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Research Article
Efficiency of 10 Compatible Isolates of *Trichoderma* spp. Against Rice Pathogens under Laboratory Conditions

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Abstract

Background and Objective: Rice (*Oryza sativa* L.) is considered as one of the oldest and most important foods for the increasing world population. Rice is attacked by many diseases such as *Bipolaris spicifera*, *Curvularia lunata*, *Fusarium* spp., *Nigrospora oryzae*, *Exserohilum rostratum*, *Alternaria* spp. and *Thanatephorus cucumeris* and caused 50% yield losses in worldwide. Current research was designed to combine between more than 1 isolate of *Trichoderma* spp. to detect their compatibility and increase their effectiveness to control rice pathogens and plant diseases in general. **Materials and Methods:** Ten *Trichoderma* isolates (T.1-T.10) were combined with each other in the petri dishes. Four *Trichoderma* isolates T.4, T.6, T.7 and T.9 were chosen among the 10 *Trichoderma* isolates. Compatible *Trichoderma* isolates, T.4+T.7, T.4+T.9, T.6+T.7 that showed 100% homologies among them were then combined in one isolate T.4679. **Results:** The antagonistic ability parameter between compatible *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7, T.4679 and 23 pathogens species of *Bipolaris spicifera*, *Curvularia lunata*, *Fusarium* spp., *Nigrospora oryzae*, *Exserohilum rostratum*, *Alternaria* spp. and *Thanatephorus cucumeris* revealed excellent potential to suppress the radial growth of pathogens after four days. **Conclusion:** Current study showed that *Trichoderma* isolate T.4679 exhibited high efficiency on reducing radial growth in *B. spicifera*, *C. lunata*, *Fusarium* spp., *N. oryzae*, *E. rostratum, Alternaria* spp. and *T. cucumeris*.

Key words: Mycoparasitism, chitinases, phytopathogens, *Trichoderma* spp., *Exserohilum rostratum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hyperparasitism (mycoparasitism) and the predation mechanism of *Trichoderma* isolates on other fungi, such as *Bipolaris spicifera*, *Curvularia lunata*, *Fusarium* spp., *Nigrospora oryzae, Exserohilum rostratum, Alternaria* spp. and *Thanatephorus cucumeris* are normal phenomena in the communities of microorganisms. The *Trichoderma* genus can attack the pathogens directly without killing the host, through the process of mycoparasitism or hyperparasitism[1]. These organisms are then further subdivided into 2 categories based on their mode of action[2,3]. Category one is the biotrophic group, which obtains their nutrient directly from the living tissues of pathogen cells by different means, such as hyphal coils or the formation of connection between *Trichoderma* genus and host through a small pore. The biotrophic of organisms are further divided into the destructive and balanced parasites[4-6]. The first group of parasites gains nutrients from hosts, whereas the second group causes slight damage to the host[7,8].

Category two is the necrotrophic group in which the *Trichoderma* genus obtains its nutrient after killing the host tissues. This group is extremely destructive to their hosts and can completely surround and cover their hosts in a few hour or days. They can dominate their host via hyphal coils and the connection between *Trichoderma* genus and host through a small pore, haustoria, hooks and appressoria. During mycoparasitism, the *Trichoderma* genus releases several lytic enzymes, such as chitinase, cellulose, glucanase and xylanase that will degrade the cell walls of phytopathogens[9]. This material produced by this microorganism has high toxicity to fungi and bacteria. The strong biodegradation and substrate colonization performances of *Trichoderma* strains are the result of their metabolic versatility and a high secretory potential, which leads to the production of a complex group of hydrolytic enzymes. Similarly, the mycoparasitic process relies on the secretion of a rich mixture of cell wall-degrading enzymes (CWDEs), which can hydrolyse the cell wall of different hosts[10,11].

Among these enzymes produced by *Trichoderma* spp. are chitinases[12], glucanases[13] and proteases[14]. These enzymes are important components of the multi-enzymatic system of *Trichoderma* strains. Some of these proteins display strong antifungal activities when they are applied *in vitro*, alone and/or in combination against plant pathogens[15,16]. Some lytic enzymes can be involved in both antagonistic and saprophytic processes, thus providing an evolutionary advantage to strains with both biodegrading and antagonistic potential.

A principal role in mycoparasitism has been attributed to chitinase and glucanase[17]. However, fungal proteases may also be substantially involved in cell wall degradation, because fungal cell walls contain chitin and glucan polymers, which are linked together to form a protein matrix[18]. The production of secondary metabolites by *Trichoderma* strains also shows great variety and application potential. *Trichoderma* strains are rich sources of antibiotics, which range from acetaldehydes, gliotoxin and viridin[19] to alpha-pyrones[20], terpenes, polyketides, isocyanide derivative, piperacines and complex families of peptaibols[21].

All these compounds have strong inhibitory activity on many fungal plant pathogen[22-25]. Additionally, *Trichoderma* spp. attacks the mycelium of pathogens on potato dextrose agar (PDA) medium, some hyphae of *Trichoderma* spp. coils around the pathogens above and the degradation their mycelium were also observed[26,27]. High costs of discovering and registering a new chemical pesticide are also the cause of the reduction in the registered chemical pesticides and are contributory to the increased reliance on biological and cultural controls. Finally, more producers are beginning to use non-pesticide control to capitalise on the demand and in the organic foods markets[28,29]. *T. cucumeris* R1, *T. cucumeris* R2, *T. cucumeris* R4, *T. cucumeris* R10, *T. cucumeris* R12, *T. cucumeris* R14, *F. solani* R3, *F. oxysporum* R5, *F. oxysporum* R6, *F. solani* R8, *F. solani* R11, *F. solani* R13, *F. solani* R16, *F. verticillioides* R17, *N. oryzae* R9, *C. lunata* R7, *C. lunata* R21, *B. spicifera* R15, *E. rostratum* R19, *A. valternata* R18, *A. alternata* R20, *A. tenuissima* R23 and *A. tenuissima* R24 were prepared via dual culture technique before use under greenhouse and natural field conditions. This study aimed to combine *Trichoderma* isolates, which showed high degree of compatibility between them in one isolate and evaluate their efficiency as biocontrol agent under laboratory conditions to control rice pathogens.

