). Chickpea crop suffers from a variety of pathogens which results in lower yields of chickpea. Among them, Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*), a soil borne plant pathogen is most widespread and important throughout the world incurring huge losses in production and productivity (Gupta *et al*., 1997).

The fungus is both seed and soil borne and may survive even in the absence of host in soil for up to six years (Haware *et al*., 1996). By considering its nature of damage and survival ability, better management can be achieved when bio-control agents were utilised along with cultural practices. Biological control provides an potential alternative to the use of synthetic fungicides. *Trichoderma* spp. were most widely used biocontrol agents for over 70 years against several plant pathogens and have gained greater public acceptance for their antifungal and anti-enduring activities (Zaidi and Singh, 2004). However, being a living agent variation exists among different isolates of *Trichoderma* in their antagonistic potential. Hence, the present investigation was conducted to evaluate isolate variation in antagonistic efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *ciceri* Bapatla isolate.

MATERIAL AND METHODS

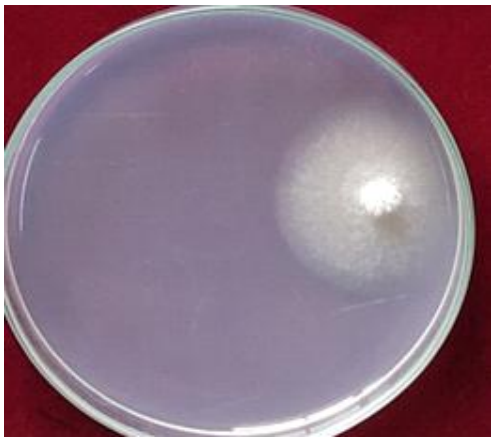
Isolation of test pathogen and sympatric *Trichoderma* spp.

The test pathogen was isolated from chickpea plants affected by Fusarium wilt in College Farm, Agricultural College, Bapatla, Guntur Dt., Andhra Pradesh. Twenty four isolates of *Trichoderma* spp. from six different chickpea growing mandals of Prakasam district, Andhra Pradesh and one isolate from College Farm, Bapatla were isolated using *Trichoderma* Selective medium (TSM) (Elad *et al.*,1981). Isolations were made from the rhizosphere soils.

Screening of *Trichoderma* spp. by dual culture method

Twenty five isolates of *Trichoderma* spp. were screened against test pathogen *Fusarium oxysporum* f. sp. *ciceri* (*Foc*) *in vitro* using dual culture method (Dhingra and Sinclair, 1985). Appropriate controls were maintained for monoculture and inoculated plates were incubated at 28±1°C. Observations on radial growth of interacting test fungi, overgrowth, zone of inhibition, pigmentation of *Foc*, sporulation of *Trichoderma* spp. were recorded.

Per cent growth inhibition was calculated by using the following formula (Dubey *et al.,* 2007):



