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Research Article

PREPARATION OF COMPATIBLE *TRICHODERMA SPP*. INOCULANT TO CONTROL *THANATEPHORUS CUCUMERIS, FUSARIUM SPP., CURVULARIA LUNATA AND ALTERNARIA TENUISSIMA* UNDER GREENHOUSE CONDITIONS

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A B S T R A C T

The current research included greenhouse studies on the evaluate efficacy of four compatible *Trichoderma* spp. (T.4, T.6, T.7 and T.9), in combination with each other in controlling five phytopathogenic fungi; (*Fusarium oxysporum* R6, *F.solani* R11, *Curvularia lunata* R7, *Alternaria tenuissima* R23 and *Thanatephorus cucumeris* R12) by using local rice variety cv. AL-Baraka. Four *Trichoderma* isolates consortium (T.4+T.7, T.4+T.9, T.6+T.7 and T. 4679), were selected based on pre-screened with pathogens via the dual culture technique to determine its compatible activity against pathogens under laboratory conditions. The experiment was carried out under uncontrolled condition in greenhouse with non-sterilized field soil, and thirteen parameters were evaluated. The results showed that rice plant inoculated with *Trichoderma* spp. triggered the highest level of chitinase, peroxidase, PAL (Phenyl alanine-ammonialyase) and chlorophyll content in plants, two months post planting. Significant differences were observed in all treatments compared to untreated control. In addition, the results showed the significant interaction between compatible *Trichoderma* spp. on growth parameters of rice plant, fresh weight of shoot and root, dry weight of shoot, root and panicle, shoot and root length. *Trichoderma* T.4679 exhibited most compatible and greater efficiency of reducing disease severity when treated with *A. tenuissima* R23 and *T. cucumeris* R12 (8.883, and 11.553%, respectively), as compared with control (pathogens alone) which gave significant increase ($p<0.05$) of (70. to 88.867 %, respectively) 120 days after transplanting. As a consequence of dual inoculation, the greenhouse experiment determined *Trichoderma* T.4679 as an effective component in an integrated pest/pathogen management (IPM) program to control rice disease.

INTRODUCTION

Trichoderma fungus belongs to the imperfect fungi which is distributed in all soils and have fast growth and produce huge spores and chlamydospores, the competition factor is very necessary to determine population density of *Trichoderma* isolates in the environment, this feature will help this fungus to be strong competitor against pathogens (Woo *et al.,* 2006; Rawat *et al.,* 2015; Hamdia *et al.,* 2016c; Barakat and Al-Masri, 2017).

Trichoderma strains produce metabolites that stimulate plants to produce and elicited their own defence compounds. Meanwhile, the application this genus leads to temporal increase in chlorophyll, chitinase, peroxidase, PAL enzyme and phenolic glucoside levels in plant (Hoel and Solhaug, 1998; Benitez *et al.,* 2004; Contreras *et al.,* 2016). Moreover, *Trichoderma* strainshave antifungal, antibacterial, antiviral activities as well as the ability to react as an antioxidant (Dennis *et al.,* 1971; De la *et al.,* 1995; Coninck *et al.*, 2012). There have been previous reports that *Trichoderma* spp. was able to promote growth under different conditions (Hamdia *et al.,* 2015, Khirallah, *et al.,* 2016) and the efficient use of available nutrients is based on the ability of Trichoderma to obtain energy from the metabolism of different sugars, such as those derived from polymers, wide-spread in fungal environments including cellulose, glucan and chitin among others (Schuster and Schmoll, 2010; Alfredo and Aleli, 2011;Gomes *et al.,* 2015;Gao, 2016). Additionally, Trichoderma has a superior capacity to mobilize and take up soil nutrients compared to other organisms. Several researchers stated that *Trichoderma* isolates demonstrated high compatibility when used in combination with each other in

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order to exploit their potential to suppress and control plant diseases successfully such as *Macrophomina phaseolina* in sesame, white root rot in avocado, *Fusarium oxysporum* in banana (Hafedh, 2001; Rosa and Herrera, 2009; Jeffries *et al.,* 2011; Mbarga *et al.,* 2012; Krauss *et al.,* 2013; Thangavelu and Gopi, 2015).

Meanwhile, *Trichoderma* isolates that have root colonization ability will stimulate leaf tissue to enhance responsive gene expression e.g. jasmonic acid and increase formation of callose which indicated to the induction of resistance mechanisms similar to the hypersensitive response (HR), systemic acquired resistance (SAR) and induced systemic resistance (ISR) in plants (Bae *et al.,* 2009; Harman and Mastouri, 2010; Spoel and Dong, 2012). These kinds of resistances occur between pathogens and plants which have a broad range of defence mechanisms e.g. chitinase, peroxidaseand PAL enzyme which correlated with the presence of fungal intracellular infection and resulted in significant reduction in the effect of pathogens, and provide them with a good chance to challenge pathogens effectively (Kuc, 1995; Dyakov *et al.,* 2007; Dildey *et al.,* 2016). In systemic acquired resistance (SAR), elicitors enhanced levels of one or more translocatable chemicals signal that in turn results in coordinated induction of genes controlling diverse defense pathways such as PAL enzymein tissues spatially distant from the initial challenge site (Kuc 1995; Kervinen *et al.,* 1998; Agrios, 2005; Singh *et al.,* 2014; Singh and Singh, 2014). The exogenous application of similar inducer chemicals leads to the induction of systemic resistance in crops (Mitchell and Walterss, 1995; Yanjun and Shiping, 2010; Yoshioka *et al.,*2012). In cereal crops like barley and rice, biotic and abiotic elicitors have been used to induce SAR against pathogens e.g. blast and powdery mildew (Manandhar, 2008).

Under Iraqi conditions as all over the world, rice (*Oryza sativa* L.) is subjected to attack by many pathogenic fungiin general (Najeeb *et al.,* 2008; Khosravi *et al.,*2011; Schwarz *et al.,*2012; Maryam *et al.,*2013), and especially *Bipolaris spicifera*, *Curvularia lunata*, *Fusarium* spp., *Nigrospora oryzae, Exserohilum rostratum, Alternaria* spp. and *Thanatephorus cucumeris* which regarded as determinant factors for crop cultivation in Iraq (Salih *et al.,*2000; Sami,2006; Hamdia *et al.,* 2016a; Hamdia *et al.,* 2016b; Hamdia *et al.,* 2016c). The rice plant, are easily infected with these pathogens at any developmental stage, so this study aimed to increase the effectiveness of our *Trichoderma* isolates through the preparation of compatible inoculants that consists of compatible combination of antagonistic strains and plant growth promoting microorganisms to reduce or replace synthetic fungicides and inorganic fertilizer.