MATERIAL AND METHODS

Study area: This research was conducted under laboratory condition, at the Integrated Pest Management Center, Agriculture Research Directorate, Ministry of Science and Technology, Iraq. The experiment was started in the November, 2017 and ended in the April, 2019.

***In vitro* antagonistic ability between ten *Trichoderma* spp. isolates for compatibility:** Ten different *Trichoderma* spp. isolates (T.1, T.2, T.3, T.4, T.5, T.6, T.7, T.8, T.9 and T.10) were used in this study. Antagonistic study was conducted using

dual culture technique under laboratory conditions. In this study one-week-old fungal culture of the *Trichoderma* isolate T.1 was placed individually on one half of the plates that was divided into two equal portions, with the other half having a 5 mm disk of *Trichoderm*a sp. T.2. The same process was repeated with all isolates. Considering the possible differences in reactivity shown on Potato Dextrose Agar (PDA) with rose bengal, the plates were incubated for five days at (28±2°C). The antagonistic activity was scored according to the scale[30]. The mean growth of three plates (9 cm diameter) was used as replicates for each treatment. The pure cultures from *Trichoderma* spp. were kept on PDA slants at 4°C for further use. Then, four *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 were chosen in retarding compatibility and more efficiency between them than the rest isolates. The PDA plates were incubated at 28±2°C and pure cultures of the fungi obtained were sub-cultured.

Antagonistic ability between compatible biocontrol agents and rice phytopathogens: Among 10 *Trichoderma* isolates (T.1, T.2, T.3, T.4, T.5, T.6, T.7, T.8, T.9 and T.10), 4 compatible *Trichoderma* isolates (T.4+T.7, T.4+T.9, T.6+T.7 and T.4679) were chosen to determine their antagonistic activity via the dual plate method based on their compatibility activity study against 23 pathogens. *T. cucumeris* R1, *T. cucumeris* R2, *T. cucumeris* R4, *T. cucumeris* R10, *T. cucumeris* R12, *T. cucumeris* R14, *F. solani* R3, *F. oxysporum* R5, *F. oxysporum* R6, *F. solani* R8, *F. solani* R11, *F. solani* R13, *F. solani* R16, *F. verticillioides* R17, *N. oryzae* R9, *C. lunata* R7, *C. lunata* R21, *B. spicifera* R15, *E. rostratum* R19, *A. alternata* R18, *A. alternata* R20, *A. tenuissima* R23 and *A. tenuissima* R24 were selected and treated under greenhouse conditions[31]. The diameter of disc implant was 5 mm and each isolate was placed 9 cm from the side of side of the plate. On the opposite end, one of the 23 pathogens was placed and then the isolates were incubated at 28±2°C. Daily follow-up was conducted on the rate of growth in each isolate and their interaction.

Antagonistic activity scale between compatible *Trichoderm*a isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 and rice pathogens: The antagonistic activity was scored according to the scale[30] that involve four degrees as following:

- Antagonistic fungus was able to grow over the pathogen and pathogen growth completely inhibited
- Pathogen growth completely inhibited, but antagonist was not able to grow over the pathogen
- Mutual inhibition initially, but antagonist was overgrown by pathogen
- Pathogen growth not inhibited, antagonist was overgrown by pathogen. The mean of three plates (9 cm diameter) was used as replicates for each treatment

RESULTS

***In vitro* antagonistic ability between 10 *Trichoderma* spp. isolates to detect their compatibility:** Two *Trichoderma* spp. isolates were cultured together inside the plate to trigger different levels of activities as presented in Fig. 1-2. The results presented in Fig.1-2 were *Trichoderma* isolates divided into the two following groups:

Group one

Incompatible *Trichoderma* spp. isolates: This group includes seven (7) subgroups as following, subgroup A comprises the cultured isolate T.1 with T.2, T.3, T.4, T.5, T.6, T.7, T.8, T.9 and T.10 (Fig. 1a), subgroup B represents isolate T.2 with T.3, T.4, T.5, T.6, T.7, T.8, T.9 and T.10 (Fig. 1b), subgroup C shows isolate T.3 with T.4, T.5, T.6, T.7, T.8, T.9 and T.10 (Fig. 1c), subgroup D shows isolate T.5 with T.6, T.7, T.8, T.9 and T.10 (Fig. 1d), subgroup E exhibits T.7 with T.8, T.9 and T.10 (Fig. 1e), subgroup F displays T.8 with T.9 and T.10 (Fig. 1f) and subgroup G shows T.9 with T.10 (Fig. 1g). As in all these groups presented in Fig. 1a-g, the mycelium growth of *Trichoderma* spp. isolates were completely inhibited and affected and resulted negative influence towards the radial growth of each other. The results did not show in a positive compatibility among *Trichoderma* isolates in these groups. Moreover, the culture of *Trichoderma* spp. isolates, also show a zone surrounding each isolate plug in the edge of the plate, also, Fig. 1a-g show a zone of interaction between *Trichoderma* spp. isolates is clearly visible.

