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In vitro SELECTION OF MOROCCAN PHOSPHATE SITES' *Trichoderma* ISOLATES ACCORDING TO THEIR ANTAGONISM AGAINST THE WHEAT AND BARLEY ROOT ROT PATHOGENS

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ABSTRACT

The biocontrol abilities of *Trichoderma* isolates isolated from soil and roots collected from adjacent sites to the Khouribga and Benguerir Phosphate mines (Morocco) were tested *in vitro* by dual culture against different pathogens responsible for root rots in wheat and barley plants (*Bipolaris, Curvularia* and *Fusarium)* in the *in vitro* assays. Under *in vitro* conditions, *Trichoderma* isolates T1; 1TH; TR-EM 2; TR-B 98 (1); TR-B 98 (2); TR-B 98 (3) were more efficacious and they give significant inhibition of both mycelial growth and germination of the seven pathogens studied through the production of volatile metabolites and mycoparasitic capacity. A maximum percentage exceeding 70% was recorded. Moreover, direct confrontation tests showed a great ability of five isolates of *Trichoderma* species to colonize the medium in competition with pathogenic fungi exhibiting colonization percentages in the range of 49.33 to 75.43%. However, all pathogen isolates were comparatively less inhibited by all the species of *Trichoderma* by producing diffusible no-volatile compounds with percentages of growth and germination inhibition lower than 29.5 and 20.5% respectively.

Keywords: Antagonistic activity; *Trichoderma* spp.; *F. graminearum*; *F. culmorum*; *F. roseum*; *C. spicifera*; *B. sorokinana*; rot root; direct confrontation; indirect confrontation.

INTRODUCTION

Soil-borne pathogens affect a broader range of susceptible plant hosts generating telluric diseases such as seed rots, seedling damping-off, root rots and root wilt disease [1,2].

These diseases are caused by the members of the genus *Fusarium*, *Bipolaris* or *Curvularia* which lead to a high yield loss in cereal production. Seed treatments by fungicides application are a widely used common tool to eradicate the seed borne pathogens and/or protect them against soil pathogens, mainly at germination time [3].

Beside, chemicals pose serious health hazards to an applicator as well as to a consumer of the treated material. In addition to target organism, pesticides also kill various beneficial organisms [4]. Their toxic forms persist in soil and contaminate the whole environment [5].

Despite the economic losses they cause, the control of soil pathogens is still limited to prophylactic measures; soil disinfection is never complete because of the difficulty of carrying it out [6] and the induction of resistant strains. *Trichoderma* species are well known for their antagonistic abilities against other phytopathogenic fungi [7,8,9,10,11] and present abundantly in almost all type of soils [12,13]. *Trichoderma* are abundant in nature and can be isolated from soil, plant roots, decaying wood or other organic matter [14]. The antagonistic properties of *Trichoderma* were firstly mentioned by Vuillemin in 1887 [15]. Thus, the genus *Trichoderma* is the most commonly used fungal biological control agent against pathogens of crop plants, both soil-borne and foliar pathogens [16,17,18,19,20,8,21,22].

Many studies have proved the potential of *Trichoderma* spp. as biological agents antagonistic to several plant pathogen species such as *Bipolaris* on sorgho [23], *Rhizoctonia solani* [16] and *Fusarium* spp. [24]. Since then, not all *Trichoderma* are effective in controlling pathogens, it is essential to find out the *Trichoderma* with biocontrol potential which is possible through *in vitro* tests subsequently followed by pot and field experiments.

The mechanisms involved in the biocontrol activity of *Trichoderma* spp. against plant pathogens are important in designing effective and safe biocontrol strategies [25]. Different proposed mechanisms include: mycoparasitism (attack and killing of pathogen) [26] and competitive inhibition for space and nutrients [27]. *Trichoderma* are also known to produce different antibiotic substances e.g. gliotoxin, gliovirin, viridin, and trichoviridin [28]. However, all isolates of *Trichoderma* spp. are not equally effective in control of the pathogen *in vitro* [29,30].

Therefore, the objective of the present study was to assess under *in vitro* conditions the antagonism of thirty three previously collected isolates of *Trichoderma* [31] in suppressing three pathogenic fungi in wheat and barley plants originating from different fields in Gharb region [32].

MATERIALS AND METHODS

Trichoderma Isolates

Isolates of *Trichoderma* spp. used included seven isolates belonging to the mycobank of the Laboratory of Botany, Biotechnology and Plant Protection (LBBPP) and twenty-three newly isolated isolates from sites adjacent to the phosphate mines. They were maintained on PSA culture medium (200 g of potato 15 g Agar-agar and 1000 ml distilled water) at 28°C in the darkness (Table 1).

Culture of Plant Pathogens

The used isolates of *F. graminearum, F. culmorum*, *F. roseum*, *C. spicifera* and *B. sorokinana* were isolated from diseased crown and root of durum and soft wheat as well as barley which collected from different fields in Gharb area during cropping season 2017-2018 [32]. They were subcultured on PSA medium at 28°C in the darkness (Table 2).

Direct Confrontation of *Trichoderma* Isolates and Pathogens

Mycelial growth inhibition: A 5 mm- plug of bio-control agents and pathogen species were placed on solidified PDA medium at 40 mm-distance and incubated at 28°C in the darkness. Three plates for each treatment for each pathogen were used as three replicates. The plates inoculated with pathogens without *Trichoderma* isolates were a control. After three days of incubation, the average colony diameters were measured at two dimensions at right angle to each other and the percentage of mycelial inhibition (%) was calculated using the equation of Sy [33]:

$$IC\ (\%) = (DT\text{-}DPA) / DT \times 100$$

DT: Radial mycelial growth of the pathogen in control;
DPA: Radial mycelial growth of the pathogen in the presence of *Trichoderma* isolate.

Evaluation of space colonization by *Trichoderma*: In the same Petri dishes previously used for mycelial inhibition assessing,

Trichoderma colonization capacity was determined by the ratio C as follows:

$$C\ (\%) = (DT/DE) \times 100$$

C%: Colonization percentage of *Trichoderma*;
DT: The distance in mm covered by *Trichoderma* front growth on the axis connecting the cuttings of the two fungi;
DE: The distance separating the two fungi.

Conidia germination inhibition: Conidial suspensions of each pathogen and bio-control agents were prepared from 7 day-old cultures on PSA plates. Petri plates were flooded with 10 mL sterile distilled water then harvested by scraping with a spatula. The suspension was then filtered through mousseline. The conidial concentration was adjusted to 10^3 conidia.mL^{-1} before spreading successively 0.2 mL of the standardized suspensions of pathogen and antagonists onto Petri plates containing 20 mL of water agar medium. Three replicate plates were inoculated for each isolates of pathogen and antagonist. Inoculated plates were incubated at 25°C for 24 h in darkness.

Petri dish containing water agar medium only inoculated with conidia suspension of the pathogen without antagonist was a control.

After 24 h, observations on the conidia germination of pathogenic fungal species were recorded and percent inhibition of spore germination (IG%) was calculated by using the following formula:

$$IG\ (\%) = (NT\text{-}NPA) / NT \times 100$$

NT: number of germinated conidia in control;
NPA: number of conidia in presence of antagonist.

Production of Volatile Metabolites

Mycelial growth inhibition: Production of volatile antibiotic study was done following the method of Dennis and Webster [34]. Thirty isolates of different *Trichoderma* species were inoculated by placing 5 mm- disc in the center of the Petri plates containing solidified sterilized PSA medium. After that the top lid of each Petri plate was replaced with bottom part of another Petri plate with same size containing PSA medium, duly inoculated with a 5 mm- mycelial disc of the pathogen. The pairs of each plate were sealed and incubated at 28±1°C for 4 days. The PSA medium without *Trichoderma* isolate in the bottom served as control. The mycelial inhibition of pathogen was calculated according to above described formula.

Conidia germination inhibition: The bottom of Petri dish containing 2 days old cultures of *Trichoderma* isolate was placed below the bottom of Petri dish containing water agar medium on which 0.2 mL of conidial suspension of the pathogen adjusted to 10^3 conidia.mL^{-1} has been spread. The two Petri plate's bottoms were sealed with parafilm, and then incubated at 28°C in darkness. After 20 hours of incubation, the percent inhibition of germination was estimated according to technique described previously.

Production of Diffusible Metabolites

Mycelial growth inhibition: PSA plates containing cellophane paper were inoculated with 5 mm-mycelial discs of 7 days old *Trichoderma* isolates cultures and incubated at 28°C. After 2 days, cellophane paper was removed and a 5 mm-disc of the pathogen was placed on the same PSA plate [34]. The cultures were further incubated for 8 days at 25°C in darkness. The mycelial inhibition of pathogen by *Trichoderma* isolates was calculated over control.

Conidia germination inhibition: After removing cellophane paper with *Trichoderma* disc adhering, 0.2 mL of conidial suspension adjusted to 10^3 conidia.mL^{-1}, was spread on Petri plates containing water agar medium. 20 hours after incubation at 25°C in the darkness, percent inhibition of conidia germination was assessed as described above.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and LSD test at 5% level. The percentages were transformed into Arcsin $\sqrt{P}$ (where P is the proportion of percentage).

RESULTS

The antagonistic activity of thirty *Trichoderma* isolates towards different pathogens was investigated through the estimation of the three biocontrol mecanisms viz competition for space, mycoparasitism and production of antifungal metabolites in inhibiting mycelial growth and conidia germination of fungal species responsible of root rots in wheat and barley.

The measurement of the antagonist colonization percentage indicated significant differences in interaction degree between *Trichoderma* isolates and pathogens. The highest colonization percentages were obtained by the *Trichoderma* isolates T1; TS-B 98; TR-B 98 (3) with maximum percentages exceeding 61.25% in presence of *F. culmorum* (Fig. 4), *F. graminearum* (Fig. 6), *F. roseum* (Fig. 5); *C. spicifera* (Fig. 2) and *B. sorokiniana* (2) (Fig. 3). The best colonization percentage of *F. graminearum* was observed in case of T1 (76.3%); TR-EM 2 (76.83%); TR-B 98 (3) (74.23%). In comparison, *B. sorokiniana* was highly colonized by TR-OL 2; TR-TB 2000 and TR-B 98 (3) with percentages exceeding 67% (Table 3).