MATERIALS AND METODS

Evaluation of compatible effectiveness of Trichoderma isolates; T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 to Control Rice Phytopathogens under Greenhouse Conditions

According to previous study four highly antagonistic *Trichoderma* isolates; T.4, T.6, T.7 and T.9 were used in this study among 10 isolates during their compatibility assayson plates (paper under publish). Though the laboratory experiments provided us with the excellent compatible ability, greenhouse experiment was conducted to evaluate the biocontrol efficiency of the following treatments; T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 against pathogens *F.oxysporum* R6*, F.solani* R11, *C.lunata* R7, *A.tenuissima* R23 and *T.cucumeris* R12.

Biocontrol Agents Preparation

*Trichoderm*a isolates were propagatedon natural medium which containing; wheat bran, corn cob and water (3: 7: 3/weight/weight/size) depending on the method of Hamdia *et al.,* (2015), the medium was sterilized inside 250 mL conical flasks. *Trichoderma* isolates (T.4+T.7, T.4+T.9, T.6+T.7 and T.4679) were individually cultured where 5 mm disc of their mycelium (obtained from PDA agar plates) placed in 250 mL flasks and incubated at (28±2 °C) with assessed visually for 5 days. Inoculum size for *Trichoderma* isolates was used in this experiment for about 2g/Kg soil was included approximately $1x10^{10}$ spore/1g.

Fungal Pathogens Preparation

Fungal pathogens were cultured on potato dextrose agar (PDA) medium and incubated at (28±2 °C) for seven days. For this experiment which was involving rice pathogens, the inoculum size was containing about (13×10^7) of *C.lunata* R7, *F.oxysporum* R6 (22×10^7), *F.solani* R11 (43×10^7), *A.tenuissima* R23 (17×105) spore/1g and *T.cucumeris* R12 was counted about (9×10^6) cfu spore/1g. The pots were inoculated separately with *F.oxysporum* R6*, F.solani* R11, *C.lunata* R7, *A.tenuissima* R23 and *T.cucumeris* R12, and left for one week. Later, the same process was repeated with *Trichoderm*a isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679, this experiment was carried out in triplicate. The pots were mixed thoroughly and watered daily. Local rice seeds ALbaraka obtained from Dr. Shatha A. Yousif /Agriculture Research Directorate were used in this study as it is the most widely grown rice cultivar in Iraq, therice seeds were sown into pots and six seeds / pot, and the experiment was conducted in triplicate.

Plant growth parameters

Fresh and dry weight of shoot, root and panicle were calculated for each replicate and the length of each root and shoot had been measured, and then analyzed the results statistically by using a randomized complete design according to the method described in Duncan's Multiple Rating Test (Duncan, 1955).

Evaluation of chitinase, peroxidase, PAL enzyme (phenylalanine-ammonialyase) and chlorophyll

In this experiment, the detection of chitinase, peroxidase, PAL enzyme and chlorophyll content were done from randomly chosen plants which were treated with fungal organisms; *F.oxysporum* R6*, F.solani* R11, *C.lunata* R7, *A.tenuissima* R23, *T.cucumeris* R12 and *Trichoderm*a isolates;T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 individually after two months post inoculation. Chitinase, peroxidase and PAL enzymes were evaluated in this study by using kits prepared by Dr. Zuhair Ali Shafeeq, Baghdad, Iraq as following;

Chitinase activity

About 1 g from fresh leaves tissue were ground in 2ml of 0.1 M sodium citrate buffer (pH 5) for several minutes by using micro-pestle, and fine solution was transferred into a 2 mL fresh centrifuge tube. The homogenate was vortexed for 10

second, and then centrifuged at 16,000 rpm for 15 minutes at room temperature, a 400 µl of supernatant was taken and transferred to a new 2 mL tubes, for each tube 0.1 ml of colloidial chitin was added plus 10 µl of 1.0 Msodium acetate buffer (pH 4). The mixture in all tubes was incubated at 37°C for 2 hours. After incubation all tubes were centrifuged at 8,000 rpm for 3 minutes at room temperature, a 300 µl of supernatant was transferred intoa new 2 mL centrifuge tubes were containing 30 µl of potassium phosphate buffer (pH 7) and two ml of p-dimethylaminobenz aldehyde reagent (DMAB). The mixture was incubated at 37 °C for 20 minutes to permit complete homogenization, then vortexed mixed and absorbency read at 585 nm against water blank which had passed through the above assay procedure. Calibration curve was obtained by assaying the following range of N-Acetylglucosamine concentrations, the average of three replicates reading for each treatment was recorded, and one unit of chitinase activity was defined as the amount of enzyme that produces 1 g mole of reducing sugar per minute under standard assay conditions.

Peroxidase activity

Fresh leaves were harvested from plants and weighed, and (1) g of leaves tissue was ground in2ml of phosphate buffer (pH 7), leaves were finely macerated transferred into freshly sterilized 2 ml micro centrifuge tube and vortexed for 10 second. Then centrifuged at 16,000 rpm for 15 minutes at room temperature, a 500 µl of supernatant was picked up and transferred to fresh tubes 10 ml, for each tube 1.5 ml of0.05 M pyrogalloland 500 µl of H_2O_2 (1%) were added. The mixture in all tubes was incubated at (28± 2°C) for 2 hours. Then vortexed mixed and absorbency read was measured by spectrophotometer at 420 nm against water blank.

Phenyl alanine-ammonialyase activity

One (1) g from fresh leaves were taken and added 3ml of 0.1 M sodium borate buffer pH 7 (must be cooled before used), finely ground leaves were transferred immediately into a sterilized 2 mL tubes and vortexed for 10 second. The samples were centrifuged at 16,000 rpm for 15 minutes at room temperature;a 400 µl of supernatant was transferred to a fresh centrifuge tube10 ml, for each tube 500 µl of 0.1 Mborate buffer (pH 8.8) was added. Enzyme activity was started after added a 500 µl of solution0.05 ML-Phenyl alanine, and all tubes were incubated at 30 °C for 30 minutes, the above assay was performed via vortexed mixed and absorbency read at 290 nm against water blank, optical density estimated every 30 second and the average of three replicate readings for each treatment was recorded. The amount of PAL enzyme activity was calculated by number of transcinnamic acid released from the substrate phenylalanine.

Chlorophyll relative

Chlorophyll content was evaluated in leaves by using chlorophyll meter SPAD–502 (Minolta, Japan). Chlorophyll analysis was conducted in three replicates for each treatment according to Minolta *et al.,* (1994). The activity of chitinase, peroxidase, PAL enzyme and chlorophyll detected through the significant difference between the values.