Group two

Compatible *Trichoderma* spp. isolates: This group divided into the two subgroups, subgroup A as shown in Fig. 2a, b. The culture of *Trichoderma* sp. isolate T.4 was cultured with T.5, T.6, T.7, T.8, T.9 and T.10 (Fig. 2a). T.4 exhibited high antagonistic activity towards T.5, T.6, T.8 and T.10 and prevented their growth, while isolate T.4 allowed the growth of T.7 and T.9 by causing 100% coverage of their growth. Also, subgroup B shows the cultured isolate T.6 with T.7, T.8, T.9 and T.10 was varied in its ability to inhibit the radial growth of T.8, T.9 and T.10 and it displayed high compatibility with isolate T.7 (Fig. 2b).

Figure 3a-g shows that different antagonist formulations have been used in laboratory experiment before usage to suppress rice phytopathogens, *T. cucumeris* R1, *T. cucumeris*

Fig. 1(a-g): *Trichoderma* isolate T.1 (left side) and T.2, T.3, T.4, T.5, T.6, T.7, T.8, T.9, T.10 (right side), (b) T.2 (left side) and T.3, T.4, T.5, T.6, T.7, T.8, T.9,T.10 (right side), (c) T.3 (left side) and T.4, T.5, T.6, T.7, T.8, T.9, T.10 (right side), (d) T.5 (left side) and T.6, T.7, T.8, T.9, T.10 (right side), (e) T.7 (left side) and T.8, T.9,T.10 (right side), (f) T.8 (left side) and T.9, T.10 (right side) and (g) T.9 (left side) and T.10 (right side)

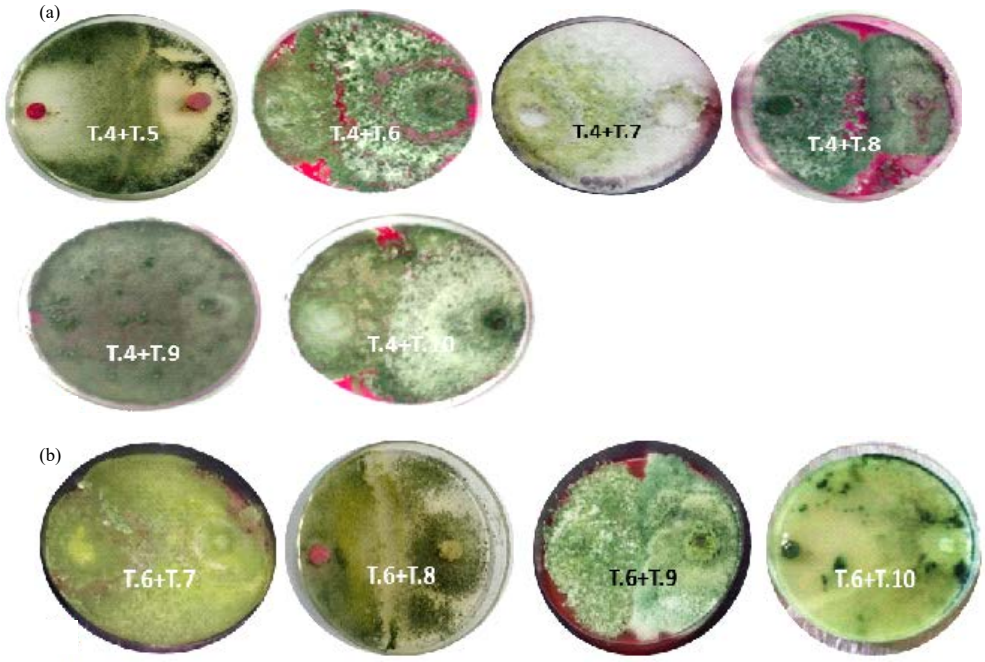

Fig. 2(a-b): *Trichoderma* isolate (a) T.4 (left side) and T.5, T.6, T.7, T.8, T.9, T.10 (right side) and (b) T.6 (left side) and T.7, T.8, T.9, T.10 (right side)

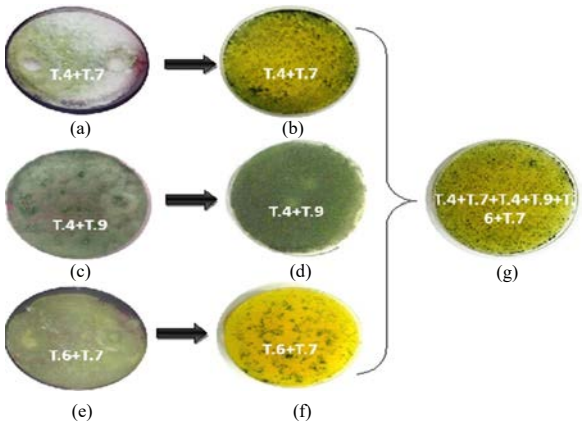

Fig. 3(a-g): *Trichoderma* isolates (a) T.4 (left side) and T.7 (right side), (b) T.4+T.7 (c) T.4 (left side) and T.9 (right side), (d) T.4+T.9 (e) T.6 (left side) and T.7 (right side), (f) T.6+T.7 and (g) T.4+T.7+ T.4+T.9+ T.6+T.7

R2, *T. cucumeris* R4, *T. cucumeris* R10, *T. cucumeris* R12, *T. cucumeris* R14, *F. solani* R3, *F. oxysporum* R5, *F. oxysporum* R6, *F. solani* R8, *F. solani* R11, *F. solani* R13, *F. solani* R16, *F. verticillioides* R17, *N. oryzae* R9, *C. lunata* R7, *C. lunata* R21, *B. spicifera* R15, *E. rostratum* R19, *A. alternata* R18, *A. alternata* R20, *A. tenuissima* R23 and *A. tenuissima* R24. Dual culture technique formula was used between *Trichoderma* spp. isolates T.4 and T.7 (Fig. 3a), T.4 and T.9 (Fig. 3c) and T.6 and T.7 (Fig. 3e). Serial dilution was used between *Trichoderma* spp. isolates T.4 and T.7 (Fig. 3b), T.4 and T.9 (Fig. 3d) and T.6 and T.7 (Fig. 3f). Then, serial dilution was continued to combine all four isolates in one isolate as seen in (Fig. 3g).