In direct confrontation tests, all tested *Trichoderma* isolates inhibited significantly the mycelial growth of pathogens at varied degrees. Of these, TR-OL1; TS-RP; TS-B98; TR-EM2; TR-B 98 (1) et TR-B 98 (3) exhibited maximum growth inhibition of *F. culmorum* overcoming 80% (Table 4) compared to those of TR-CB 2000 (2); TS-B-2000 (2); TR-TB 2000; TR-EM 2 et TR-B 98 (3) which reduced the growth of *F. gaminearum* by 81.19%, 83.33%, 80.55%, 83.33% and 81.94 respectively (Table 4). The isolates T1; TR-OL1; TR-EM 2; TR-B 98 (1); TR-B 98 (2) and TR-B 98 (3) presented the inhibition percents of mycelial growth of *F. roseum* comprised between 71.15% and 76.92% (Table 4). In direct confrontation with *B. sorokiniana* (1) (Fig. 1), a great reduction of the mycelial growth varied from 70.17% to 78.94% were obtained by twelve isolates viz T1; 1TH; TR-B 98/2002 (2); TR-OL 2; TS-B-2000 (2); TR-CB 2000 (1); TR-TB 2000; TR-OL 1; TS-B 98; TR-EM 2; TR-B 98 (1) et TR-B 98 (3) (Table 4). Similarly, the isolates T1; 1TH; TOL; T30; TY; TR-OL 2; TS-B-2000 (2); TR-TB 2000; TS-B 98; TR-EM 2; TR-B 98 (1); TR-B 98 (3) of *Trichoderma* performed well in inhibiting the mycelial growth of *C. spicifera* with a IC_{CD}% comprised between 71.37 and 76.14% (Table 4). While, in presence of *B. sorokiniana* (2), the highest growth repression were obtained by T1 (78.5%) followed by that of TR-B 98 (3) (76.19%) not significatly different from the inhibition percentage (71.42%) reached by TR-OL 2; TR-TB 2000; TR-OL 1; TS-B 98 ; TR-EM.

In dual culture, a satisfactory result was achieved by the *Trichoderma* isolates in stopping conidia germination of tested fungal species (Table 5). The most of the *Trichoderma* isolates exhibited significant inhibition surpassing 70% (Table 5). Some of the tested isolates differed in their ability to suppress the germination of various pathogens (Table 6). However, the isolates T1; TR-OL 1; TR-EM 2; TR-B 98 (1); TR-B 98 (2) et TR-B 98 (3) had equal efficiency with a value of IGCD% not significantly different which was superior to 70.5% (Table 6).

Concerning the *in vitro* inhibitory effect of volatile metabolites produced by the majority of *Trichoderma* isolates, the mycelial growth of the six pathogens were strongly reduced and higher percent inhibition were recorded (Table 6). Among isolates, there were seven which showed a identical performance in restricting the mycelial growth of all test pathogens. It was between 70.17% and 81.80% (Table 6). Moreover, volatile products of almost *Trichoderma* isolates had heavily affected the conidia germination of pathogenic fungi. The isolates T1; TR-OL 1; TR-B 98 (1); TR-B 98 (2) et TR-B 98 (3) showed inhibition rates of germination in the range of 70.5% to 77% (Table 7). Diffusible metabolites produced by all *Trichoderma* isolates were found no efficacious in reducing neither mycelial growth (Table 8) nor germination of whole test pathogens. The mycelial growth inhibition rates were low not exceeding 29.5% compared to 20.5% for as germination (Table 9).

Table 1. Origins and isolation sources of *Trichoderma* spp. isolates

***Trichoderma* spp. Isolates**	**Isolation sources**	**Localities (country)**
T1 (BankIt1902509 SMis1 KU987252) *Trichoderma asperellum*	TTC Compost	Missour/ Morocco
1TH	Bananas/ Mnasra	Kenitra region / Morocco
TH2	Bananas/Mnasra	Kenitra region / Morocco
T27(BankIt1902509 SDLA27 KU987250)*Trichoderma asperellum*	Strawberry, Festival variety	Dlalha / My Bouselham / Morrocco
T 30	Strawberry, Sabrina variety	Gnafda / My Bouselham / Morocco
TOL	Roots of an olive tree	Sidi Kacem/ Morocco
TY	Rhizosphere of an olive tree's roots	Sidi Kacem/ Morocco
TS-BG	Soil of Bengurir region,	Bengurir region /Morocco
TS-ML	Soil of Mrah Lahrech site	Khouribga region /Morocco
TS-H	Hattan site soil	
TS-RP	Pure phosphate rock	
TR-OL 1	Rhizosphere of the roots of an olive tree	
TR-OL 2	Rhizosphere of the roots of an olive tree	
TR-CB 2000 (2)	Root rhizosphere of a *Crucifera* agriculture, sludge 2000	
TR-TB 2000	Roots of *Tamarix*, sludge 2000	
TS-B 98	Sludge soil 1998	
TS-EM 98 (1)	Sludge soil 1998	
TS-EM 98 (2)	Sludge soil 1998	
TR-EM 1	Roots of mixed plant samples	
TR-EM 2	Roots of mixed plant samples	
TR-B 98 (1)	Sludge roots 1998	
TR-B 98 (2)	Sludge roots 1998	
TR-B 98 (3)	Sludge roots 1998	
TS-B 98/2002 (1)	Sludge roots 1998/2002	
TS-B 98/2002 (2)	Sludge roots 1998/2002	
TR-B 98/2002 (1)	Sludge roots 1998/2002	
TR-B 98/2002 (2)	Sludge roots 1998/2002	
TS-B 2000 (1)	Sludge soil 2000	
TS-B 2000 (2)	Sludge soil 2000	
TR-C B 2000 (1)	Sludge cruciferous roots 2000	

Table 2. Origins and isolation sources of fungal species responsible of root rots of wheat and barley

Pathogenic fungal species	**Codes**	**Isolation sources**	**Localities (countries)**
Fusarium culmorum	FC-RBD-SL	Roots / Durum wheat	Souk Larbaa/ Morocco
Fusarium graminearum	FG-RBT-SAT	Roots / Soft wheat	Sidi Allal tazi/ Morocco
Fusarium roseum	FR-RBD-O	Roots / Durum wheat	Ouazzane/ Morocco
Bipolaris sorokiniana 1	BS-CO-ST	Collar / Barley	Souk Tlat / Morocco
Bipolaris sorokiniana 2	BS-CO-OS	Collar / Barley	Oulad Sellam / Morocco
Curvularia spicifera	CS-CBD-M	Collar / Durum wheat	Mogren / Morocco

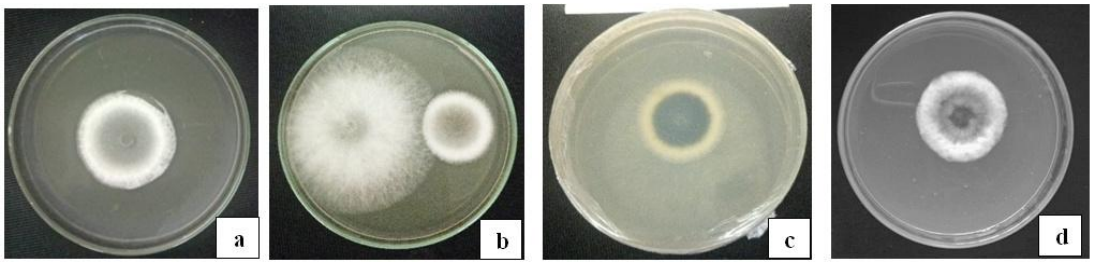

Fig. 1. *In vitro* effect of TR-B98(3) against *Bipolaris sorokiniana*(1) a: Control; b: direct confrontation of TR-B98(3) with *B. sorokiniana*; c: indirect confrontation by producing volatile compounds, d: indirect confrontation by producing diffusible compounds

Table 3. Colonization percentage during mycoparasitism process by thirty *Trichoderma* isolates on fungal growth of species causing root rots in wheat and barley (C%)