Fusarium oxysporum* f. sp. *ciceri

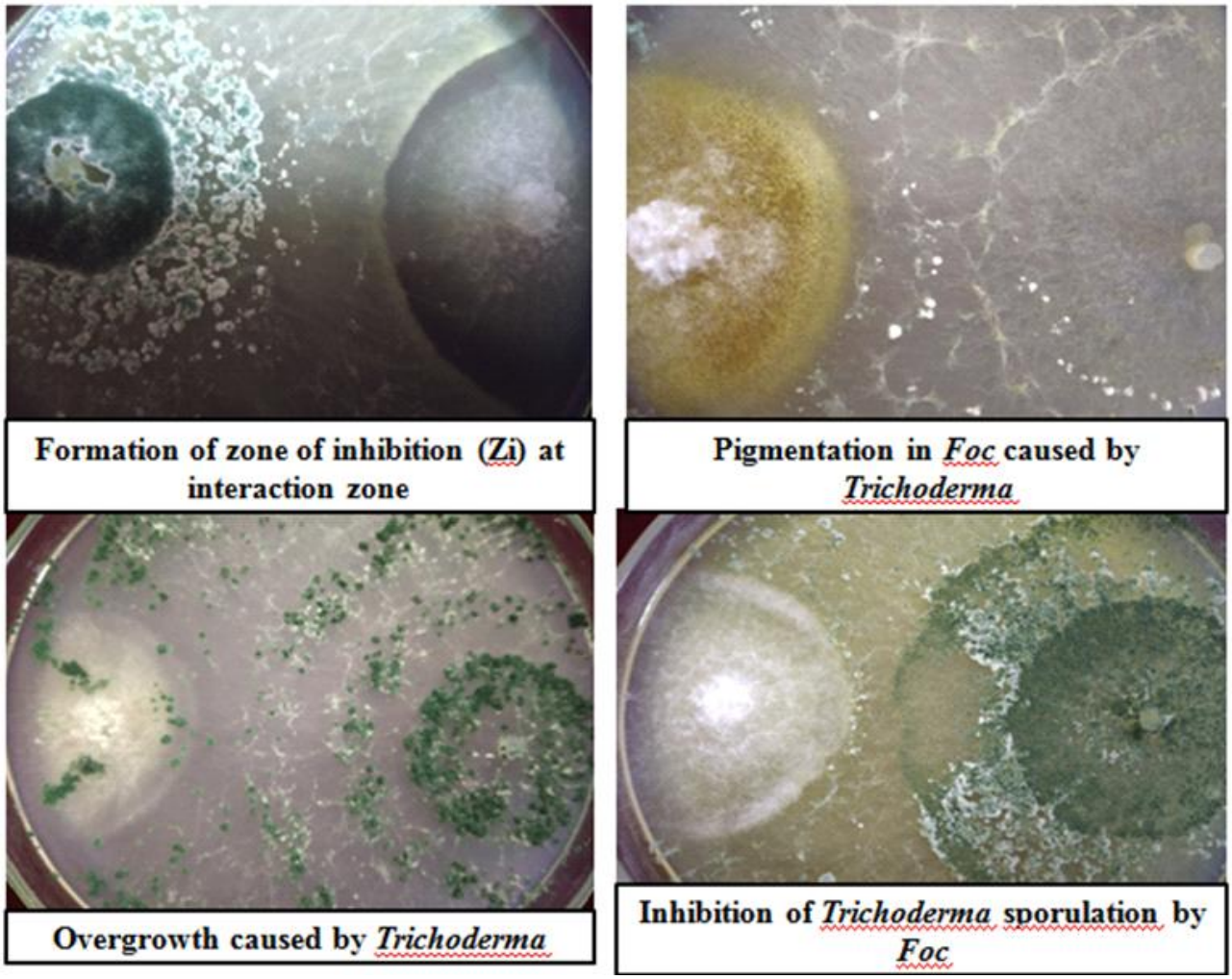


Plate 1. Interaction effects of *Fusarium oxysporum* f. sp. *ciceri* and *Trichoderma* spp. in dual culture five days after inoculation