Antagonistic ability between compatible *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 and rice pathogens: Figure 4-7, show the results of the antagonistic activity of compatible *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 against 23 rice pathogens including: *T. cucumeris* R1, *T. cucumeris* R2, *T. cucumeris* R4, *T. cucumeris* R10, *T. cucumeris* R12, *T. cucumeris* R14, *F. solani* R3, *F. oxysporum* R5, *F. oxysporum* R6, *F. solani* R8, *F. solani* R11, *F. solani* R13, *F. solani* R16, *F. verticillioides* R17, *N. oryzae* R9, *C. lunata* R7, *C. lunata* R21, *B. spicifera* R15, *E. rostratum* R19, *A. alternata* R18, *A. alternata* R20, *A. tenuissima* R23 and *A. tenuissima* R24, which were analysed via the dual culture technique. The compatible *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 were varied in terms of their ability under laboratory conditions through dual culture technique to reduce and suppress the radial growth of rice. The compatible *Trichoderma* isolates were grown very fast and the mycelium covered the whole petri dish within 4 days at (28±2°C) as analysed in Fig. 4-7.

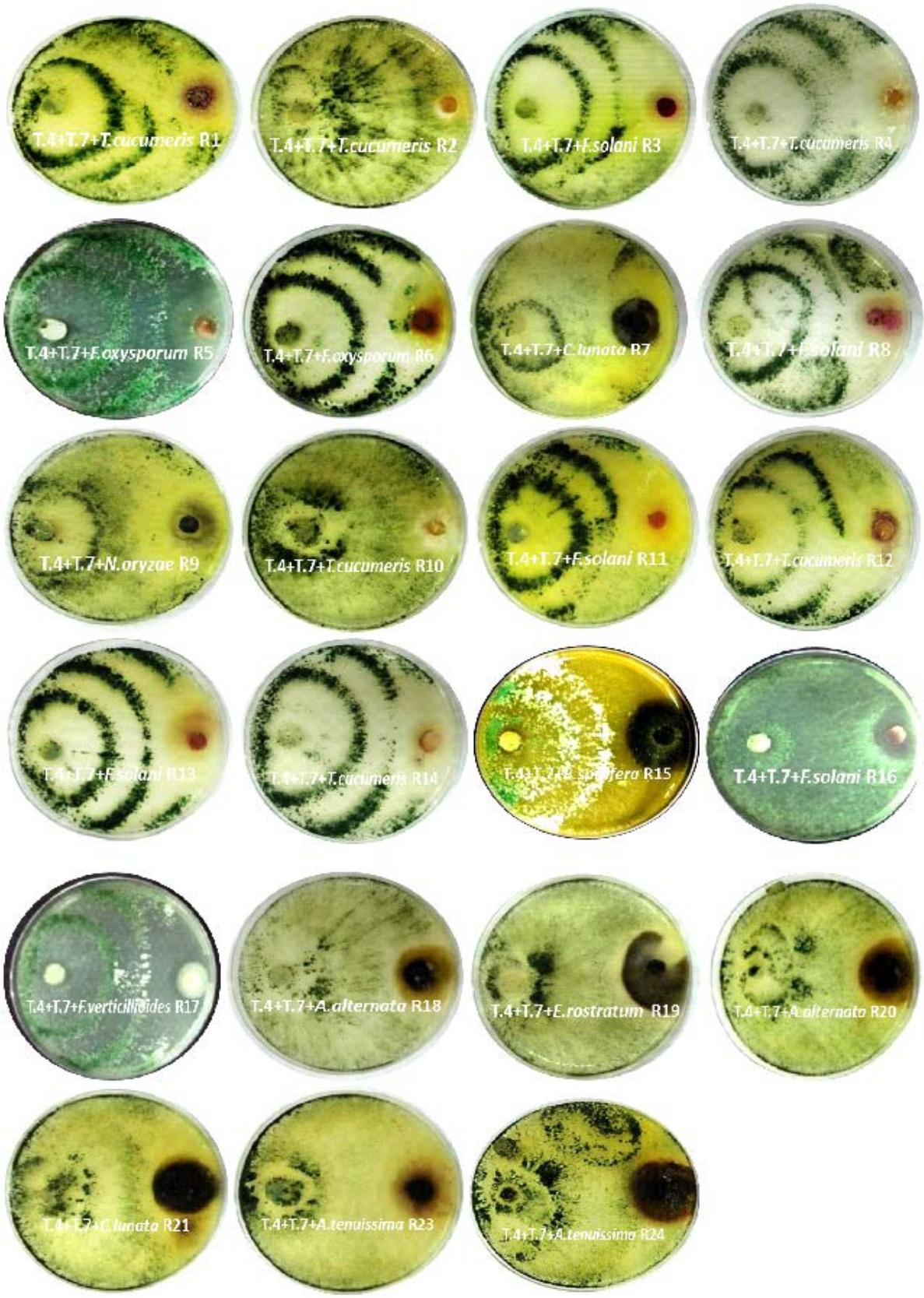

Fig. 4: Antagonistic activity between *Trichoderma* isolates T.4+T.7 (left side) and rice pathogens (right side)