Antagonists	Pathogenic species					
	Fusarium culmorum	***Fusarium graminearum***	***Fusarium roseum***	***Bipolaris sorokiniana 1***	***Curvularia spicifera***	***Bipolaris sorokiniana 2***
T1	72.6^{a}_{a}	76.3^{a}_{a}	72.5^{a}_{a}	63^{b}_{b}	72.5^{a}_{a}	67.73^{b}_{a}
1TH	50^{b}_{cd}	54^{b}_{c}	47.5^{c}_{d}	62.5^{a}_{b}	51.25^{b}_{c}	51.06^{b}_{c}
TH_2	57.5^{a}_{c}	53.2^{a}_{c}	50^{a}_{c}	46.63^{b}_{d}	39^{c}_{e}	47.45^{b}_{d}
TOL	65^{a}_{b}	62.03^{a}_{bc}	47.5^{c}_{d}	55^{b}_{c}	39.75^{d}_{e}	51.06^{b}_{c}
T27	62.5^{b}_{b}	67.73^{a}_{b}	60^{b}_{b}	47.7^{c}_{d}	47.5^{c}_{d}	45.53^{c}_{d}
T30	54.25^{b}_{c}	52.67^{b}_{c}	62.5^{a}_{b}	55^{b}_{c}	37^{d}_{e}	49.75^{c}_{d}
TY	30^{c}_{e}	55.32^{a}_{c}	45^{b}_{d}	46.63^{b}_{d}	52.17^{a}_{c}	54.25^{a}_{c}
TS-ML	42.5^{c}_{d}	52.06^{b}_{c}	60^{a}_{b}	55^{b}_{c}	53.25^{b}_{c}	45.53^{c}_{d}
TS-H	37.5^{b}_{d}	56.5^{a}_{c}	55^{a}_{c}	55^{a}_{c}	52.25^{a}_{c}	51.06^{a}_{c}
TS-B 2000 (1)	65^{a}_{b}	54.39^{b}_{c}	57.5^{b}_{c}	46.63^{c}_{d}	55^{b}_{e}	47.5^{c}_{d}
TS-B 98/2002 (1)	28.5^{c}_{e}	47.9^{b}_{d}	55^{a}_{c}	49.13^{b}_{d}	53^{ab}_{c}	47.45^{b}_{d}
TS-B 98/2002 (2)	28.7^{c}_{e}	46.3^{a}_{d}	25^{c}_{e}	32.46^{b}_{e}	46.5^{a}_{d}	34.12^{b}_{e}
TR-B 98/2002 (1)	63.2^{a}_{b}	67.54^{a}_{b}	30^{c}_{e}	46.63^{b}_{d}	47.5^{b}_{d}	35.45^{c}_{e}
TR-B 98/2002 (2)	69.15^{a}_{b}	65.34^{a}_{b}	62.5^{a}_{b}	64^{a}_{b}	52.5^{b}_{c}	47.45^{c}_{d}
TR-OL 2	40^{c}_{d}	52.7^{b}_{c}	65^{a}_{b}	67.42^{a}_{a}	57.5^{b}_{c}	45.53^{c}_{d}
TS-EM-98 (1)	31.25^{c}_{e}	42.19^{b}_{d}	35^{c}_{c}	47.5^{b}_{d}	54.4^{a}_{c}	32.74^{c}_{e}
TR-CB 2000 (2)	55.75^{a}_{c}	52.76^{a}_{c}	40^{b}_{d}	45.98^{b}_{d}	47.5^{b}_{d}	35.77^{c}_{e}
TS-B-2000 (2)	58.75^{b}_{c}	58.63^{b}_{c}	62.5^{a}_{b}	61.42^{a}_{b}	53.25^{bc}_{c}	47.87^{c}_{d}
TR-CB 2000 (1)	35^{d}_{d}	48^{c}_{d}	67.5^{a}_{b}	64.13^{a}_{b}	57.5^{b}_{c}	49.75^{c}_{d}
TS-EM-98 (2)	54.5^{b}_{c}	56.8^{ab}_{c}	45^{c}_{d}	31.63^{d}_{e}	64^{a}_{b}	31.17^{d}_{e}
TR-TB 2000	62.5^{a}_{b}	57.5^{b}_{c}	60^{ab}_{b}	67.45^{a}_{a}	61.25^{a}_{b}	51.06^{b}_{c}
TS-BG	61.75^{a}_{bc}	53.53^{b}_{c}	55^{b}_{c}	53.16^{b}_{c}	53.25^{b}_{c}	50.2^{b}_{cd}
TR-OL 1	67.5^{a}_{b}	56.57^{b}_{c}	67.5^{a}_{b}	61.42^{a}_{b}	59.5^{ab}_{c}	47.60^{c}_{d}
TS-RP	68.5^{a}_{b}	58.58^{a}_{c}	57.5^{a}_{c}	55^{a}_{c}	54.5^{a}_{c}	47.5^{c}_{d}
TR-EM 1	58.5^{a}_{c}	56.58^{a}_{c}	57.5^{a}_{c}	53.85^{a}_{c}	58.5^{a}_{c}	40.13^{b}_{e}
TS-B 98	70^{a}_{ab}	58.32^{c}_{c}	72.5^{a}_{a}	61.42^{b}_{b}	67.5^{a}_{a}	67.60^{a}_{a}
TR-EM 2	69^{a}_{b}	76.83^{a}_{a}	67.5^{b}_{b}	64.13^{b}_{b}	55^{c}_{c}	49.33^{d}_{d}
TR-B 98 (1)	68.5^{a}_{b}	66.8^{a}_{b}	62.5^{a}_{b}	64.13^{a}_{b}	52.5^{b}_{c}	47.53^{b}_{d}
TR-B 98 (2)	67.75^{b}_{b}	57.05^{c}_{c}	57.5^{c}_{c}	62.4^{b}_{b}	52.17^{c}_{c}	37.5^{d}_{e}
TR-B 98 (3)	75.43^{a}_{a}	74.23^{a}_{a}	72.5^{a}_{a}	67.45^{b}_{a}	70.25^{a}_{a}	65.06^{a}_{a}

* The results of the same column followed by different index letters differ significantly at 5%.
* The values with different superscript letters in a line are significantly different at 5%.

Table 4. Mycelial growth inhibition ((IC_{CD}%) of some fungal species causing root rots in wheat and barly in the presence of *Trichoderma* isolates in tests of direct confrontation

Antagonists	Fungal species					
	Fusarium culmorum	***Fusarium graminearum***	***Fusarium roseum***	***Bipolaris sorokiniana 1***	***Curvularia spicifera***	***Bipolaris sorokiniana 2***
T1	79.12^{a}_{a}	78.04^{a}_{a}	76.92^{a}_{a}	78.94^{a}_{a}	76.14^{a}_{a}	78.51^{a}_{a}
1TH	67.14^{b}_{b}	57.87^{c}_{b}	61.53^{b}_{a}	72.87^{a}_{a}	72.47^{a}_{a}	61.19^{b}_{c}
TH_2	72.45^{a}_{a}	60.18^{b}_{b}	65.38^{b}_{a}	53.50^{c}_{c}	65.13^{b}_{a}	57.14^{c}_{d}
TOL	74.46^{a}_{a}	65.27^{b}_{b}	63.34^{b}_{a}	62.63^{b}_{b}	72.47^{a}_{a}	64.28^{b}_{c}
T27	73.26^{a}_{a}	62.73^{a}_{b}	53.45^{c}_{b}	56.14^{c}_{c}	72.47^{a}_{a}	61.90^{b}_{c}
T30	66.13^{b}_{b}	61.80^{b}_{b}	67.30^{b}_{b}	62.63^{b}_{b}	71.37^{a}_{a}	67.14^{b}_{b}
TY	24.53^{d}_{e}	63.88^{b}_{b}	53.96^{c}_{a}	58.66^{c}_{c}	72.47^{a}_{a}	61.19^{b}_{c}
TS-ML	46.52^{b}_{c}	68.05^{a}_{b}	68.45^{a}_{b}	62.98^{a}_{b}	54.86^{b}_{b}	61.19^{a}_{c}
TS-H	42.95^{c}_{c}	63.88^{a}_{b}	53.45^{b}_{a}	58.77^{b}_{c}	66.14^{a}_{a}	57.14^{b}_{d}
TS-B 2000 (1)	78.60^{a}_{a}	66.66^{b}_{b}	61.53^{b}_{a}	50.87^{c}_{cd}	65.13^{b}_{a}	61.90^{b}_{c}
TS-B 98/2002 (1)	24.24^{d}_{e}	55.55^{b}_{b}	63.34^{a}_{a}	42.30^{c}_{d}	51.32^{b}_{b}	54.76^{b}_{d}
TS-B 98/2002 (2)	26.91^{c}_{e}	42.73^{a}_{c}	38.56^{b}_{b}	36.53^{b}_{e}	45.59^{a}_{b}	33.3^{b}_{e}
TR-B 98/2002 (1)	80.65^{a}_{a}	79.16^{b}_{a}	42.17^{d}_{b}	41.22^{d}_{d}	56.60^{c}_{b}	40.47^{d}_{e}
TR-B 98/2002 (2)	82.19^{a}_{a}	77.7^{b}_{a}	69.23^{c}_{a}	77.19^{b}_{a}	64.14^{c}_{a}	61.19^{c}_{c}

Antagonists	Fungal species					
	Fusarium culmorum	*Fusarium graminearum*	*Fusarium roseum*	*Bipolaris sorokiniana 1*	*Curvularia spicifera*	*Bipolaris sorokiniana 2*
TR-OL 2	34.12^{d}_{d}	59.72^{c}_{b}	61.53^{b}_{a}	75.43^{a}_{a}	72.84^{a}_{a}	71.42^{a}_{b}
TS-EM-98 (1)	37.6^{b}_{d}	37.5^{b}_{c}	42.17^{a}_{b}	42.10^{a}_{d}	45.5^{a}_{b}	40.47^{a}_{e}
TR-CB 2000 (2)	71.47^{b}_{ab}	81.19^{a}_{a}	47.36^{c}_{b}	48.24^{c}_{d}	41.28^{c}_{b}	35.71^{d}_{e}
TS-B-2000 (2)	$78\ .60^{b}_{a}$	83.33^{a}_{a}	69.23^{c}_{a}	71.92^{bc}_{a}	73.85^{b}_{a}	61.19^{c}_{c}
TR-CB 2000 (1)	20.52^{e}_{e}	36.11^{d}_{c}	75^{a}_{a}	70.36^{a}_{a}	49.5^{c}_{b}	64.28^{b}_{c}
TS-EM-98 (2)	67.91^{a}_{b}	63.42^{a}_{b}	37.57^{bc}_{b}	36.84^{c}_{e}	69.35^{a}_{a}	40.47^{b}_{e}
TR-TB 2000	79.64^{a}_{a}	80.55^{a}_{a}	69.23^{b}_{a}	77.8^{a}_{a}	71.59^{b}_{a}	71.42^{b}_{b}
TS-BG	64.34^{a}_{b}	66.66^{a}_{b}	50^{b}_{b}	63.15^{a}_{b}	64.22^{a}_{a}	54.76^{b}_{d}
TR-OL 1	82.17^{a}_{a}	77.7^{b}_{a}	73.03^{b}_{a}	70.89^{b}_{a}	69.88^{b}_{a}	71.42^{b}_{b}
TS-RP	81.4^{a}_{a}	65.27^{b}_{b}	68.45^{b}_{a}	64.91^{b}_{b}	69.17^{b}_{a}	50^{c}_{d}
TR-EM 1	67.91^{a}_{b}	61.11^{a}_{b}	61.53^{a}_{a}	57.01^{b}_{c}	63.45^{a}_{a}	54.76^{b}_{d}
TS-B 98	81.80^{a}_{a}	73.61^{b}_{a}	69.23^{b}_{a}	72.63^{b}_{a}	72.11^{b}_{a}	71.42^{b}_{b}
TR-EM 2	82.60^{a}_{a}	83.33^{a}_{a}	71.15^{b}_{a}	70.87^{b}_{a}	71.37^{b}_{a}	71.42^{b}_{b}
TR-B 98 (1)	83.31^{a}_{a}	76.38^{b}_{a}	73.03^{b}_{a}	70.17^{b}_{a}	73.39^{b}_{a}	69.04^{b}_{b}
TR-B 98 (2)	74.23^{a}_{a}	61.11^{b}_{b}	75^{a}_{a}	65.14^{b}_{b}	67.2^{b}_{a}	57.14^{b}_{d}
TR-B 98 (3)	86.47^{a}_{a}	81.94^{a}_{a}	76.84^{b}_{a}	71.97^{b}_{a}	76.14^{b}_{a}	76.19^{b}_{a}

* The results of the same column followed by different index letters differ significantly at 5%.
* The values with different superscript letters in a line are significantly different at 5%.