RESULTS

Evaluation of Compatible Effectiveness of Trichoderma Isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 To Control Rice Diseases Under Greenhouse Conditions

The effect on chitinase, peroxidase enzymes and chlorophyll content

Table 1 shows the results of the effect of the four *Trichoderma* isolates combination T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 which were added to the non-autoclaved soil on chitinase, peroxidase and chlorophyll in rice plant after two months of planting. We can see the *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.6+T.7, *Trichoderma* T.4+T.9, *Trichoderma* T.4+T.7+*F.oxysporum* R6, *Trichoderma* T.4+T.9+*T.cucumeris* R12, *Trichoderma* T.4679+*C.lunata* R7, *Trichoderma* T.4679+*T.cucumeris* R12 and *Trichoderma* T.4+T.7+*A.tenuissima* R23 as they yielded high value in chitinase level by approximately 0.356, 0.222, 0.182, 0.159, 0.159, 0.122, 0.107, 0.107 and 0.107µg/mLrespectively,as compared to *Trichoderma* T.4+T.9+*F.oxysporum* R6 and *F.oxysporum* R6 which revealed lowest value 0.005 and 0.007µg/mL respectively (Table 1). As for peroxidase enzyme level was higher in *Trichoderma* T.4679+*F.solani* R11, *Trichoderma* T.4+T.7, *Trichoderma* T.4+T.7+*F. solani* R11, *Trichoderma* T.4+T.9+*C.lunata* R7, *Trichoderma* T.4+T.9,*Trichoderma* T.4+T.7+*T.cucumeris* R12, *Trichoderma* T.6+T.7+*A .tenuissima* R23, *Trichoderma* T.4679+*F.oxysporum* R6 were gave value roughly 1.703, 1.451, 1.393, 1.374, 1.394, 1.366, 1.334,1.326 and 1.333 unit/mL respectively as comparison with *F.oxysporum* R6,*C.lunata* R7 which exhibited 1.091 and 1.040 unit/mL respectively. The content of chlorophyll was increased in *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.4679+*C.lunata* R7, *Trichoderma* T.6+T.7+*F.oxysporum* R6, *Trichoderma* T.4+T.7, *Trichoderma* T.4+T.9+*T.cucumeris* R12, *Trichoderma* T.4679+*T.cucumeris* R12 which exhibited 10.466, 10.233, 10.133, 9.567, 8.833, 8.367 and 8.367 SPAD respectively, and the lowest chlorophyll content was observed in *F.oxysporum* R6, *A.tenuissima* R23, *F.solani* R11 which gave 2.700, 2.100, 1.567 SPAD espectively as indicated in (Table 1).

Table 1.The effect of compatible *Trichoderma* T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 as biological control agents on chitinase, peroxidase activity and chlorophyll content of rice plant under greenhouse conditions

Treatments	Chitinase* µg/mL	Peroxidase* unit/mL	Chlorophyll* /SPAD
Natural Control	0.010 c	1.136 def	4.800 bcdefg
F.oxysporum R6	0.005 c	1.091 ef	2.700 efg
C.lunata R7	0.012 c	1.040 f	3.967 defg
F.solani R11	0.007 c	1.136 def	1.567 g
T.cucumeris R12	0.031 c	1.176 cdef	4.233 cdefg
A.tenuissima R23	0.024 c	1.183 cdef	2.100 fg
Trichoderma T.4+T.7	0.062 bc	1.451 b	8.833 abcd
Trichoderma T.4+T.9	0.182 bc	1.366 bcd	5.867 abcdefg
Trichoderma T.6+T.7	0.222 ab	1.305 bcde	6.400 abcdefg
Trichoderma T.4679	0.072 bc	1.219 bcdef	7.100 abcdef
Trichoderma T.4+T.7+*F.oxysporum* R6	0.159 bc	1.245 bcdef	5.900 abcdefg
Trichoderma T.4+T.7+*C.lunata* R7	0.059 bc	1.349 bcd	5.567 abcdefg
Trichoderma T.4+T.7+*F.solani* R11	0.085 bc	1.393 bc	5.167 abcdefg
Trichoderma T.4+T.7+*T.cucumeris* R12	0.065 bc	1.334 bcd	7.600 abcde
Trichoderma T.4+T.7+*A.tenuissima* R23	0.107 bc	1.284 bcde	4.867 bcdefg
Trichoderma T.4+T.9+*F.oxysporum* R6	0.029 c	1.171 cdef	4.267 cdefg
Trichoderma T.4+T.9+*C.lunata* R7	0.072 bc	1.394 bc	4.767 bcdefg
Trichoderma T.4+T.9+*F.solani* R11	0.085 bc	1.206 cdef	6.567 abcdefg
Trichoderma T.4+T.9+*T.cucumeris* R12	0.159 bc	1.264 bcdef	8.367 abcd
Trichoderma T.4+T.9+*A.tenuissima* R23	0.052 bc	1.264 bcdef	6.300 abcdefg
Trichoderma T.6+T.7+*F.oxysporum* R6	0.071 bc	1.222 bcdef	9.567 abc
Trichoderma T.6+T.7+*C.lunata* R7	0.059 bc	1.313 bcde	4.767 bcdefg
Trichoderma T.6+T.7+*F.solani* R11	0.067 bc	1.271 bcde	10.133 ab
Trichoderma T.6+T.7+*T.cucumeris* R12	0.082 bc	1.287 bcde	5.400 abcdefg
Trichoderma T.6+T.7+*A.tenuissima* R23	0.050 bc	1.326 bcd	7.300 abcdef
Trichoderma T.4679+*F.oxysporum* R6	0.044 bc	1.333 bcd	6.333 abcdefg
Trichoderma T.4679+*C.lunata* R7	0.122 bc	1.374 bc	10.233 ab
Trichoderma T.4679+*F.solani* R11	0.107 bc	1.703 a	6.600 abcdefg
Trichoderma T.4679+*T.cucumeris* R12	0.107 bc	1.282 bcde	8.367 abcd
Trichoderma T.4679+*A.tenuissima* R23	0.356 a	1.270 bcde	10.466 a

Mean* of three replicates of each treatment
Numbers in each column that have same letter do not differ significantly from each other at p<0.05 according to Duncan's multiple range test.