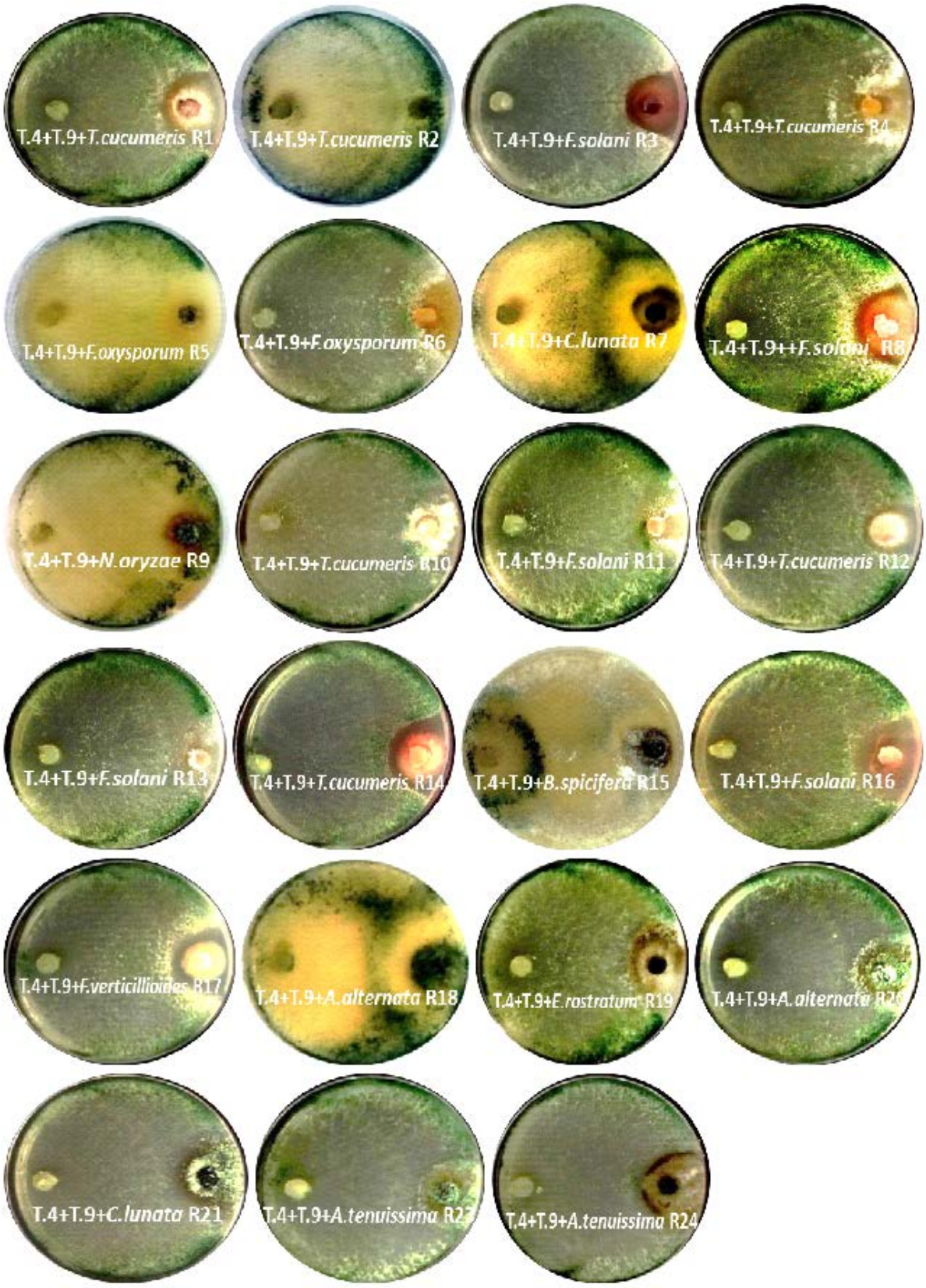

Fig. 5: Antagonistic activity between *Trichoderma* isolates T.4+T.9 (left side) and rice pathogens (right side)