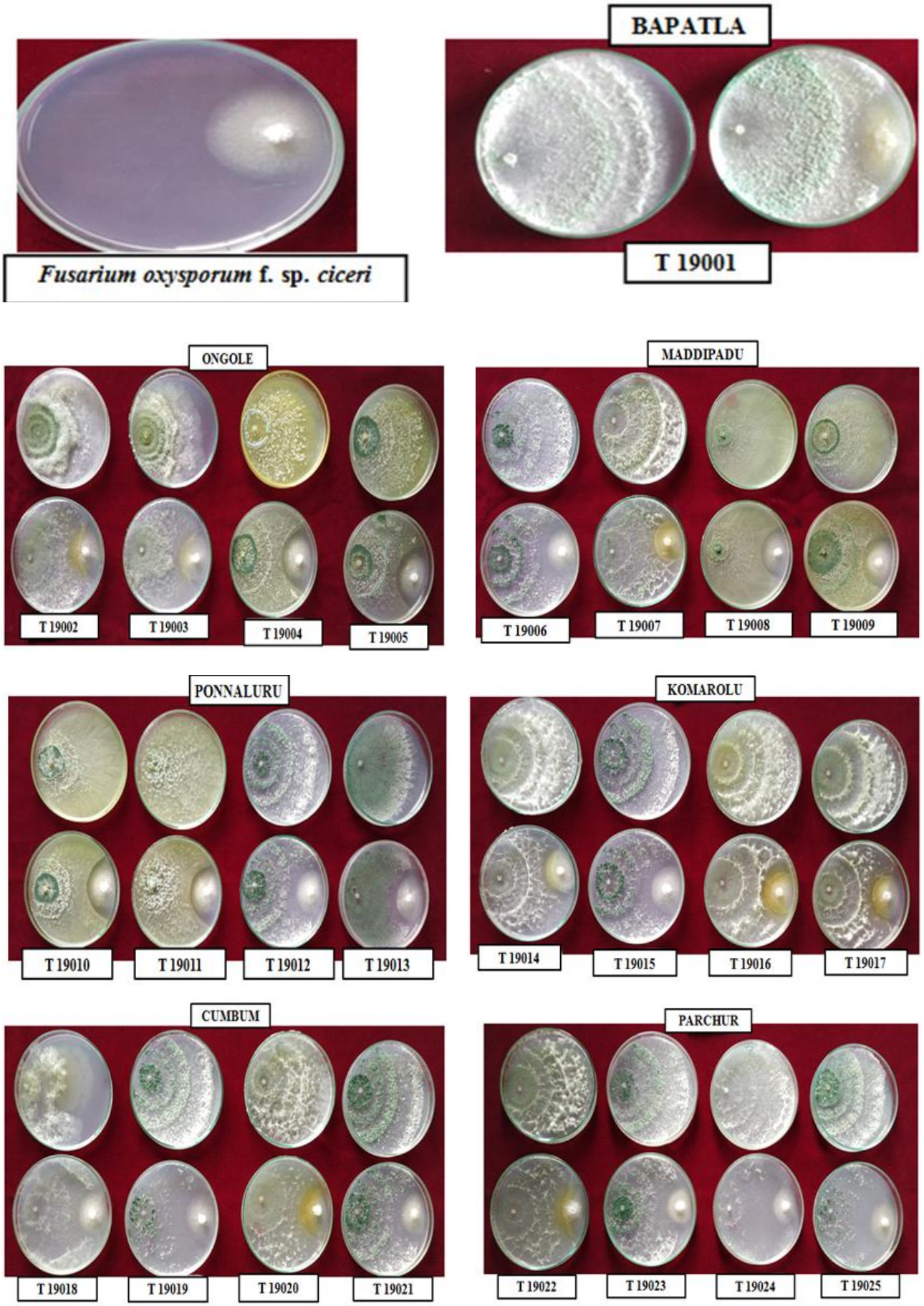


Plate 2. Interactions of isolates of *Trichoderma* against *Foc* in dual culture

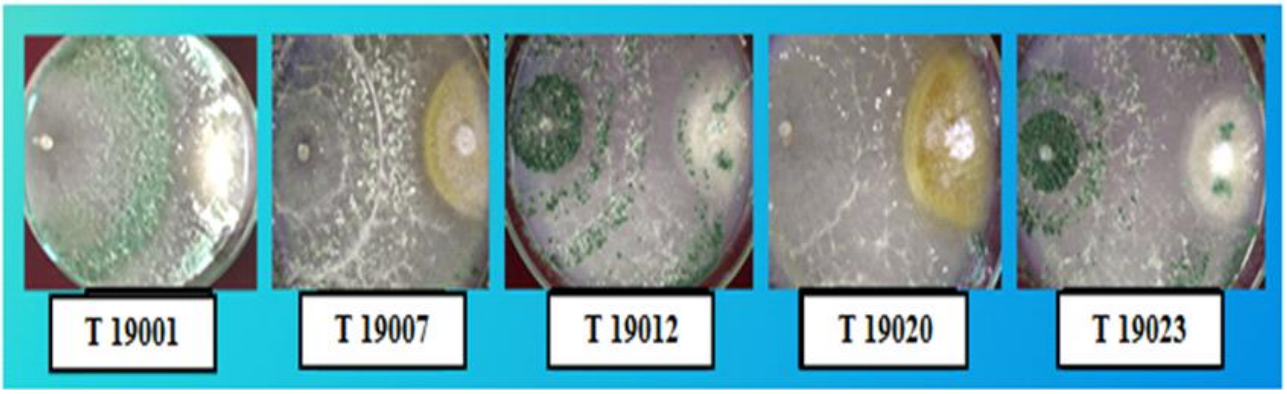


Plate 3. Five *Trichoderma i*solates considered potential for antagonistic efficacy against *Fusarium oxysporum* f. sp. *ciceri*