Table 5. Conidia germination inhibition (IG_{CD} %) of fungi causing root rot diseases in wheat and barly in the presence of *Trichoderma* isolates in tests of direct confrontation

Antagonists	Pathogenic species					
	Fusarium culmorum	*Fusarium graminearum*	*Fusarium Roseum*	*Bipolaris sorokiniana 1*	*Curvularia spicifera*	*Bipolaris sorokiniana 2*
T1	73.5^{a}_{a}	73.25^{a}_{a}	76.5^{a}_{a}	74.25^{a}_{a}	77.25^{a}_{a}	75.5^{a}_{a}
1TH	79.5^{a}_{a}	70.5^{a}_{a}	70.5^{a}_{a}	72.25^{a}_{a}	77.5^{a}_{a}	70.25^{a}_{a}
TH_2	63.5^{a}_{a}	68.5^{a}_{b}	67.5^{a}_{b}	68.5^{a}_{a}	67.5^{a}_{b}	61.25^{a}_{b}
TOL	62.25^{b}_{a}	63.25^{b}_{b}	65.5^{b}_{b}	64.5^{b}_{a}	74.5^{a}_{a}	64^{b}_{b}
T27	67.5^{b}_{a}	61.5^{b}_{b}	53.25^{c}_{c}	57.5^{c}_{a}	73.75^{a}_{a}	67^{b}_{b}
T30	65.5^{b}_{a}	57.25^{c}_{c}	65.25^{b}_{b}	66.5^{b}_{a}	73.75^{a}_{a}	64.75^{b}_{b}
TY	41.25^{c}_{b}	62.25^{a}_{b}	52.5^{b}_{c}	55^{b}_{b}	67.5^{a}_{b}	39.5^{c}_{d}
TS-ML	45.25^{c}_{b}	65.25^{a}_{b}	65.25^{a}_{b}	64.5^{a}_{a}	53.75^{b}_{c}	62^{a}_{b}
TS-H	43.25^{c}_{b}	62.25^{a}_{b}	50.75^{b}_{c}	50^{b}_{b}	52.5^{b}_{c}	42^{c}_{d}
TS-B 2000 (1)	63.5^{b}_{a}	65.25^{b}_{b}	60.5^{b}_{b}	60^{b}_{a}	73.75^{a}_{a}	64^{b}_{b}
TS-B 98/2002 (1)	45.5^{c}_{b}	50.5^{b}_{c}	60.5^{a}_{b}	60^{a}_{a}	44.25^{c}_{c}	42^{c}_{d}
TS-B 98/2002 (2)	45.25^{a}_{b}	38.25^{b}_{d}	35.75^{bc}_{d}	31.5^{c}_{c}	36.25^{b}_{d}	44.5^{a}_{d}
TR-B 98/2002 (1)	75.5^{a}_{a}	73.25^{a}_{a}	37.5^{c}_{d}	35.25^{c}_{c}	54.5^{b}_{c}	75^{a}_{a}
TR-B 98/2002 (2)	75.5^{a}_{a}	65.25^{b}_{b}	67.5^{b}_{b}	65^{b}_{a}	76.25^{a}_{a}	75^{a}_{a}
TR-OL 2	45.25^{d}_{b}	56.25^{c}_{c}	60.5^{b}_{b}	60^{b}_{a}	74.25^{a}_{a}	64^{b}_{b}
TS-EM-98 (1)	45.5^{a}_{b}	38.25^{b}_{d}	37.25^{b}_{d}	36.5^{b}_{c}	47.75^{a}_{c}	44^{a}_{d}
TR-CB 2000 (2)	63.5^{b}_{a}	77.25^{a}_{a}	45.5^{c}_{c}	46^{c}_{b}	43.75^{c}_{c}	61.75^{b}_{b}
TS-B-2000 (2)	73.25^{a}_{a}	73.25^{a}_{a}	67.5^{b}_{b}	70.5^{a}_{a}	73.5^{a}_{a}	75^{a}_{a}
TR-CB 2000 (1)	45.25^{c}_{b}	35.5^{d}_{d}	76.5^{a}_{a}	78.5^{a}_{a}	44.25^{c}_{c}	66^{b}_{b}
TS-EM-98 (2)	63.5^{a}_{a}	63.25^{a}_{b}	35.5^{b}_{d}	32^{b}_{c}	67.5^{a}_{b}	66^{a}_{b}
TR-TB 2000	73.25^{a}_{a}	75.25^{a}_{a}	65.5^{b}_{b}	67^{b}_{a}	78^{a}_{a}	73^{a}_{a}
TS-BG	63.5^{a}_{a}	63.25^{a}_{b}	50.5^{b}_{c}	50^{b}_{b}	67.5^{a}_{b}	66^{a}_{b}
TR-OL 1	73.5^{a}_{a}	75.25^{a}_{a}	75.5^{a}_{a}	77^{a}_{a}	73.75^{a}_{a}	75^{a}_{a}
TS-RP	75.25^{a}_{a}	63.25^{b}_{b}	67.5^{b}_{b}	68.25^{b}_{a}	67.5^{b}_{b}	75^{a}_{a}
TR-EM 1	63.5^{b}_{a}	63.25^{b}_{b}	60.5^{b}_{b}	62^{b}_{a}	73.75^{a}_{a}	61.75^{b}_{b}
TS-B 98	73.25^{a}_{a}	66.25^{b}_{b}	67.5^{b}_{b}	67^{b}_{a}	71.5^{a}_{a}	73^{a}_{a}
TR-EM 2	75.25^{a}_{a}	73.25^{a}_{a}	77.5^{a}_{a}	77^{a}_{a}	72.5^{a}_{a}	75^{a}_{a}
TR-B 98 (1)	73.5^{a}_{a}	73.25^{a}_{a}	75.5^{a}_{a}	76.25^{a}_{a}	78^{a}_{a}	73.25^{a}_{a}
TR-B 98 (2)	72.5^{a}_{a}	76.25^{a}_{a}	76.5^{a}_{a}	76.25^{a}_{a}	73.75^{a}_{a}	74.5^{a}_{a}
TR-B 98 (3)	75.25^{a}_{a}	77.25^{a}_{a}	76.5^{a}_{a}	78^{a}_{a}	78^{a}_{a}	77^{a}_{a}

* The results of the same column followed by different index letters differ significantly at 5%.
* The values with different superscript letters in a the same line are significantly different at 5%.

Table 6. Inhibition of mycelial growth (IG_{SV}%) of fungi causing root rot in wheat and barly through volatile metabolites produced by different *Trichoderma* isolates