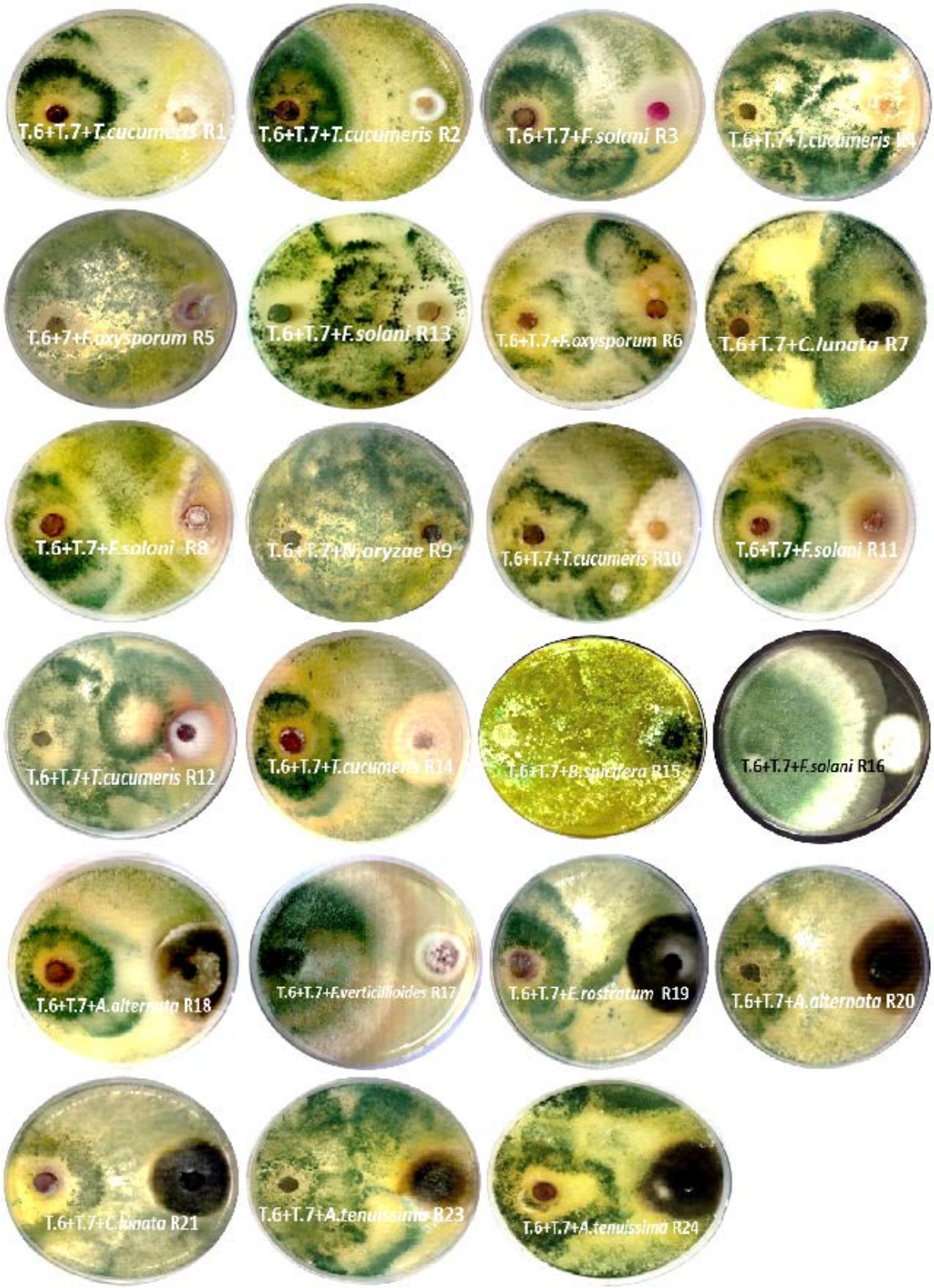

Fig. 6: Antagonistic activity between *Trichoderma* isolates T.6+T.7 (left side) and rice pathogens (right side)

Fig. 7: Antagonistic activity between *Trichoderma* isolates T.4679 (left side) and rice pathogens (right side)

Table 1: Antagonistic ability between compatible *Trichoderma* isolate T.4+T.7 and rice phytopathogens

Treatments	*Degree of antagonism (cm) after 4 days
T.4+T.7+*T. cucumeris* R1	2.5
T.4+T.7+*T. cucumeris* R2	1.0
T.4+T.7+*F. solani* R3	2.5
T.4+T.7+*T. cucumeris* R4	2.0
T.4+T.7+*F. oxysporum* R5	2.5
T.4+T.7+*F. oxysporum* R6	2.5
T.4+T.7+*C. lunata* R7	2.4
T.4+T.7+*F. solani* R8	2.3
T.4+T.7+*N. oryzae* R9	1.0
T.4+T.7+*T. cucumeris* R10	1.0
T.4+T.7+*F. solani* R11	2.0
T.4+T.7+*T. cucumeris* R12	2.5
T.4+T.7+*F. solani* R13	2.5
T.4+T.7+*T. cucumeris* R14	3.0
T.4+T.7+*B. spicifera* R15	3.9
T.4+T.7+*F. solani* R16	1.0
T.4+T.7+*F. verticillioides* R17	2.4
T.4+T.7+*A. alternata* R18	2.0
T.4+T.7+*E. rostratum* R19	2.5
T.4+T.7+*A. alternata* R20	2.0
T.4+T.7+*C. lunata* R21	2.5
T.4+T.7+*A. tenuissima* R23	2.0
T.4+T.7+*A. tenuissima* R24	2.0

*Mean of 3 plates (9 cm diameter) was used as replicates for each treatment

Table 2: Antagonistic ability between compatible *Trichoderma* isolate T.4+T.9 and rice phytopathogens

Treatments	*Degree of antagonism (cm) after 4 days
T.4+T.9+*T. cucumeris* R1	2.0
T.4+T.9+*T. cucumeris* R2	1.0
T.4+T.9+*F. solani* R3	2.2
T.4+T.9+*T. cucumeris* R4	2.5
T.4+T.9+*F. oxysporum* R5	2.1
T.4+T.9+*F. oxysporum* R6	2.9
T.4+T.9+*C. lunata* R7	2.3
T.4+T.9+*F. solani* R8	2.1
T.4+T.9+*N. oryzae* R9	1.0
T.4+T.9+*T. cucumeris* R10	2.4
T.4+T.9+*F. solani* R11	1.7
T.4+T.9+*T. cucumeris* R12	2.5
T.4+T.9+*F. solani* R13	2.9
T.4+T.9+*T. cucumeris* R14	2.3
T.4+T.9+*B. spicifera* R15	2.6
T.4+T.9+*F. solani* R16	2.9
T.4+T.9+*F. verticillioides* R17	2.2
T.4+T.9+*A. alternata* R18	1.1
T.4+T.9+*E. rostratum* R19	2.7
T.4+T.9+*A. alternata* R20	1.0
T.4+T.9+*C. lunata* R21	1.5
T.4+T.9+*A. tenuissima* R23	1.5
T.4+T.9+*A. tenuissima* R24	2.0