Table 1. Effect of *Trichoderma* isolates on the radial growth of *Fusarium oxysporum* f. sp. *ciceri*

S. No.	Treatments	5 DAI	
		Radial growth (cm)*	Inhibition (%)
1	T 19001	1.20 (1.09)	50
2	T 19002	1.37 (1.47)	43.1
3	T 19003	1.20 (1.09)	50
4	T 19004	1.13 (1.06)	52.8
5	T 19005	1.43 (1.20)	40.2
6	T 19006	1.30 (1.14)	45.8
7	T 19007	1.07 (1.03)	55.5
8	T 19008	1.30 (1.14)	45.8
9	T 19009	1.40 (1.18)	41.7
10	T 19010	1.27 (1.12)	47.2
11	T 19011	1.40 (1.18)	41.7
12	T 19012	1.33 (1.15)	44.4
13	T 19013	1.00 (1.00)	58.3
14	T 19014	1.23 (1.11)	48.6
15	T 19015	1.40 (1.18)	41.7
16	T 19016	1.20 (1.10)	50
17	T 19017	1.30 (1.14)	45.8
18	T 19018	1.33 (1.18)	45.8
19	T 19019	1.33 (1.15)	44.4
20	T 19020	1.30 (1.14)	45.8
21	T 19021	1.40 (1.18)	41.7
22	T 19022	1.17 (1.08)	51.4
23	T 19023	1.30 (1.14)	45.8
24	T 19024	1.30 (1.14)	45.8
25	T 19025	1.43 (1.20)	40.3
26	*FOC* control	2.4 (1.55)	
SEm ±		0.03	
C.D. (0.05)		0.08	
CV%		4.46	

Each treatment replicated thrice.
DAI - Days after inoculation
*Values in the parenthesis are square root transformed values

Table 2. Qualitative parameters of *Trichoderma* antagonistic potential against *Fusarium oxysporum* f. sp. *ciceri in vitro* 5 days after inoculation

S. No.	Treatments	Zone of inhibition (Zi)	Lysis		Sporulation in *Trichoderma* at Zi	Over growth of *Trichoderma*	Pigmentation in *Foc*
			T	*Foc*			
1	T 19001	-	-	+	+	+	-
2	T 19002	-	-	+	-	+	+
3	T 19003	-	-	+	-	+	+
4	T 19004	+	-	-	+	-	-
5	T 19005	+	-	-	+	-	-
6	T 19006	-	-	-	-	+	-
7	T 19007	-	-	+	-	+	+
8	T 19008	+	-	-	-	-	-
9	T 19009	+	-	+	+	-	-
10	T 19010	+	-	-	-	-	-
11	T 19011	+	-	-	-	-	-
12	T 19012	-	-	-	+	+	-
13	T 19013	+	-	-	-	-	-
14	T 19014	-	-	+	-	+	+
15	T 19015	-	-	+	+	+	-
16	T 19016	-	-	+	-	+	+
17	T 19017	-	-	+	-	+	+
18	T 19018	-	-	-	-	+	-
19	T 19019	-	+	+	-	-	-
20	T 19020	-	-	+	-	+	+
21	T 19021	-	-	+	+	+	-
22	T 19022	-	-	+	-	+	+
23	T 19023	-	-	-	+	+	-
24	T 19024	-	+	-	-	-	-
25	T 19025	-	+	-	-	-	-

+ : Positive for the character over other isolates
- : Negative for the character over other isolates

T : *Trichoderma*
Foc : *Fusarium oxysporum* f. sp. c*iceri*

Per cent inhibition (%) =

$$\frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \times 100$$

RESULTS AND DISCUSSIONS

In monocultured control plates, *Foc* grew to an extent of 2.4 cm in five days of inoculation (Table 1). Variation existed among *Trichoderma* isolates in interactions with *Foc*. Though there was no physical contact between the two interacting fungi up to 5 DAI, significant reduction in *Foc* radial growth was observed when compared to its respective control plates. The radial growth of *Foc* in dual cultured plates ranged between 1.0-1.43 cm at 5 DAI which was significantly lower than that in monocultured plates (2.4 cm).