The effect of Trichoderma isolates;T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 on plant growth parameters

The data shows in Table 2 theeffect ofT.4+T.7,T.4+T.9, T.6+T.7 and T.4679 were added to the non-autoclaved soil on plant growth parameters e.g. fresh weight of shoot and root, dry weight of shoot and root and height shoot and root of the rice plant of the end of the season. We can see the result of fresh weight of shoot increased in *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.4679+*T.cucumeris* R12, *Trichoderma* T.6+T.7, *Trichoderma* T.4+T.7+*F.solani* R11, *Trichoderma* T.4+T.7+*C.lunata* R7, *Trichoderma* T.4679+*C.lunata* R7 were gave optimum rate at(34.760, 30.447, 29.833, 28.722, 29.016 and 28.305 g respectively). However, *T.cucumeris* R12, *F.solani* R11, *A.tenuissima* R23, *Trichoderma* T.6+T.7+*F.oxysporum* R6 gave the lowest value by approximately 5.184, 6.293, 6.396 and 6.606g respectively (Table 2). The treatment *Trichoderma* T.4679+*A.tenuissima* R23 which was valued at (34.760 g) as in comparison with *A.tenuissima* R23 without biocontrol agent (6.396 g). Fresh root weight results showed high value in *Trichoderma* T.4679+*T.cucumeris* R12, *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.4+T.7+*T.cucumeris* R12, *Trichoderma* T.4+T.9+*F.solan i*R11 which were valued at (14.560, 13.323, 13.976 and 11.190 g respectively**)** as comparison with *T.cucumeris* R12*, C.lunata* R7, *A.tenuissima* R23, *F.oxysporum* R6 and *F.solani* R11which obtained the lowest value 1.895, 1.693, 1.522, 1.515 and 1.187 g respectively.

Dry weight of shoot of the rice plant which inoculated with compatible *Trichoderm* a isolates T.4+T.7, T.4+T.9, T.6+T.7 was increased in *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.4679+*T.cucumeris* R12, *Trichoderma* T.4+T.7+*F.oxysporum* R6, *Trichoderma* T.6+T.7, *Trichoderma* T.4+T.7+*C.lunata* R7*, Trichoderma* T.4679+*C.lunata* R7*, Trichoderma* T.4+T.7+*F .solani* R11 were gave12.397, 11.498, 10.616, 10.482, 10.386, 10.279 and 9.216 g respectively. While *F.oxysporum* R6 evealed the lowest value (1.950 g). The data presented in (Table 2) shows dry weight of root exhibited that *Trichoderma* T.4679+*T.cucumeris* R12, T.4+T.7+*T.cucumeris* R12, *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.6+T.7, *Trichoderma* T.4+T.7+*C.lunata* R7, *Trichoderma* T.4+T.9+*F.solani* R11 had significantly ($p<0.05$) increased dry weight of root *(*4.199,4.087, 3.959, 3.725, 3.368 and 2.935g respectively) compared to *Trichoderma* T.6+T.7+*A.tenuissima* R23, *Trichoderma* T.6+T.7+*F.oxysporum* R6, *Trichoderma* T.6+T.7+*F.solani* R11, *Trichoderma* T.6+T.7+*T.cucumeris* R12, *A.tenuissima* R23, *Trichoderma* T.4+T.9+*F.oxysporum* R6, *T.cucumeris* R12, *C.lunata* R7, *F.oxysporum* R6, *F.solani* R11 which were gave the lowest values (0.691, 0.654, 0.606, 0.589, 0.547, 0.541, 0.483, 0.442, 0.395 and 0.370 g respectively (Table 2).

As for shoot height, data obtained from this study indicates that *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.4679+*C.lunata* R7, *Trichoderma*, T.4679+*F.solani* R11 were gave the best value by approximately (61.550, 61.220, 60.550cm respectively) as in comparison with *Trichoderma* T.6+T.7+*F.solani* R11*, T.cucumeris* R12, *C.lunata* R7*, F.oxysporum* R6*,A.tenuissima* R23 which resulted (49.500, 48.443, 48.500, 47.777 and 42.887cm respectively). Results of the root height showed *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.4+T.7+*T.cucumeris* R12, *Trichoderma* T.4679+*F.solani* R11, *Trichoderma* T.4+T.7+*F.solani* R11, *Trichoderma* T.4+T.9+*F.solani* R11, *Trichoderma* T.4+T.9+*C.lunata* R7 exhibited the highest value (28.773, 24.500, 24.440, 23.886, 23.773 and 23.550cm respectively), while *F.solani* R11, *F.oxysporum* R6 and *T.cucumeris* R12 were gave the lowest value by approximately 11.773, 11.496 and 9.500cm respectively (Table 2).

Table 2.The effect of compatible *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 as biological control agents on fresh and dry weight of shoot and root, high shoot and root under greenhouse conditions

Treatments	Plant Growth Parameters					
	*Fresh weight of shoot (g)	*Fresh weight of root (g)	*Dry weight of shoot (g)	*Dry weight of root (g)	*Plant shoot length (cm)	*Plant root length (cm)
Natural Control	19.234 bcdefghijk	6.964 cdefg	7.051 bcdefg	1.401 cde	54.220 abcd	17.883 bcdefg
F.oxysporum R6	7.029 jkl	1.515 g	1.950 h	0.395 e	47.777 de	11.773 gh
C.lunata R7	6.606 kl	1.693 g	2.389 gh	0.442 e	48.443 de	12.610 fgh
F.solani R11	6.293 kl	1.187 g	2.938 fgh	0.370 e	50.163 cde	9.500 h
T.cucumeris R12	5.184 l	1.895 g	2.719 fgh	0.483 e	48.500 de	11.496 gh
A.tenuissima R23	6.396 kl	1.522 g	2.871 fgh	0.547 e	42.887 e	13.996 fgh
T.4+T.7	21.949 abcdefgh	6.418 cdefg	7.156 bcdef	1.753 bcde	55.330 abcd	19.773 bcdef
T.4+T.9	20.381 bcdefghi	4.872 defg	7.067 bcdefg	1.468 cde	50.110 cde	17.610 bcdefg
T.6+T.7	29.833 ab	8.876 abcde	10.482 abc	3.725 ab	55.550 abcd	19.106 bcdefg
T.4679	11.143 fghijkl	2.678 efg	3.640 efgh	0.757 de	56.440 abcd	15.000 efgh
T.4+T.7+*F.oxy.* R6	26.058 abcd	2.341 fg	10.616 abc	4.087 a	51.943 abcde	22.386 abcde
T.4+T.7+*C.lun.*R7	28.722 abc	9.854 abcd	10.386 abc	3.368 abc	53.610 abcde	20.110 bcdef
T.4+T.7+*F.sol.*R11	29.016 abc	7.213 cdefg	9.216 abc	2.573 abcde	50.440 bcde	23.773 abc
T.4+T.7+*T.cuc.*R12	19.752 bcdefghij	13.976 ab	6.872 bcdefg	2.424 abcde	55.167 abcd	24.500 ab
T.4+T.7+*A.ten.*R23	9.595 ghijkl	6.033 cdefg	4.525 defgh	2.383 abcde	53.940 abcd	16.106 cdefgh
T.4+T.9+*F.oxy.*R6	11.590 efghijkl	5.754 cdefg	2.686 fgh	0.541 e	55.610 abcd	13.996 fgh
T.4+T.9+*C.lun.*R7	19.086 bcdefghijk	8.360 bcdef	7.292 bcdef	2.266 abcde	51.883 abcde	23.550 abc
T.4+T.9+*F.sol.*R11	22.393 abcdefg	11.190 abc	8.233 abcde	2.935 abcd	58.107 abcd	23.886 abc
T.4+T.9+*T.cuc.*R12	16.359 cdefghijkl	6.042 cdefg	6.072 cdefgh	2.148 abcde	55.163 abcd	19.163 bcdefg
T.4+T.9+*A.ten.*R23	14.181 defghijkl	5.914 cdefg	6.196 cdefgh	1.469 cde	53.773 abcd	23.163 abcd
T.6+T.7+*F.oxy.*R6	7.859 ijkl	2.744 efg	3.192 fgh	0.654 e	52.883 abcde	14.886 efgh
T.6+T.7+*C.lun.*R7	9.071 hijkl	3.014 efg	3.469 fgh	0.835 de	49.993 cde	15.326 defgh
T.6+T.7+*F.sol.*R11	9.624 ghijkl	2.759 efg	3.117 fgh	0.606 e	49.500 de	15.333 defgh
T.6+T.7+*T.cuc.*R12	8.981 hijkl	2.177 fg	3.264 fgh	0.589 e	51.330 abcde	15.330 defgh
T.6+T.7+*A.ten.*R23	7.793 ijkl	2.452 fg	4.467 defgh	0.691 e	51.777 abcde	15.443 defgh
T.4679+*F.oxy.*R6	23.297 abcdef	5.235 cdefg	8.582 abcd	1.310 cde	56.330 abcd	17.440 bcdefg
T.4679+*C.lun.*R7	28.305 abc	5.337 cdefg	10.279 abc	1.624 bcde	61.220 ab	19.886 bcdef
T.4679+*F.sol.*R11	24.410 abcde	9.509 abcd	8.738 abcd	2.506 abcde	60.550 abc	24.440 ab
T.4679+*T.cuc.*R12	30.447 ab	14.560 a	11.498 ab	4.199 a	58.773 abcd	22.856 abcde
T.4679+*A.ten.*R23	34.760 a	13.323 ab	12.397 a	3.959 a	61.550 a	28.773 a