Antagonists	Fungal species					
	Fusarium culmorum	***Fusarium graminearum***	***Fusarium roseum***	***Bipolaris sorokiniana 1***	***Curvularia spicifera***	***Bipolaris sorokiniana 2***
T1	75.12^{a}_{a}	73.61^{a}_{a}	75^{a}_{a}	73.1^{a}_{a}	73.4^{a}_{a}	72.25^{a}_{a}
1TH	64.88^{a}_{a}	61.75^{a}_{b}	65.38^{a}_{a}	64.3^{a}_{a}	62.96^{a}_{b}	67.14^{a}_{b}
TH_2	67.11^{b}_{a}	73.14^{a}_{a}	61.53^{b}_{a}	54.8^{c}_{b}	64.81^{b}_{b}	65.55^{b}_{b}
TOL	72.56^{ab}_{a}	66.20^{b}_{b}	61.53^{b}_{a}	56.1^{c}_{b}	68.51^{b}_{b}	69.04^{b}_{b}
T27	68.59^{a}_{a}	66.20^{a}_{b}	53.38^{b}_{b}	56.14^{b}_{b}	62.96^{a}_{b}	64.81^{a}_{b}
T30	64.88^{b}_{a}	72.22^{a}_{a}	65.38^{b}_{a}	54.38^{c}_{b}	64.81^{b}_{b}	65.55^{b}_{b}
TY	26.4^{c}_{c}	75^{a}_{a}	59.61^{c}_{a}	66.66^{b}_{a}	64.81^{b}_{b}	62.96^{b}_{b}
TS-ML	37.6^{e}_{c}	72.22^{a}_{a}	65.38^{b}_{a}	52.63^{c}_{b}	46.48^{d}_{d}	62.96^{b}_{b}
TS-H	35.66^{d}_{c}	70.83^{a}_{a}	51.19^{c}_{b}	55.2^{c}_{b}	64.81^{b}_{b}	54.38^{c}_{c}
TS-B 2000 (1)	71.56^{a}_{a}	68.33^{b}_{b}	53.96^{c}_{b}	56.64^{c}_{b}	50^{c}_{c}	61.11^{b}_{b}
TS-B 98/2002 (1)	28.10^{c}_{c}	55.55^{b}_{c}	61.53^{a}_{a}	50.8^{b}_{b}	55.55^{b}_{c}	55.55^{b}_{c}
TS-B 98/2002 (2)	28.69^{d}_{c}	59.7^{a}_{b}	42.30^{b}_{b}	52.63^{a}_{b}	44.4^{b}_{d}	32.25^{c}_{e}
TR-B 98/2002 (1)	78.26^{a}_{a}	77.77^{a}_{a}	32.69^{d}_{c}	52.63^{b}_{b}	42.59^{c}_{d}	37.6^{d}_{e}
TR-B 98/2002 (2)	78.4^{a}_{a}	75^{a}_{a}	65.38^{b}_{a}	60.52^{b}_{a}	68.51^{b}_{b}	64.81^{b}_{b}
TR-OL 2	31.66^{d}_{c}	51.11^{c}_{c}	53.96^{c}_{b}	68.42^{b}_{a}	70.37^{a}_{a}	69.04^{ab}_{b}
TS-EM-98 (1)	30.77^{c}_{c}	50^{a}_{c}	46.15^{b}_{b}	53.50^{a}_{b}	50^{a}_{c}	37.6^{c}_{e}
TR-CB 2000 (2)	63.78^{b}_{a}	73.6^{a}_{a}	42.30^{d}_{b}	50.87^{c}_{b}	44.4^{d}_{d}	32.25^{e}_{e}
TS-B-2000 (2)	73.07^{a}_{a}	79.16^{a}_{a}	75.38^{a}_{a}	70.17^{a}_{a}	70.37^{a}_{a}	72.96^{a}_{a}
TR-CB 2000 (1)	23.12^{e}_{c}	45.8^{d}_{c}	73.07^{a}_{a}	71.92^{a}_{a}	55^{c}_{c}	64.81^{b}_{b}
TS-EM-98 (2)	64.24^{a}_{a}	61.11^{a}_{b}	46.15^{b}_{b}	50.87^{b}_{b}	64.40^{a}_{b}	47.61^{b}_{d}
TR-TB 2000	76.56^{a}_{a}	73.61^{a}_{a}	65.38^{b}_{a}	66.14^{b}_{a}	70.37^{a}_{a}	69.04^{ab}_{b}
TS-BG	56.92^{b}_{b}	62.27^{a}_{b}	53.96^{b}_{b}	55.2^{b}_{b}	67.40^{a}_{b}	50^{b}_{c}
TR-OL 1	77.10^{a}_{a}	75^{a}_{a}	73.07^{a}_{a}	70.17^{a}_{a}	64.81^{b}_{b}	69.04^{ab}_{b}
TS-RP	75.33^{a}_{a}	65.04^{b}_{b}	65.38^{b}_{a}	57.89^{c}_{b}	67.40^{b}_{b}	50^{c}_{c}
TR-EM 1	60.74^{a}_{a}	62.27^{a}_{b}	53.96^{b}_{b}	52.63^{b}_{b}	65^{a}_{b}	55.55^{b}_{c}
TS-B 98	75.98^{a}_{a}	75^{a}_{a}	75.38^{a}_{a}	70.17^{a}_{a}	70.37^{a}_{ab}	73.04^{a}_{a}
TR-EM 2	74.15^{a}_{a}	76.38^{a}_{a}	72.23^{a}_{a}	73.68^{a}_{a}	73.51^{a}_{a}	71.42^{a}_{a}
TR-B 98 (1)	77.42^{a}_{a}	77.77^{a}_{a}	72.23^{a}_{a}	70.17^{a}_{a}	72.22^{a}_{a}	73.09^{a}_{a}
TR-B 98 (2)	73.15^{a}_{a}	65.27^{b}_{b}	69.23^{b}_{a}	62.63^{b}_{a}	65.5^{b}_{b}	55.55^{c}_{c}
TR-B 98 (3)	81.06^{a}_{a}	80.55^{a}_{a}	73.07^{b}_{a}	72.80^{b}_{a}	70.37^{b}_{a}	73.80^{b}_{a}

* The results of the same column followed by different index letters differ significantly at 5%.
* The values with different superscript letters in the same line are significantly different at 5%.

Table 7. Condia germination inhibition (IG_{SV}%) of fungi causing root rot in wheat and barly through volatile metabolites produced by different *Trichoderma* isolates

Antagonists	Fungal species					
	Fusarium culmorum	***Fusarium graminearum***	***Fusarium roseum***	***Bipolaris sorokiniana 1***	***Curvularia spicifera***	***Bipolaris sorokiniana 2***
T1	72.25^{a}_{a}	72.5^{a}_{a}	75.5^{a}_{a}	76^{a}_{a}	75^{a}_{a}	76^{a}_{a}
1TH	67.5^{a}_{b}	51^{c}_{c}	60.25^{b}_{b}	64^{b}_{b}	53.75^{c}_{b}	65.75^{a}_{a}
TH_2	62.5^{a}_{b}	56.5^{b}_{c}	67.5^{a}_{b}	66.5^{a}_{b}	66.5^{a}_{a}	63.75^{a}_{a}
TOL	64.5^{a}_{b}	64.25^{a}_{b}	60.5^{a}_{b}	60^{a}_{b}	61.5^{a}_{a}	62.25^{a}_{a}
T27	65.25^{a}_{b}	61.5^{a}_{b}	48.75^{b}_{c}	50^{b}_{c}	66.5^{a}_{a}	61.5^{a}_{a}
T30	63.25^{a}_{b}	58.5^{b}_{c}	63.25^{a}_{b}	63^{a}_{b}	53.75^{b}_{b}	67.25^{a}_{a}
TY	43.5^{b}_{c}	64.5^{a}_{b}	60.5^{a}_{b}	62^{a}_{b}	67.25^{a}_{a}	62.25^{a}_{a}
TS-ML	43.5^{c}_{c}	66.5^{a}_{b}	65.25^{a}_{b}	63^{a}_{b}	50^{b}_{b}	63.75^{a}_{a}
TS-H	43.5^{c}_{c}	64.5^{a}_{b}	48.5^{b}_{c}	50^{b}_{c}	66.5^{a}_{a}	51.5^{b}_{b}
TS-B 2000 (1)	62.25^{a}_{b}	63.5^{a}_{b}	58.5^{b}_{c}	57^{b}_{b}	63.25^{a}_{a}	65^{a}_{a}
TS-B 98/2002 (1)	43.5^{c}_{c}	50^{b}_{c}	60.5^{a}_{b}	60^{a}_{b}	50^{b}_{b}	62.25^{a}_{a}
TS-B 98/2002 (2)	43.5^{a}_{c}	$40^{a}_{\mathbf{d}}$	40.75^{a}_{d}	45^{a}_{c}	42.5^{a}_{b}	47^{a}_{b}
TR-B 98/2002 (1)	72.5^{a}_{a}	72.25^{a}_{a}	40.5^{c}_{d}	44^{c}_{c}	53.25^{b}_{b}	44.5^{c}_{b}
TR-B 98/2002 (2)	73.5^{a}_{a}	63.5^{b}_{b}	65.5^{b}_{b}	64.25^{b}_{b}	68.5^{b}_{a}	68^{b}_{a}
TR-OL 2	43.5^{d}_{c}	57.5^{c}_{c}	60.75^{b}_{b}	64.25^{b}_{b}	72.5^{a}_{a}	68^{b}_{a}

Antagonists	Fungal species					
	Fusarium culmorum	*Fusarium graminearum*	*Fusarium roseum*	*Bipolaris sorokiniana 1*	*Curvularia spicifera*	*Bipolaris sorokiniana 2*
TS-EM-98 (1)	$43.5^{a}{}_{c}$	$39.5^{a}{}_{d}$	$42.75^{a}{}_{d}$	$40.5^{a}{}_{c}$	$48.5^{a}{}_{b}$	$44.5^{a}{}_{b}$
TR-CB 2000 (2)	$62.5^{b}{}_{b}$	$76.5^{a}{}_{a}$	$40.5^{c}{}_{d}$	$40.5^{c}{}_{c}$	$46.5^{c}{}_{b}$	$44.5^{c}{}_{b}$
TS-B-2000 (2)	$73^{a}{}_{a}$	$76.5^{a}{}_{a}$	$65.5^{b}{}_{b}$	$65^{b}{}_{b}$	$76.25^{a}{}_{a}$	$67^{b}{}_{a}$
TR-CB 2000 (1)	$43.5^{b}{}_{c}$	$35^{c}{}_{d}$	$75.5^{a}{}_{a}$	$72.5^{a}{}_{a}$	$45.5^{b}{}_{b}$	$75.25^{a}{}_{a}$
TS-EM-98 (2)	$62.5^{a}{}_{b}$	$63.5^{a}{}_{b}$	$42.25^{b}{}_{d}$	$45^{b}{}_{c}$	$63.25^{a}{}_{a}$	$47^{b}{}_{b}$
TR-TB 2000	$72.5^{a}{}_{a}$	$74.5^{a}{}_{a}$	$65.5^{b}{}_{b}$	$63.5^{b}{}_{b}$	$74.25^{a}{}_{a}$	$65.75^{b}{}_{a}$
TS-BG	$62.25^{a}{}_{b}$	$62.5^{a}{}_{\mathbf{b}}$	$50.5^{b}{}_{c}$	$50^{b}{}_{c}$	$64.5^{a}{}_{a}$	$55^{b}{}_{b}$
TR-OL 1	$72.5^{a}{}_{a}$	$75.25^{a}{}_{a}$	$75.5^{a}{}_{a}$	$73.5^{a}{}_{a}$	$73.25^{a}{}_{a}$	$75.25^{a}{}_{a}$
TS-RP	$72.5^{a}{}_{a}$	$63.5^{b}{}_{b}$	$68.5^{b}{}_{b}$	$67^{b}{}_{b}$	$63.25^{b}{}_{a}$	$67^{b}{}_{a}$
TR-EM 1	$62.5^{a}{}_{b}$	$63.5^{a}{}_{b}$	$50.25^{b}{}_{c}$	$50^{b}{}_{c}$	$64.75^{a}{}_{a}$	$55^{b}{}_{b}$
TS-B 98	$72.5^{a}{}_{a}$	$68.25^{b}{}_{b}$	$65.5^{b}{}_{b}$	$67^{b}{}_{b}$	$68.5^{b}{}_{a}$	$66^{b}{}_{a}$
TR-EM 2	$72.5^{a}{}_{a}$	$72.5^{a}{}_{a}$	$67.75^{b}{}_{b}$	$67^{b}{}_{b}$	$76^{a}{}_{a}$	$69.5^{b}{}_{a}$
TR-B 98 (1)	$72.5^{a}{}_{a}$	$72.5^{a}{}_{a}$	$71.5^{a}{}_{a}$	$74^{a}{}_{a}$	$72^{a}{}_{a}$	$73.25^{a}{}_{a}$
TR-B 98 (2)	$72.5^{a}{}_{a}$	$73.5^{a}{}_{a}$	$72.5^{a}{}_{a}$	$72.5^{a}{}_{a}$	$70.5^{a}{}_{a}$	$75.25^{a}{}_{a}$
TR-B 98 (3)	$73.5^{a}{}_{a}$	$76.5^{a}{}_{a}$	$75.5^{a}{}_{a}$	$77^{a}{}_{a}$	$76.25^{a}{}_{\mathbf{a}}$	$76^{a}{}_{a}$

* The results of the same column followed by different index letters differ significantly at 5%.
* The values with different superscript letters in the same line are significantly different at 5%.