After five days of inoculation, when there was a physical contact between the isolates, maximum reduction in radial growth of the pathogen was observed in isolate T19013 (1.00 cm) with 58.3 % inhibition which was on par with T 19007 (1.07 cm) with 55.5 % inhibition over control which were further on par with T 19004, T 19022 (1.13 cm and 1.17 cm) with 52.8 % and 51.4% inhibition over control. Least reduction in the *Foc* mycelial growth was observed in interaction involving T 19025 (1.43 cm) with 40.3 % inhibition which was on par with T 19005, T 19021, T 19018, T 19015, T 19011, T 19009, T 19002, T 19012, T 19019, T 19024, T 19017, T 19020, T 19008,T 19006, T 19023, T 19010 isolates (Table 1).

Rudresh *et al.* (2005) tested nine isolates of *Trichoderma* spp. against wilt complex in chickpea and found that *T. virens*-PDBCTVs 12 as a potential isolate against *Foc* over other isolates. Srivastava *et al.* (2014) reported that the antagonistic variability of the isolates of *T. atroviride* revealed significant suppression in the radial growth of *F. o.* f. sp. *ciceri*. Maximum inhibition (55.08%) of mycelial growth was recorded in case of TH3 isolate against *F. o.* f. sp. *ciceri* (*Foc*) which was isolated from the soil sample of Bilgram (Hardoi), followed by TS6 (53.65%) isolated from Kadipur (Sultanpur) and isolate TSi4 (37.69%) from Misrikh (Sitapur) was found to be least effective against *Foc*.

According to Patibanda and Sen (2004), Zone of inhibition (Zi) signifies the end of "initial interaction stage" and beginning of "interaction stage" between the interacting fungi. Continued incubation beyond five days resulted in overgrowth making it clear that short periods (five days) of incubation may not be sufficient to determine the overgrowing / antagonistic potential of the pathogen. The present investigation also revealed that incubation up to five days was insufficient to categorize an isolate as potential or not *in vitro*. Hence, other qualitative parameters such as zone of inhibition, overgrowth potential, lysis, sporulation in *Trichoderma* at Zi and pigmentation in *Foc* were also considered for screening and selection of potential isolates (Table 2, Plate 1). Accordingly the following groups were formed.

1. Either Zi or lysis of *Foc* or both but without overgrowth of *Trichoderma* on *Foc*: T 19004, T 19005, T 19008, T 19009, T 19010, T 19011 and T 19013. These isolates were considered least potential (Plate 2).
2. Either Zi or lysis of *Foc* or both and overgrowth of *Trichoderma* on *Foc* but sporulation in *Trichoderma* was inhibited: T 19019, T 19024 and T 19025. These isolates were considered less potential (Plates 1 and 2).
3. Either Zi or lysis of *Foc* or both, overgrowth of *Trichoderma* on *Foc*, sporulation in *Trichoderma* not inhibited, with or without change in *Foc* medium pigmentation, faster radial growth in monoculture: T 19001, T 19002, T 19003, T 19006, T 19007, T 19012, T 19014, T 19015, T 19016, T 19017, T 19018, T 19020, T 19021, T 19022 and T 19023. These isolates were considered as having high antagonistic potential against *Foc* (Plates 1,2 and 3).

In *Phoma* sp. / *F. solani* interaction, dark purple pigmentation was observed on the test pathogen indicating the growth of the test endophyte was unaffected while that of the pathogen was reduced (Hamzah *et al.*, 2018). Blanchette (2018) reported that *Foc* grown in dual culture with *T. atroviride* from delayed inoculation of *T. atroviride* developed a darkened orange brown "frontline" within *Foc* that could be interpreted as the leading edge of *T. atroviride* growth overlapping into the *Foc* culture. Hyphal barrier morphology also differed depending on the *Trichoderma* species used in dual culture, timing of inoculation, and growth forms. This barrier was visible from both the top and reverse of the plate before sporulation of *T. atroviride*.

CONCLUSION

Currently, the control of Fusarium wilt is a major problem in endemic areas and biological control using *Trichoderma* could be an alternative. However, our study demonstrates that variation existed among the sympatric isolates of *Trichoderma* in their antagonistic potential against *Foc*. Hence, care need to be taken while selecting the isolate for biocontrol purpose.

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