*Mean of 3 plates (9 cm diameter) was used as replicates for each treatment

The best antagonistic results were exhibited with compatible *Trichoderma* isolate T.4+T.7 against *T. cucumeris* R2, *N. oryzae* R9, *T. cucumeris* R10 and *F. solani* R16 by causing 100% coverage/over growth of the 9 cm plates as shown in (Fig. 4). In (Fig. 5) the interaction between *Trichoderma* isolate T.4+T.9 and rice pathogens and this *Trichoderma* isolate completely prevented the growth of *T. cucumeris* R2, *N. oryzae* R9 and *A. alternata* R20. Also, in (Fig. 6) the influenced of *Trichoderma* isolate T.6+T.7 on *T. cucumeris* R2, *N. oryzae* R9 and *B. spicifera* R15 by causing 100% coverage/over growth of the 9 cm petri dishes. The *Trichoderma* isolate T.4679 revealed excellent potential to stop the growth of *T. cucumeris* R2, *C. lunata* R7 and *A. alternata* R18 by causing 100 % coverage/over growth of the 9 cm petri dishes (Fig. 7).

Table 3: Antagonistic ability between compatible *Trichoderma* isolate T.6+T.7 and rice phytopathogens

Treatments	*Degree of antagonism (cm) after 4 days
T.6+T.7+*T. cucumeris* R1	3.1
T.6+T.7+*T. cucumeris* R2	1.0
T.6+T.7+*F. solani* R3	2.5
T.6+T.7+*T. cucumeris* R4	2.5
T.6+T.7+*F. oxysporum* R5	1.5
T.6+T.7+*F. oxysporum* R6	2.5
T.6+T.7+*C. lunata* R7	1.8
T.6+T.7+*F. solani* R8	2.6
T.6+T.7+*N. oryzae* R9	1.0
T.6+T.7+*T. cucumeris* R10	2.2
T.6+T.7++*F. solani* R11	2.1
T.6+T.7+*T. cucumeris* R12	2.7
T.6+T.7+*F. solani* R13	1.0
T.6+T.7+*T. cucumeris* R14	2.4
T.6+T.7+*B. spicifera* R15	1.0
T.6+T.7+*F. solani* R16	2.9
T.6+T.7+*F. verticillioides* R17	3.5
T.6+T.7+*A. alternata* R18	2.9
T.6+T.7+*E. rostratum* R19	2.4
T.6+T.7+*A. alternata* R20	2.5
T.6+T.7+*C. lunata* R21	2.4
T.6+T.7+*A. tenuissima* R23	2.5
T.6+T.7+*A. tenuissima* R24	2.8

*Mean of 3 plates (9 cm diameter) was used as replicates for each treatment

Antagonistic ability score between compatible *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 and rice pathogens: Table 1-3 and 4 display the results of the antagonistic ability of compatible *Trichoderma* spp. isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 against rice pathogens. As presented in Table 1, among the 23 pathogenic isolates, *Trichoderma* isolate T.4+T.7 yielded the highest score of antagonistic activity against causal agents *T. cucumeris* R2, *N. oryzae* R9, *T. cucumeris* R10 and *F. solani* R16, which yielded high value of 1° degree (for all pathogens respectively). Table 2 represents *Trichoderma* isolate T.4+T.9 which it is obtained high value (1° degree) of antagonistic activity against *T. cucumeris* R2, *N. oryzae* R9 and *A. alternata* R20 as compared with the rest of the pathogens.

Table 3 represents that although *Trichoderma* isolate T.6+T.7 displayed high inhibitory activity against *T. cucumeris*

Table 4: Antagonistic ability between compatible *Trichoderma* isolate T.4679 and rice phytopathogens

Treatments	*Degree of antagonism (cm) after 4 days
T.4679+*T. cucumeris* R1	1.6
T.4679+*T. cucumeris* R2	1.0
T.4679+*F. solani* R3	2.5
T.4679+*T. cucumeris* R4	1.7
T.4679+*F. oxysporum* R5	2.8
T.4679+*F. oxysporum* R6	2.5
T.4679+*C. lunata* R7	1.0
T.4679+*F. solani* R8	2.6
T.4679+*N. oryzae* R9	1.0
T.4679+*T. cucumeris* R10	2.6
T.4679+*F. solani* R11	2.6
T.4679+*T. cucumeris* R12	2.5
T.4679+*F. solani* R13	2.8
T.4679+*T. cucumeris* R14	2.4
T.4679+*B. spicifera* R15	2.6
T.4679+*F. solani* R16	2.8
T.4679+*F. verticillioides* R17	3.6
T.4679+*A. alternata* R18	1.0
T.4679+*E. rostratum* R19	1.5
T.4679+*A. alternata* R20	1.4
T.4679+*C. lunata* R21	2.8
T.4679+*A. tenuissima* R23	1.7
T.4679+*A. tenuissima* R24	1.7

* Mean of 3 plates (9 cm diameter) was used as replicates for each treatment

R2, *N. oryzae* R9 and *B. spicifera* R15, it had maximum reduction in the mycelial growth of pathogen by 1° degree as indicated in Table 3 in comparison with *T. cucumeris* R1 and *F. verticillioides* R17, which obtained the lowest value of (3.1 and 3.5°, respectively). The compatible *Trichoderma* isolate T.4679 had differences in its inhibitory effect to suppress the radial growth of rice pathogens Table 4. Among the 23 pathogenic isolates, *T. cucumeris* R2, *C. lunata* R7 and *A. alternata* R18 exhibited the highest antagonistic results, which yielded 1° degree of all above pathogens compared with the rest of the pathogens. The *Trichoderma* isolate T.4679 displayed moderate inhibition of certain pathogens, such as *F. solani* R3, *F. oxysporum* R6, *T. cucumeris* R12 and *T. cucumeris* R14, which yielded by approximately (2.5, 2.5, 2.5 and 2.4°), respectively. Although *Trichoderma* isolate T.4679 exhibited some inhibitory activity against *F. verticillioides* R17, it recorded the lowest level of inhibition and was scored as 3.6° (Table 4).