Mean* of three replicate of each treatment. Numbers in each column that have same letter do not differ significantly from each other at $p<0.05$ according to Duncan's multiple range test.

The effect of compatibleTrichoderma isolates;T.4+T.7, T.4+T.9, T.6+T.7 and T.4679on PAL (phenyl alanine-ammonialyase) enzyme and dry weight of panicle (g)

Table 3 shows the results of effect *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 on PAL enzyme and dry weights of panicles of rice plants which were varied as shown by each of these isolates. The PAL enzyme was recorded in two times, the first period after two months of transplanting, and found *A.tenuissima* R23 was gave high score (3.841 unit/mL) in comparison with *Trichoderma* T.4+T.9+*T.cucumeris* R12 which gave only (3.059 unit/mL). Moreover, *Trichoderma* T.6+T.7+*A.tenuissima* R23 and *Trichoderma* T.6+T.7+*T.cucumeris* R12 gave a high level in PAL enzyme by approximately (3.545 and 3.543 unit/mL). However, the statistical analysis did not show any significant difference ($p<0.05$) in the level of PAL enzyme between *F.oxysporum* R6, *C.lunata* R7, *F.solani* R11, *T.cucumeris* R12 and *Trichoderma* T.4+T.7, *Trichoderma* T.4+T.7 combination with *C.lunata* R7, *F.solani* R11 and *T.cucumeris* R12*, Trichoderma* T.4+T.9 combination with *F.oxysporum* R6, *C.lunata* R7, *F.solani* R11 and *A.tenuissima* R23, *Trichoderma* T.6+T.7 combination with *C.lunata* R7, *F.oxysporum* R6 and *F.solani* R11, *Trichoderma* T.4679 combination with *T.cucumeris* R12 and *F.solani* R11 which yielded 3.421, 3.387, 3.374, 3.381, 3.394, 3.392, 3.395, 3.435,

3.364, 3.486, 3.359, 3.363, 3.399, 3.396, 3.453, 3.407 and 3.319unit/mL respectively (Table 3). However, the second reading of PAL enzyme in the end of experiment, and found this enzyme was reduced in all treatments especially in *Trichoderma* T.4+T.7+*F.solani* R11 and *Trichoderma* T.4+T.7+*T.cucumeris* R12 which gave the lowest rate (1.184 and 1.063 unit/mL respectively) as compare with the rest pathogens. The level of PAL enzyme was increased only in *F.solani* R11, *Trichoderma* T.4+T.9 and *Trichoderma* T.6+T.7+*A.tenuissima* R23, *Trichoderma* T.4679+*F.oxysporum* R6 to the 2.380, 2.484, 2.276 and 2.380 unit/mL respectively (Table 3). Dry weight of panicle was showed a significant increase in *Trichoderma* T.4679+*F.solani* R11, *Trichoderma* T.4679+*T.cucumeris* R12 and *Trichoderma* T.4679+*A.tenuissima* R23 were exhibited high value at 3.264, 2.509 and 2.087 g respectively (Table 3). While *F.oxysporum* R6, *C.lunata* R7, *F.solani* R11, *T.cucumeris* R12, *A.tenuissima* R23, *Trichoderma* T.4679, *Trichoderma* T.6+T.7+*F.oxysporum* R6,*Trichoderma* T.6+T.7+*C.lunata* R7, *Trichoderma* T.6+T.7+*T.cucumeris* R12 and *Trichoderma* T.6+T.7+*A.tenuissima* R23 which revealed the lowest value 0.232,0.286, 0.244, 0.315, 0.260, 0.562,0.442, 0.423, 0.428 and 0.363 g respectively (Table 3).