Table 8. Inhibition of mycelial growth (IC_{SD}%) of fungi causing root rot in wheat and barly through diffusible metabolites produced by different *Trichoderma* isolates

Antagonists	Fungal species					
	Fusarium culmorum	*Fusarium graminearum*	*Fusarium roseum*	*Bipolaris sorokiniana 1*	*Curvularia spicifera*	*B.bipolaris sorokiniana 2*
T1	$28.47^{a}{}_{a}$	$27.12^{a}{}_{a}$	$29.50^{a}{}_{a}$	$27.54^{a}{}_{a}$	$28.10^{a}{}_{a}$	$20.33^{a}{}_{a}$
1TH	$23.05^{a}{}_{a}$	$21.66^{a}{}_{a}$	$23^{a}{}_{a}$	$26.47^{a}{}_{a}$	$25.13^{a}{}_{a}$	$26.16^{a}{}_{a}$
TH_2	$27.1^{a}{}_{a}$	$23.05^{a}{}_{a}$	$26.25^{a}{}_{a}$	$23\ .14^{a}{}_{a}$	$22,19^{a}{}_{a}$	$24.29^{a}{}_{a}$
TOL	$25.8^{a}{}_{a}$	$28.75^{a}{}_{a}$	$26.50^{a}{}_{a}$	$29.28^{a}{}_{a}$	$16,75^{b}{}_{b}$	$22.73^{a}{}_{a}$
T27	$28.10^{a}{}_{a}$	$25.41^{a}{}_{a}$	$22.50^{a}{}_{a}$	$26.16^{a}{}_{a}$	$22^{a}{}_{a}$	$22.19^{a}{}_{a}$
T30	$23.94^{a}{}_{a}$	$21.38^{a}{}_{a}$	$24.55^{a}{}_{a}$	$29.54^{a}{}_{a}$	$23.94^{a}{}_{a}$	$25.41^{a}{}_{a}$
TY	$16.75^{b}{}_{b}$	$26.80^{a}{}_{a}$	$27.25^{a}{}_{a}$	$26,72^{a}{}_{a}$	$23.94^{a}{}_{a}$	$24.19^{a}{}_{a}$
TS-ML	$25.13^{a}{}_{a}$	$21.66^{a}{}_{a}$	$22.50^{a}{}_{a}$	$24.61^{a}{}_{a}$	$27.66^{a}{}_{a}$	$23.49^{a}{}_{a}$
TS-H	$27.66^{a}{}_{a}$	$21.38^{ab}{}_{a}$	$26.25^{a}{}_{a}$	$29.6^{a}{}_{a}$	$19.32^{b}{}_{ab}$	$24.29^{a}{}_{a}$
TS-B 2000 (1)	$26.52^{a}{}_{a}$	$25.78^{a}{}_{a}$	$27.25^{a}{}_{a}$	$23.14^{a}{}_{a}$	$26.52^{a}{}_{a}$	$26.42^{a}{}_{a}$
TS-B 98/2002 (1)	$25.6^{a}{}_{a}$	$21.94^{a}{}_{a}$	$24.61^{a}{}_{a}$	$22.65^{a}{}_{a}$	$22^{a}{}_{a}$	$22.19^{a}{}_{a}$
TS-B 98/2002 (2)	$22^{a}{}_{a}$	$26.11^{a}{}_{a}$	$26.50^{a}{}_{a}$	$23.19^{a}{}_{a}$	$16,75^{b}{}_{b}$	$22.73^{a}{}_{a}$
TR-B 98/2002 (1)	$22.19^{a}{}_{a}$	$26.33^{a}{}_{a}$	$24.50^{a}{}_{a}$	$24.43^{a}{}_{a}$	$19.32^{b}{}_{ab}$	$23.49^{a}{}_{a}$
TR-B 98/2002 (2)	$24.55^{a}{}_{a}$	$20.88^{a}{}_{ab}$	$26.25^{a}{}_{a}$	$26.35^{a}{}_{a}$	$25.63^{a}{}_{a}$	$24.43^{a}{}_{a}$
TR-OL 2	$22^{a}{}_{a}$	$19.58^{b}{}_{b}$	$23.50^{a}{}_{a}$	$25.12^{a}{}_{a}$	$25.84^{a}{}_{a}$	$26.35^{a}{}_{a}$
TS-EM-98 (1)	$15.32^{b}{}_{b}$	$17.36^{b}{}_{b}$	$24.50^{a}{}_{a}$	$20.12^{a}{}_{a}$	$24.55^{a}{}_{a}$	$24.19^{a}{}_{a}$
TR-CB 2000 (2)	$23.94^{b}{}_{a}$	$22.77^{b}{}_{a}$	$22^{b}{}_{a}$	$26.42^{b}{}_{a}$	$22^{b}{}_{a}$	$24.29^{b}{}_{a}$
TS-B-2000 (2)	$21.07^{a}{}_{a}$	$22.70^{a}{}_{a}$	$25.50^{a}{}_{a}$	$23.71^{a}{}_{a}$	$24.43^{a}{}_{a}$	$26.42^{a}{}_{a}$
TR-CB 2000 (1)	$26.98^{a}{}_{a}$	$15.97^{b}{}_{b}$	$24.50^{a}{}_{a}$	$24.19^{a}{}_{a}$	$23.14^{a}{}_{a}$	$24.59^{a}{}_{a}$
TS-EM-98 (2)	$27.21^{a}{}_{a}$	$25.78^{a}{}_{a}$	$27.25^{a}{}_{a}$	$25.41^{a}{}_{a}$	$19.32^{b}{}_{ab}$	$20.33^{b}{}_{a}$
TR-TB 2000	$28.34^{a}{}_{a}$	$22.77^{a}{}_{a}$	$22.50^{a}{}_{a}$	$26.74^{a}{}_{a}$	$25.41^{a}{}_{a}$	$16.66^{b}{}_{b}$
TS-BG	$24.5^{a}{}_{a}$	$25.78^{a}{}_{a}$	$26.50^{a}{}_{a}$	$24.19^{a}{}_{a}$	$22.73^{a}{}_{a}$	$24.29^{a}{}_{a}$
TR-OL 1	$26.42^{a}{}_{a}$	$20.88^{a}{}_{ab}$	$23^{a}{}_{a}$	$25.\ 21^{a}{}_{a}$	$21.66^{a}{}_{a}$	$20,0^{a}{}_{a}$
TS-RP	$21.66^{a}{}_{a}$	$27.17^{a}{}_{a}$	$23.50^{a}{}_{a}$	$24.62^{a}{}_{a}$	$24.62^{a}{}_{a}$	$22.73^{a}{}_{a}$
TR-EM 1	$22^{a}{}_{a}$	$27.32^{a}{}_{a}$	$27.25^{a}{}_{a}$	$22.19^{a}{}_{a}$	$21.44^{a}{}_{a}$	$24.59^{a}{}_{a}$
TS-B 98	$20.49^{ab}{}_{a}$	$21.26^{a}{}_{a}$	$23.25^{a}{}_{a}$	$24.29^{a}{}_{a}$	$23.74^{a}{}_{a}$	$18.33^{b}{}_{b}$
TR-EM 2	$22.53^{a}{}_{a}$	$26.33^{a}{}_{a}$	$24.50^{a}{}_{a}$	$23.73^{a}{}_{a}$	$23.05^{a}{}_{a}$	$25.64^{a}{}_{a}$
TR-B 98 (1)	$25.13^{a}{}_{a}$	$22.04^{a}{}_{a}$	$26.50^{a}{}_{a}$	$22.73^{a}{}_{a}$	$25.8^{a}{}_{a}$	$20.33^{a}{}_{a}$
TR-B 98 (2)	$28.62^{a}{}_{a}$	$26.62^{a}{}_{a}$	$27.25^{a}{}_{a}$	$24.43^{a}{}_{a}$	$23.41^{a}{}_{a}$	$18.33^{b}{}_{b}$
TR-B 98 (3)	$27.5^{a}{}_{a}$	$25.64^{a}{}_{a}$	$26.25^{a}{}_{a}$	$25.64^{a}{}_{a}$	$26.24^{a}{}_{a}$	$25.20^{a}{}_{a}$

* The results of the same column followed by different index letters differ significantly at 5%.
* The values with different superscript letters in the same line are significantly different at 5%.