DISCUSSION

The ecological efficiency of our *Trichoderma* spp. isolates was increased and the possibility of increasing their effectiveness was studied through synthesizing with other compatible isolates. The results of the compatibility study between *Trichoderma* spp. isolates revealed that isolate T.4 was compatible with isolate T.7 and T.9 and *Trichoderma* isolate T.6 was compatible with isolate T.7 which indicated the possibility of being used together to increase their biogenic efficiency.

For the results of the rest of the isolates, the interaction growth between them appeared as line presented between the isolates *Trichoderma* sp. T.1+T.2, T.2 +T.6, T.3+T.4, T.4 +T.8 and T.8 +T.9. This finding indicates the difficultly in combining them to prepare the double combination of these isolates. Data obtained from this study also indicated the natural interaction between *Trichoderma* isolates when they met on the centre of the Petri dishes. It is also possible to distinguish two types of relationships (Fig. 1c, d), which are the passive type format (Fig. 1c) characterized by the existence of a dividing line between the isolates. This growth reflects the state of antagonism (incompatible). The second positive form (Fig. 1d) in the case of overlapping harmonious growth of mycelium showed an expression of consensus between the isolates (compatible). The results of this test provided an opportunity to test more than one isolate as shown in Fig. 1-2 to prepare the bio-fungicide, which is an effective biowider and can overcome the harshest environmental conditions by combining between two or more isolates having different ecological characteristics, but they are compatible with each other. Among the ten *Trichoderma* isolates, isolates T.4+T.7, T.4+T.9 and T.6+T.7 were more compatible than the rest of the isolates in retarding growth. In addition, *Trichoderma* isolates consortium T.4+T.7, T.4+T.9 and T.6+T.7 which were then combined in one isolate T.4679 showed 100% homology among them, which agrees with the observations made on the dual culture assays conducted *in vitro*[32].

Previous studies have indicated that Certain *Trichoderma* isolates were able to produce effective volatile and non-volatile toxic metabolites that impede colonization by antagonized microorganisms[33]. Among these metabolites, the production of harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others have been described[33]. Apart from the gliotoxin and gliviridin, the peptaibols are another class of antibiotics secreted by *Trichoderma*. These are linear peptides that have strong antimicrobial activity against Gram-positive bacteria and fungi, act synergistically with cell wall degrading enzymes (CWDEs) to inhibit the growth of fungal pathogens and elicit plant resistance response to pathogens[34].

The most efficient inhibition by *Trichoderma* isolate T.4679 was observed in *T. cucumeris* R2, *C. lunata* R7 and *A. alternata* R18, which yielded 1 degree (Fig. 7). The

Trichoderma isolates inhibited pathogenic infection of the rice plant as compared to those that gave the least. In Fig. 7, however, the overgrowth and hyphal coiling are accompanied by extensive degradation and the inhibition of *Trichoderma* isolate T.4679 was higher in dual inoculation. *Trichoderma* isolate T.4679 was the best biocontrol concoction against all 23 phytopathogens in petri dish test.

Trichoderma strongly inhibited *T. cucumeris* R2, *N. oryzae* R9, *T. cucumeris* R10 and *F. solani* R16 (Fig. 4), *T. cucumeris* R2, *N. oryzae* R9 and *A. alternata* R20 (Fig. 5), *T. cucumeris* R2, *N. oryzae* R9 and *B. spicifera* R15 (Fig. 6) and *T. cucumeris* R2, *C. lunata* R7 and *A. alternata* R18 (Fig. 7) when cultured in the same PDA medium. The inhibitory action was associated with high rate and extent of CO_2 accumulation in comparison with the plant pathogenic fungi[35]. This test also showed *Trichoderma* sp. T.4 and T.7 to 100% coverage/overgrowth of the 9 cm petri dishes (Fig. 1c), completely mixed and could not be re-isolated from these plates. The interaction between *Trichoderma* isolate T.4679 and rice pathogens was the best example of the influence of metabolites produced by both the organisms on each other as seen in Fig. 7, where the inhibition zone of interaction between antagonists and pathogens was observed clearly in all treatments.

CONCLUSION

This study was combined between more than one isolate of *Trichoderma* spp. to control rice pathogens *B. spicifera*, *C. lunata*, *Fusarium* spp., *N. oryzae, E. rostratum, Alternaria* spp. and *T. cucumeris* via dual culture technique. This strategy of new control is ecologically compatible with different models of agriculture: organic, biological and integrated pest/pathogen management (IPM) programs.

SIGNIFICANCE STATEMENT

This study discover the compatibility between *Trichoderma* isolates having different ecological characteristics that can be beneficial for preparing effective bio-fungicide and increase their effectiveness to control rice pathogens. This study will help the researcher to uncover the critical areas of alternative method to reduce chemical pesticides that many researchers were not able to explore. Thus a new theory on combination between more than one isolate of *Trichoderma* spp. may be arrived at.

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