Table 3.The effect of compatible *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 as biological control agents on Pal enzyme and dry weight of panicle under greenhouse conditions

Treatments	*Pal enzyme after two months (unit/mL)	*Pal enzyme in the end of season (unit/mL)	*Dry weight of Panicle* (g)
Natural Control	3.421 bc	1.809 b	0.663 cd
F.oxysporum R6	3.421 bc	1.744 bc	0.232 d
C.lunata R7	3.387 bc	1.784 b	0.286 d
F.solani R11	3.374 bc	2.380 a	0.244 d
T.cucumeris R12	3.381 bc	1.751bc	0.315 d
A.tenuissima R23	3.841 a	1.348 cde	0.260 d
Trichoderma T.4+T.7	3.394 bc	1.497 bcd	0.818 cd
Trichoderma T.4+T.9	3.196 cd	2.484 a	1.042 cd
Trichoderma T.6+T.7	3.312 bcd	1.837 b	1.481 bcd
Trichoderma T.4679	3.312 bcd	1.502 bcd	0.562 d
Trichoderma T.4+T.7+*F.oxysporum* R6	3.282 bcd	1.651 bc	1.311 bcd
Trichoderma T.4+T.7+*C.lunata* R7	3.392 bc	1.858 b	1.022 cd
Trichoderma T.4+T.7+*F.solani* R11	3.395 bc	1.184 de	1.388 bcd
Trichoderma T.4+T.7+*T.cucumeris* R12	3.435 bc	1.063 de	1.205 bcd
Trichoderma T.4+T.7+*A.tenuissima* R23	3.230 cd	1.634 bc	0.884 cd
Trichoderma T.4+T.9+*F.oxysporum* R6	3.364 bc	1.893 b	0.585 cd
Trichoderma T.4+T.9+*C.lunata* R7	3.486 bc	1.579 bc	0.729 cd
Trichoderma T.4+T.9+*F.solani* R11	3.359 bc	1.605 bc	0.886 cd
Trichoderma T.4+T.9+*T.cucumeris* R12	3.059 d	1.726 bc	0.820 cd
Trichoderma T.4+T.9+*A.tenuissima* R23	3.363 bc	1.647 bc	0.592 cd
Trichoderma T.6+T.7+*F.oxysporum* R6	3.399 bc	1.752 bc	0.442 d
Trichoderma T.6+T.7+*C.lunata* R7	3.396 bc	1.772 b	0.423 d
Trichoderma T.6+T.7+*F.solani* R11	3.453 bc	1.707 bc	0.689 cd
Trichoderma T.6+T.7+*T.cucumeris* R12	3.543 b	1.638 bc	0.428 d
Trichoderma T.6+T.7+*A.tenuissima* R23	3.545 b	2.276 a	0.363 d
Trichoderma T.4679+*F.oxysporum* R6	3.318 bcd	2.380 a	0.997 cd
Trichoderma T.4679+*C.lunata* R7	3.332 bcd	1.780 b	1.724 bcd
Trichoderma T.4679+*F.solani* R11	3.407 bc	1.473 bcd	3.264 a
Trichoderma T.4679+*T.cucumeris* R12	3.319 bc	1.680 bc	2.509 ab
Trichoderma T.4679+*A.tenuissima* R23	3.313 bcd	1.742 bc	2.087 abc

Mean* of three replicate of each treatment. Numbers in each column that have same letter do not differ significantly from each other at p<0.05 according to Duncan's multiple range test.

The effect of compatible Trichoderma isolates; T.4+T.7, T.4+T.9, T.6+T.7 and T.4679on disease infection and severity

Table 4 shows the results of disease incidence and severity parameters of a local rice variety (cv.) *Al-Baraka* against causal agents *F.oxysporum* R6, *F.solani* R11, *C.lunata* R7, *A.tenuissima* R23 and *T.cucumeris* R12 which showed a significant differences (p<0.05) level of stimulation and increasing rice plant growth when treated with *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 (Figure 1).The greenhouse experiment showed that *Trichoderma* T.6+T.7 and *Trichoderma* T.4679+*A.tenuissima* R23 were effective in inducing significant decrease (p<0.05) in disease infection and gave lowest value 33% of both (Table 4). However, *F.oxysporum* R6, *C.lunata* R7, *F.solani* R11, *T.cucumeris* R12 and *A.tenuissima* R23 had same level in disease infection at approximately 100% respectively (Table 4). Visual ratings of disease severity parameter decreased significantly in *Trichoderma* T.4679 which exhibited most compatible and greater efficiency of reducing disease severity in *Trichoderma* T.4679+*A.tenuissima* R23 and *Trichoderma* T.4679+*T.cucumeris* R12 (8.883 and 11.553% respectively), as compared with all pathogens which gave significant increase (p<0.05) of (70. to 88.867 % respectively) after 120 days post transplanting.

Table 4.Antagonistic effect between compatible *Trichoderma* isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 and rice phytopathogens under greenhouse conditions

Treatments	*Disease infection (percentage, %)	**Disease severity (percentage, %)
Natural Control	88.887 ab	44.444 c
F.oxysporum R6	100.00 a	83.300 ab
C.lunata R7	100.00 a	75.567 ab
F.solani R11	100.00 a	78.867 ab
T.cucumeris R12	100.00 a	70.000 b
A.tenuissima R23	100.00 a	88.867 a
Trichoderma T.4+T.7	61.100 cd	19.997 efgh
Trichoderma T.4+T.9	38.667 de	18.887 fgh
Trichoderma T.6+T.7	33.000 e	17.777 fgh
Trichoderma T.4679	55.440 cde	39.997 cd
Trichoderma T.4+T.7+*F.oxysporum* R6	38.667 de	17.777 fgh
Trichoderma T.4+T.7+*C.lunata* R7	60.900 cd	24.443 defgh
Trichoderma T.4+T.7+*F.solani* R11	44.333 cde	17.777 fgh
Trichoderma T.4+T.7+*T.cucumeris* R12	61.133 cd	22.217 efgh
Trichoderma T.4+T.7+*A.tenuissima* R23	44.333 cde	24.440 defgh
Trichoderma T.4+T.9+*F.oxysporum* R6	44.233 cde	35.553 cde
Trichoderma T.4+T.9+*C.lunata* R7	38.667 de	26.663 defg
Trichoderma T.4+T.9+*F.solani* R11	55.553 cde	15.550 fgh
Trichoderma T.4+T.9+*T.cucumeris* R12	61.220 cd	19.997 efgh
Trichoderma T.4+T.9+*A.tenuissima* R23	55.550 cde	22.220 efgh
Trichoderma T.6+T.7+*F.oxysporum* R6	44.220 cde	26.663 defg
Trichoderma T.6+T.7+*C.lunata* R7	49.887 cde	28.887 def
Trichoderma T.6+T.7+*F.solani* R11	61.120 cd	27.550 defg
Trichoderma T.6+T.7+*T.cucumeris* R12	55.453 cde	24.440 defgh
Trichoderma T.6+T.7+*A.tenuissima* R23	38.777 de	24.443 defgh
Trichoderma T.4679+*F.oxysporum* R6	55.467 cde	17.773 fgh
Trichoderma T.4679+*C.lunata* R7	55.333 cde	13.330 fgh
Trichoderma T.4679+*F.solani* R11	60.900 cd	15.553 fgh
Trichoderma T.4679+*T.cucumeris* R12	50.000 cde	11.553 gh
Trichoderma T.4679+*A.tenuissima* R23	33.000 e	8.883 h

Numbers in each column that have same letter do not differ significantly from each other at p<0.05 according to Duncan's multiple range tests.*Disease infection and **Severity of rice plants, the experiments were conducted in triplicate for each pathogen. *Disease infection and **Severity were scored after 4 months from sowing according to Hamdia (2014).