Table 9. Conidia germination inhibition (IC_{SD}%) of fungi causing root rot in wheat and barly through diffusible metabolites produced by different *Trichoderma* isolates

Antagonists	Species of fungal pathogens					
	Fusarium culmorum	***Fusarium graminearum***	***Fusarium roseum***	***Bipolaris sorokiniana 1***	***Curvularia spicifera***	***Bipolaris sorokiniana 2***
T1	20.5^{a}_{a}	20.5^{a}_{a}	20^{a}_{a}	19^{a}_{a}	19.5^{a}_{a}	23^{a}_{a}
1TH	13.5^{a}_{a}	19.5^{a}_{a}	16.25^{a}_{a}	16.5^{a}_{a}	15.5^{a}_{a}	17.75^{a}_{a}
TH_2	17.5^{a}_{a}	16.5^{a}_{a}	17.25^{a}_{a}	19.5^{a}_{a}	19.5^{a}_{a}	13.25^{a}_{a}
TOL	12.25^{a}_{a}	14.25^{a}_{a}	14.5^{a}_{a}	15.25^{a}_{a}	14^{a}_{a}	15.5^{a}_{a}
T27	13.5^{a}_{a}	14.5^{a}_{a}	15^{a}_{a}	17.5^{a}_{a}	16^{a}_{a}	17.75^{a}_{a}
T30	13.5^{a}_{a}	15.5^{a}_{a}	14.5^{a}_{a}	15.25^{a}_{a}	19.5^{a}_{a}	15.5^{a}_{a}
TY	12.25^{a}_{a}	13.25^{a}_{a}	14.5^{a}_{a}	17.5^{a}_{a}	14^{a}_{a}	16.25^{a}_{a}
TS-ML	12.25^{a}_{a}	16.25^{a}_{a}	15.5^{a}_{a}	17.5^{a}_{a}	15.5^{a}_{a}	17.75^{a}_{a}
TS-H	12.25^{a}_{a}	15.25^{a}_{a}	14.5^{a}_{a}	15.25^{a}_{a}	14^{a}_{a}	13.25^{a}_{a}
TS-B 2000 (1)	15.5^{a}_{a}	17.5^{a}_{a}	16.5^{a}_{a}	19^{a}_{a}	15.5^{a}_{a}	14.75^{a}_{a}
TS-B 98/2002 (1)	12.25^{a}_{a}	14.25^{a}_{a}	15.25^{a}_{a}	15.25^{a}_{a}	19.5^{a}_{a}	13.75^{a}_{a}
TS-B 98/2002 (2)	12.25^{a}_{a}	15.25^{a}_{a}	14.5^{a}_{a}	16.5^{a}_{a}	14^{a}_{a}	16.25^{a}_{a}
TR-B 98/2002 (1)	15.5^{a}_{a}	17.5^{a}_{a}	19.25^{a}_{a}	19^{a}_{a}	16.5^{a}_{a}	13.25^{a}_{a}
TR-B 98/2002 (2)	15.5^{a}_{a}	17.5^{a}_{a}	15.5^{a}_{a}	15.25^{a}_{a}	15.5^{a}_{a}	11.75^{a}_{a}
TR-OL 2	13.5^{a}_{a}	15.5^{a}_{a}	17.5^{a}_{a}	17^{a}_{a}	19.5^{a}_{a}	13.25^{a}_{a}
TS-EM-98 (1)	12.25^{a}_{a}	14.5^{a}_{a}	19.5^{a}_{a}	20^{a}_{a}	16^{a}_{a}	16.25^{a}_{a}
TR-CB 2000 (2)	16.25^{a}_{a}	18.5^{a}_{a}	20^{a}_{a}	18^{a}_{a}	14^{a}_{a}	20.5^{a}_{a}
TS-B-2000 (2)	14.5^{a}_{a}	17.5^{a}_{a}	19^{a}_{a}	18^{a}_{a}	15.5^{a}_{a}	13.25^{a}_{a}
TR-CB 2000 (1)	12.25^{a}_{a}	15.5^{a}_{a}	18^{a}_{a}	18^{a}_{a}	12.5^{a}_{a}	14.75^{a}_{a}
TS-EM-98 (2)	13.5^{a}_{a}	19.5^{a}_{a}	17^{a}_{a}	19^{a}_{a}	19.5^{a}_{a}	17.75^{a}_{a}
TR-TB 2000	14.5^{a}_{a}	18.5^{a}_{a}	16.25^{a}_{a}	16^{a}_{a}	15.5^{a}_{a}	14.75^{a}_{a}
TS-BG	13.5^{a}_{a}	18.5^{a}_{a}	17.5^{a}_{a}	17^{a}_{a}	14^{a}_{a}	15.5^{a}_{a}
TR-OL 1	15.5^{a}_{a}	17.5^{a}_{a}	19.25^{a}_{a}	21.5^{a}_{a}	16.5^{a}_{a}	16.25^{a}_{a}
TS-RP	15.5^{a}_{a}	17.5^{a}_{a}	15.25^{a}_{a}	17^{a}_{a}	19.5^{a}_{a}	13.25^{a}_{a}
TR-EM 1	14.5^{a}_{a}	16.5^{a}_{a}	18.5^{a}_{a}	18^{a}_{a}	14^{a}_{a}	14.75^{a}_{a}
TS-B 98	14.5^{a}_{a}	16.5^{a}_{a}	19.5^{a}_{a}	19^{a}_{a}	12.5^{a}_{a}	18^{a}_{a}
TR-EM 2	15.5^{a}_{a}	19.5^{a}_{a}	12.5^{a}_{a}	14^{a}_{a}	16.5^{a}_{a}	17.25^{a}_{a}
TR-B 98 (1)	15.5^{a}_{a}	19.5^{a}_{a}	20^{a}_{a}	21.5^{a}_{a}	16.5^{a}_{a}	19.25^{a}_{a}
TR-B 98 (2)	14.5^{a}_{a}	17.5^{a}_{a}	19^{a}_{a}	21.5^{a}_{a}	14^{a}_{a}	14^{a}_{a}
TR-B 98 (3)	15.5^{a}_{a}	20.5^{a}_{a}	20.5^{a}_{a}	23^{a}_{a}	19.5^{a}_{a}	17.25^{a}_{a}

* The results of the same column followed by different index letters differ significantly at 5%.
* The values with different superscript letters in the same line are significantly different at 5%.

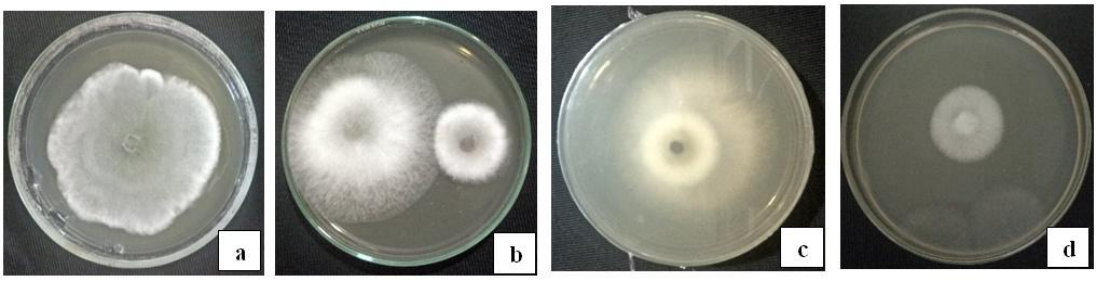

Fig. 2. *In vitro* effect of TR-B98(3) against *Curvularia spicifera,* a: Control; b: direct confrontation, c: indirect confrontation by producing volatile substances, d: indirect confrontation by producing diffusible compounds

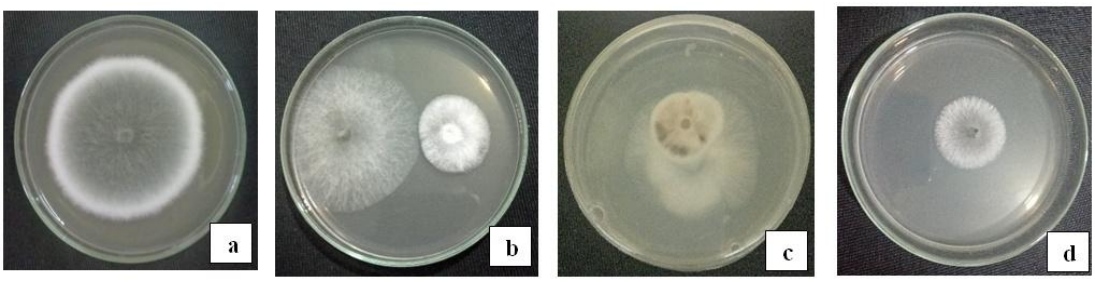

Fig. 3. *In vitro* effect of TR-B98(3) against *Bipolaris sorokiniana*(2) a: control, b: direct confrontation, c: indirect confrontation by producing volatile substances, d: indirect confrontation by producing diffusible compounds

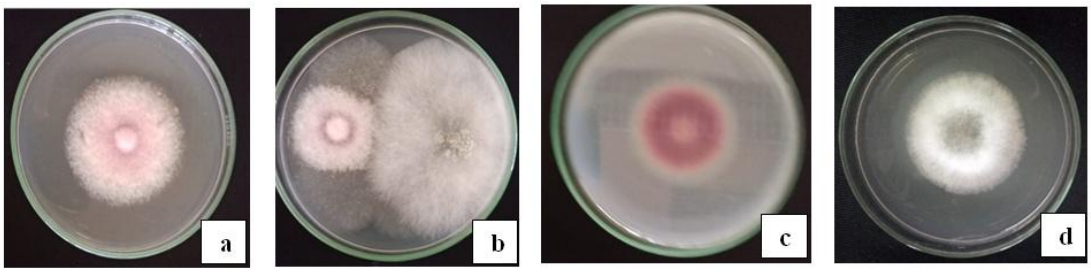

Fig. 4. Antagonistic activity of TR-B98(3) against *Fusarium culmorum.* a: control; b: direct confrontation; c: indirect confrontation by producing volatile substances; d: indirect confrontation by producing diffusible compounds