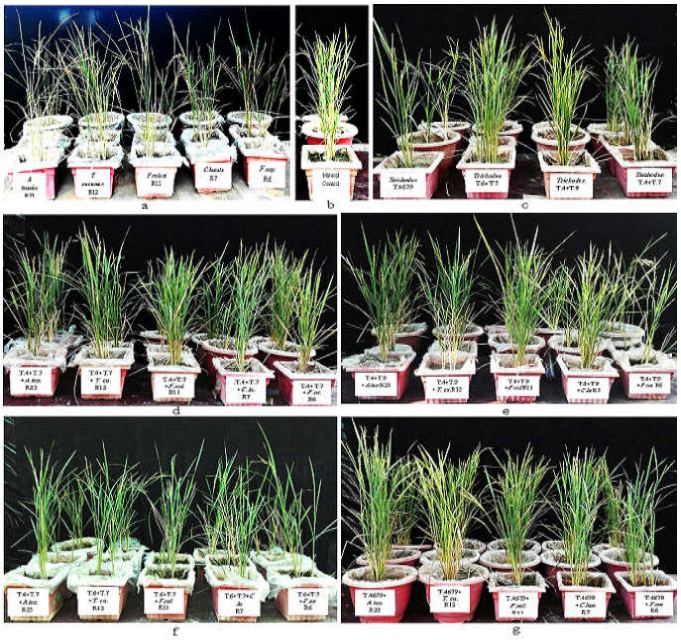

Figure 1. Evaluation of compatible effectiveness of *Trichoderma* isolates (T.4+T.7, T.4+T.9, T.6+T.7 and T.4679) to control rice phytopathogens under greenhouse conditions. (a) Pathogens treatments;) *F. oxysporum* R6, *C.lunata* R7, *T.cucumeris* R12, .(b) Control untreated (natural infection). (c) Plants inoculated with *Trichoderma* isolates only (T.4+T.7, T.4+T.9, T.6+T.7 and T.4679), without pathogens. (d) Plants inoculated with *Trichoderma* isolates (T.4+T.9) and *Fusarium oxysporum* R6, *F.solani* R11, *Curvularia lunata* R7, *Alternaria tenuissima* R23. (e) Plants inoculated with *Trichoderma* isolates (T.6+T.7) and *Fusarium oxysporum* R6, *F.solani* R11, *Curvularia lunata* R7, *Alternaria tenuissima* R23. (f) Plants inoculated with *Trichoderma* isolates (T.4+T.7) and pathogens; *Fusarium oxysporum* R6, *F.solani* R11, *Curvularia lunata* R7, *Alternaria tenuissima* R23. (g) Plants inoculated with *Trichoderma* compatible isolate (T.4679) and *Fusarium oxysporum* R6, *F.solani* R11, *Curvularia lunata* R7, *Alternaria tenuissima* R23. All labels Started from right side to the left.

Growth and development of compatible Trichoderma isolates population in the end of seasonunder greenhouse conditions

The results of compatible *Trichoderma* isolates which were taken in the end of experiment were observed population development increased in *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.6+T.7, *Trichoderma* T.4679+*T.cucumeris* R12, *Trichoderma*T.4+T.9+*A.tenuissima* R23, *Trichoderma* T.4+T.7+*C.lunata* R7**,** *Trichoderma* T.4679+*F.solani* R11, *Trichoderma* T.4679+*C.lunata* R7 and *Trichoderma* T.4+T.9+*F.solani* R11which were gave a high value 225×10^4, 220×10^4, 197×10^4, 148×10^4, 140×10^4, 135×10^4, 133×10^4 and 132×0^4 unit consisting of the colony (CFU) /1g of soil respectively as compared with all treatments as denoted in(Figure 2).

However clear decrease was showed in the treatments of *Trichoderma* T.4+T.9+*F.oxysporum* R6**,** *Trichoderma* T.4+T.9+*T.cucumeris* R12, *Trichoderma* T.6+T.7+*C.lunata* R7**,** *Trichoderma* T.6+T.7+*F.solani* R11, *Trichoderma* T.6+T.7+*A.tenuissima* R23 which were given the lowest number in population density by approximately 40×10^4, 40×10^4,47×10^4, 49×10^4, 43×10^4cfu /1g of soil respectively. While decreased slightly in the treatments of *Trichoderma* T.4+T.9 and *Trichoderma* T.4679+*F.oxysporum* R6 (110×10^4 and 108×10^4cfu) /1g of soil whereas decreased to become double decreasing in the treatments; *Trichoderma* T.4+T.7, *Trichoderma* T.4679, *Trichoderma* T.4+T.7+*F.solani* R11 and T.4+T.7+*A.tenuissima* R23 were displayed 74×10^4, 80×10^4,65×10^4 and 80×10^4cfu/1g of soil respectively (Figure 2)..

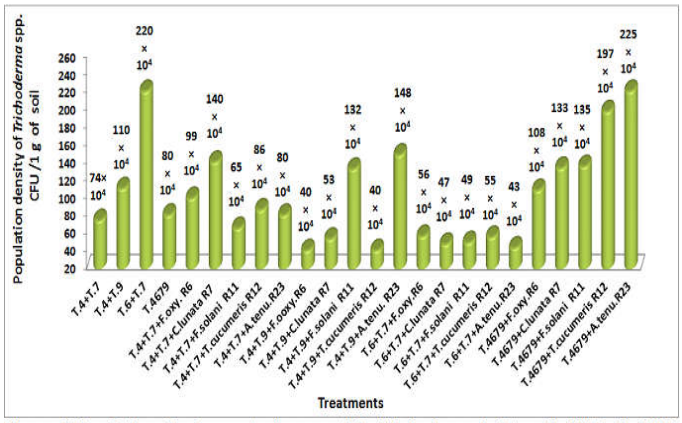

Figure 2.Population development of compatible *Trichoderma* isolates; T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 in the end of season under greenhouse conditions.

DISCUSSION

In greenhouse studies to determine the interactions between compatible *Trichoderma* isolates;T.4+T.7, T.4+T.9, T.6+T.7 and T.4679 and five fungal pathogens of rice plant; *F.oxysporum* R6*, F.solani* R11, *C.lunata* R7, *A.tenuissima* R23 and *T.cucumeris* R12.