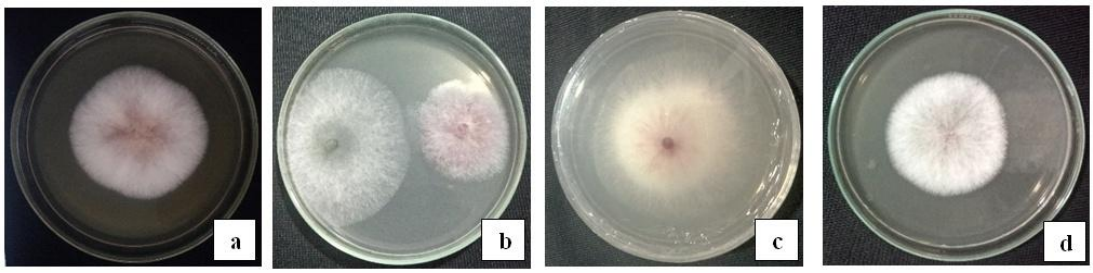

Fig. 5. Antagonistic activity of TR-B98(3) against *Fusarium roseum.* a: control, b: direct confrontation; c: indirect confrontation by producing volatile substances, d: indirect confrontation by producing diffusible compounds

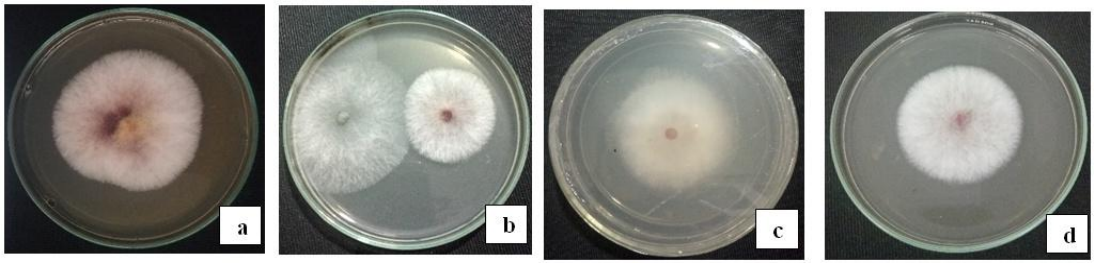

Fig. 6. Antagonistic activity of TR-B98(3) against *Fusarium graminearum.* a: control; b: direct confrontation; c: indirect confrontation by producing volatile substances, d: indirect confrontation by producing diffusible compounds

DISCUSSION

Microbial biocontrol agents express several mecanisms towards the plant pathogens [35,36]. By their capability to parasite directly the pathogen, producing antifungal substances, competition for nutrients and space and release of enzymes which hydrolyzes cellular components of pathogen, they can wear down or destroy it [37]. *Trichoderma* species can act as biocontrol agents against fungal phytopathogens through several mechanisms including competition for nutrients and space, antibiosis and induction of plant defensive mechanisms and mycoparasitism [38,39,40,41,42,27,43,44,45]. Confrontation plate assay is frequently used as preliminary test when biological control agents are selected [46,47,48]. It allows observe hyperparasitism, non-volatile metabolites effects and nutrient competition [49]. For instance, *Trichoderma* mycelium can invade several genera and fungal species as for *Pythium ultimum* [50], *Helminthosporium teres*, *Rhizoctonia solan*i or *Sclerotium rolfsii* [16,51].

The results revealed that the thirty used *Trichoderma* isolates showed varying degrees of antagonism throught the various mecanisms challenged to suppress the mycelial growth and conidial germinnation of *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium roseum*, *Bipolaris sorokiniana* 1, *Curvularia spicifera*, *Bipolaris sorokiniana* 2. Likewise, *Trichoderma* species clearly exhibited varying levels of antagonism towards *P. aphanidermatum* [52]. Observations on the growth and colonization of *Fusarium oxysporum* f. sp. *lycopersici*, *Alternaria solani*, *Aspergillus niger* and *Macrophomina phaseolina* in dual culture screening by the antagonistic isolates proved their varying ability to suppress the growth of the test pathogens [53]. The antagonistic action of *Trichoderma* species towards many pathogen agents has been reported by different workers [54,55,56,57,8,58,59,60,35]. It is worth mentioned that the tested *Trichoderma* isolates displayed a strong antagonism. Amin et al. [61] had placed the isolate of *T. viride* (Tv-2) in class I according to Bell et al. [57] scale for scoring degree of antagonism. This isolate had completely overgrown *Rhizoctonia solani* in dual culture and showed maximum inhibition of 71.41 per cent over control in *R. solani* compared to 60.05% by *T. harzianum* (Th-1). In line with our findings, Koka et al. [62] had also noted a significant inhibition in mycelial growth of *Alternaria alternata* (Fr.) Keissl., *Pencillium* sp. and *Aspergillus niger* van Tiegh in presence of different local isolates of *Trichoderma*. According to Jabnoun-khiareddine et al. [63], the mycelial growth reduction of *Verticillium* spp. was mainly due to the important competitive potential of the antagonists used. For Bastakoti et al. [64], the inhibition shown by the antagonists may be due to release of antibiotic or antibiotic like substances or hyphal parasitism which results in direct inhibition of growth of the pathogen by disintegrating the hyphal wall resulting in the penetration, absorption and lysis of the mycelium. In case of *Pythium aphanidermatum*, the production of antibiotics, cell wall degrading enzymes and competition for space and nutrients by *Trichoderma* spp., were solely responsible for its suppression [65,66]. In addition, Schirmböck et al. [67] attributed the inhibition of radial growth to secretion of extracellular lytic enzymes, production of antibiotic compounds and cell wall degrading enzymes such as chitinase, glucanase decomposing polysaccharides, chitins and the glucans by destroying cell wall integrity [59]. These enzymes can play a key role in mycoparasitism due to changes in cell wall integrity before physical contact. *Trichoderma* isolates are able of invading and sporulating on the pathogen's colonies. This action was reported by Daami-Remadi [68]. Likewise, the mycelial invasion of fungus by *Trichoderma* was observed by four to five days after inoculation, and cells of *P. ultimum* exhibited changes characterized by a local retraction of the plasma membrane which was accompanied by deposits heavily labeled by the b-1,4-exoglucanase–gold complex [6].

According to Maslouhi [69], the inhibitory effect was due to chemical products released by *Trichoderma* isolates through the antibiosis process. Indeed, many studies reported on the significance of *Trichoderma* metabolites in the antagonistic action of *Trichoderma* spp. against the pathogenic fungi [54,34,70,71,72,73,74]. For instance, *Trichoderma* volatile compounds have proved their effectivness against *Fusarium oxysporum* [75]. Also, *T. viride* and *T. polysporum* produced diverse metabolites which reduced

efficiently the mycelial growth of *Ceratocystis paradoxa* the causal agent of black seed rot disease in oil palm [76]. The *in vitro* assay confirmed the efficacy of volatile metabolites produced by different *Trichoderma* for controlling the growth of *Fusarium*, *Curvularia* and *Bipolaris* isolates. Similar results have been reported for *T. viride* isolates against *P. aphanidermatum* [52]. Volatile metabolites from *T. viride* (Tv-1) caused maximum reduction in mycelial growth and sclerotial production in *S. rolfsii* and *S. sclerotiorum*, whereas, in *Helminthosporium oryzae*, *T. harzianum* (Th-1) accounted for maximum reduction in mycelial growth in the order of 37.16% [61]. Rathore et al. [77] reported volatile activity of *T. viride* against *F. solani* which vacuolated most hyphae of pathogen and that the hyphae of pathogen were comparatively thin as compared to control. From other experiments, the inhibition varied from 2.0% to 64% in volatile metabolites, depending on the *Trichoderma* species producing the metabolites [76]. On *F. oxysporum* f. sp. *Phaseoli,* the percentages of inhibition were between 23 and 40% in presence of volatile metabolites from *T. harzianum* [49]. Outcomes from Stazzonelli et al. [78] study indicated a lower inhibitory effect of volatile metabolites produced by isolates of *T. koningiopsis*, T. *harzianum, T. atroviridi, T. longibrachiatum* species against *Sclerotinia sclerotiorum* [78] whose the percentage of growth inhibition ranged from 0% to 29.17%. By contrast, these strains showed a best performance of no-volatile products in inhibiting the mycelial growth of *Sclerotinia sclerotiorum* [78]. According to Hajlaoui et al. [79], *Trichoderma* species act by competition and mycoparasitism vs *S. sclerotiorum* whereas *Gliocladium roseum* attacks by antibiosis. Therefore, Li et al. [80] demonstrated that the interaction of enzymes and secondary metabolites of *T. asperellum* strains enhanced the antagonist activity against *Fusarium graminearum*. Also, it was suggested that *Trichoderma* sp., are able to repress pathogen through synchronization of mycoparasitism and antibiotic production [81,82,83].

Indeed, *Trichoderma* isolates exhibited active action against phytopathogenic fungi by producing diffusible no volatile substances such as antibiotics [73] and enzymes [84,81,60]. However, in the present study, all of tested *Trichoderma* isolates were shown less ability in inhibiting the growth and the conidia germination of six pathogens by mean of diffusible metabolites. In contrary, Tapwal et al. [85] have reported the efficacy of diffusible antibiotics released by *T. viride* on mycelial growth of pathogens in dual culture. The same trend was observed in the interaction of *T. hamatum* T614, *T. hamatum* T612, *T. harzianum* T447, *T. harzianum* T969, *Trichoderma virens* T523 and *Trichoderma* sp. towards *F. gaminearum*, *R. solani* and *Macrophomina phaseolina* [86], in the case of *T. viride* on *Fusarium oxysporum* f.sp. *radicis-lycopersici* [87] and *T. harzianum* on *Phytophthora capsici* [88].

CONCLUSION

In the present study, the isolates of *Trichoderma* species had varied antagonistic action. They were found to be moderately to highly effective in inhibiting the mycelial growth and conidial germination of the seven fungal species viz, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium roseum*, *Bipolaris sorokiniana*, *Curvularia spicifera*, *Bipolaris sorokiniana*. The *in vitro* assays allowed the selection of *Trichoderma* species possessing an antagonistic activity which was partially associated with having mycoparasitic capability and production of volatile metabolites. Among the *Trichoderma* isolates tested, T1; TS-B-2000 (2); TR-TB 2000; TR-B 98 (1); TR-B 98 (2); TR-B 98 (2); TR-B 98 (3) showed the highest percentages of mycelial growth and conidial germination inhibition against all pathogens studied. But further research is needed to validate the most prominent isolates under field conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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