As we can see from the data analyzed in (Table 1), the combination between *Trichoderma* isolates is very important to plant activity and growth, and the compatibility between *Trichoderma* isolates was led to a regular increase ofchitinase in the rice plant, as in the same case of peroxidase and chlorophyll after two months, and showed high increased in treatments; *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.4679+*F.solani* R11 and *Trichoderma* T.4679+*A.tenuissima* R23 were gave (0.356 µg/mL 1.703 unit/mL and 10.466 SPAD) respectively. While *F.oxysporum* R6, *C.lunata* R7 and *F.solani*R11 treatments recorded the lowest level by approximately 0.005 µg/mL, 1.040 unit/mL and 1.567 SPAD respectively (Table 1). These results were in agreement with several researchers who pointed out that the chitinase, peroxidase and chlorophyll had excellent potential to suppress pathogens, and this fact indicated to high compatibility between *Trichoderma* isolates and hence we correlated this to chitinase, peroxidase enzymes and chlorophyll activity (Nadarajah *et al.,* 2014; Mustafa, 2016; Zhang *et al.,* 2016).

The results of chitinase, peroxidase and chlorophyll activity were significantly influenced on plant growth such as fresh weight of shoot and root. *Trichoderma* T.4679+*A.tenuissima* R23 and *Trichoderma* T.4679+*T.cucumeris* R12 were gave high value (34.760 and 14.560 g) respectively as compare with *T.cucumeris* R12 and *F.solani* R11 which gave the lowest value 5.184 and 1.187 g respectively (Table 2). Dry weight of shoot, root and panicle was showed a significant increase in *Trichoderma* T.4679+*A.tenuissima* R23, *Trichoderma* T.4679+*T.cucumeris* R12, *Trichoderma* T.4+T.7+ *F.oxysporum* R6, *Trichoderma* T.4679+*A.tenuissima* R23 and *Trichoderma* T.4679+*F.solani* R11 were gave the highest valueat 12.397, 4.199, 4.087, 3.959 and 3.264 g respectively as indicated in (Table 2 and 3), as in comparison with *F.oxysporum* R6, *F.solani* R11 and *F.oxysporum* R6 which gave only 1.950, 0.370 and 0.232 g respectively (Table 2 and 3). The results of high shoot and root parameters exhibited *Trichoderma* T.4679+*A.tenuissima* R23 gave high rate by approximately 61.550 and 28.773 cmas compared with *A.tenuissima* R23 and *F.solani* R11 which recorded the lowest value at 42.887 and 9.500cm(Table 4). As we can see from above results, the increasing in plant growth and productivity gave us evidenced with the isolates that showed excellent compatibility between *Trichoderma* isolates especially when combined in one isolate T.4679 and used against pathogens as denoted in (Table 1 and 4). Also give clear indicator of the importance role of *Trichoderma* isolates on effect in the growth and development of the root system, which will reflect positively on the level of chitinase, peroxidase and chlorophyll (Figure 1). The results of this study were agreed with several studies that point out to combination between *Trichoderma* isolates to increase plant growth parameters and production (Susana, 2006; Shahbazi *et al.,* 2014; Samuelian, 2016)

The result of PAL (Phenyl alanine-ammonialyase) enzyme was recorded after two months from culture and found significant differences ($p<0.05$) between treatments e.g.*A.tenuissima* R23 was recorded high score (3.841 unit/mL), as compared with *Trichoderma* T.4+T.9+*T.cucumeris* R12 which gave lowest value (3.059 unit/mL), however, the PAL enzyme concentration decreased occurred after four months from sowing at any treatment of compatible *Trichoderma* isolates added that maybe explains a large part of the PAL enzyme in the plant was induced this stage of plant growth. PALenzyme in the end of experiment found *F.solani* R11, *Trichoderma* T.4+T.9, *Trichoderma* T.6+T.7+*A.tenuissima* R23 and *Trichoderma* T.4679+*F.oxysporum* R6 were gave 2.380, 2.484, 2.276and 2.380unit/mL respectively (Table 3). Certain researchers stated that PAL activity concentration of plants is produced in all plants in response to pathogen attack. However the speed at which this enzymeis expressed to

contain the infection determines the level of resistance of the host, and impact on plant growth and production (Mustafa, 2016).

When we refer to the greenhouse experiment results of disease infection and severity as shown in Table 4 and Figure 1, the best *Trichoderma* isolates potential were displayed by *Trichoderma* T.6+T.7 and *Trichoderma* T.4679+*A.tenuissima* R23 were effective in inducing significant decrease ($p<0.05$) in disease infection and gave lowest value (of both 33%). However, *F.oxysporum* R6, *C.lunata* R7, *F.solani* R11, *T.cucumeris* R12, *A.tenuissima* R23 were gave high value in disease infection at approximately 100% respectively (Table 4)**.** Disease severity parameter was reduced significantly in *Trichoderma* T.4679 which exhibited most compatible and greater efficiency of reducing disease severity when applied pathogens with *A.tenuissima* R23 and *T.cucumeris* R12 by approximately 8.883 and 11.553% respectivelyas compared with all pathogens which gave significant increase ($p<0.05$) of (70. to 88.867 % respectively**)** after 120 days. Figure 2 shows the viability of compatible *Trichoderma* isolates used in this study, and influence on the plant also the population density to the fungal biomass of *Trichoderm*a isolates T.4+T.7, T.4+T.9, T.6+T.7 and T.4679.*Trichoderma* isolate T.4679 which gave high colonization in *A.alternata* R23 at approximately 225×10^4cfu /g of soil (Figure 2) in the end of season was correlated positively with growth parameter maybe due to the activities of *Trichoderma* enzymes (Harman, 2004; Nandani *et al.,*2012; Yoshioka *et al.,* 2012; Shahbazi *et al.,* 2014; Hamdia *et al.,* 2016c; Nakazawa *et al.,*2016). Overall, *Trichoderma* is a saprophytic fungus, and the non-sterilized soil contained a rich source of several microorganisms from which it may obtain food and other chemical exudates to increase its growth, sustenance and effectiveness (Hamdia, 2014).

Ultimately, all *Trichoderm*a isolates had high level in suppression disease severity, but results showed *Trichoderma* isolates T.4+T.7, T.4+T.9 and T.6+T.7 were further compatible when these isolates were used in combination to produce one isolate T.4679 which recorded the highest growth parameters, and production than dual inoculation of *Trichoderma* isolates T.4+T.7, T.4+T.9and T.6+T.7 (Figure 1g).

CONCLUSION

We had identified the compatible effectiveness of *Trichoderma* isolates as biocontrol agent under greenhouse conditions, and found *Trichoderm* a isolate T.4679 was the best combination among *Trichoderma* isolates T.4+T.7, T.4+T.9 and T.6+T.7. This therefore indicates to make valid concentrations of efficiency isolate T.4679 against *B.spicifera*, *C.lunata*, *Fusarium* spp., *N.oryzae, E.rostratum, Alternaria* spp. And *T.cucumeris* that had been studied previously; may be developed further as commercial agent as well as soil conditioners that may induce growth of crops.

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