Pak. J. Bot., 51(1): 289-295, 2019. DOI: 10.30848/PJB2019-1(6)

A NOVEL NATIVE BIO-CONTROL AGENT *TRICHODERMA BREVICOMPACTUM* PROMOTES GROWTH AND RESISTANCE TO THE POWDERY MILDEW IN *IMPATIENS BALSAMINA*

ABDUL MAJEED BALOCH[1Θ], TONGTONG ZHAI[1Θ], ABDUL WAHID BALOCH[2], ZHIHUA LIU[3], XINGTANG YANG[1], CHUANYING JIANG[1], ZHAOYING LIU[1], AND RONGSHU ZHANG[1]*

[1]College of Landscape Architecture, Northeast Forestry University, 26 Hexing Road, 150040 Harbin, China,
[2]Department of Plant Breeding and Genetics, Sindh Agriculture University Tandojam, Pakistan; [3]School of Forestry, Northeast Forestry University, 26 Hexing Road, 150040 Harbin, China
[*]Corresponding author's email: zrs_nefu@163.com
[Θ]Authors contributed equally to this work

Abstract

Ornamental plants are very important to control environmental pollution and ultimately contribute in beautification of urban areas. Native *Trichoderma brevicompactum* Tb-50 isolated from rhizosphere soil of ornamental plant, *Salvia splendens* Ker-Gawler was identified *via* combination of morphological and molecular methods based on rDNA internal transcribed spacer region gene sequences. The antagonist action of Tb-50 against five soil-borne plant pathogens, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria alternate* and *Cytospora chrysosperma*, was respectively investigated on dual culture in petri dishes. Moreover, the present study focuses to promote flower production, improvement of vegetative parts, diseases resistance and early blossoming flowers of *Impatiens balsamina* by the applications of Tb-50. The presented results showed positive effects on leaves, stem height, seeds setting and plant height when compared with control group (CK). Besides, it is also found that the applications of Tb-50 controlled powdery mildew on *I. balsamina*.

Key words: *Trichoderma brevicompactum, Impatiens balsamina,* dual culture, antagonist, growth promotion

Introduction

Rose balsam (*Impatiens balsamina*) is mainly grown as ornamental plant, and annually grows up to 25 to 75 cm tall. These plants tend to have flowers of different colours such as red, pink and white (Rajendran *et al.,* 2014). Rose balsam also possesses medicinal properties *i.e.,* Tib-e-Unani, and have been used for different purposes such as snake bite, jaundice in elderly and in adults patients (Meenu *et al.,* 2015). In addition, anthocyanin, saponins and flavonoids have also been isolated from *I. balsamina* (Suk-Nam *et al.,* 2013). Increase in human population presents great challenges, and to cope with this, it is needed to improve agricultural products. However, increases in use of pesticides have negatively affected certain crop species and pose risk for human health (Gerhardson *et al.,* 2002). Certain economically important crop varieties have become extinct due to harmful plant pathogens that ultimately resulted disturbance in human living standards because of organic food crops food (Mamta *et al.,* 2013). Soils contain several types of chemicals waste, metals, and environment contamination by gases, these all result in a great amount of reduction in plants development, growth rate and low defense system in plants (Pereira *et al.,* 2014).

Trichoderma spp. were used for the first time in 1930 in several parts of world against different plant pathogens and became one of the most important biological agents (Goudjal *et al.,* 2014; Shafique *et al.,* 2015). Recent researches have proved that part of *T. longibrachiatum* can fight against plant pathogen and improve both vegetative and reproductive plant structures (Kredics *et al.,* 2003; Jaklitsch, 2009). Many *Trichoderma* spp. strains have been proved very effective bio-protect for crops, and used to control plants pathogens *viz.*, *Fusarium oxysporum, Alternaria alternata, Cytospora chrysosperma, Rhizoctonia solani, Sclerotinia sclerotiourm* (Midhun & Sobita, 2017). Different phenomena observed by different plant scientists on several plants treated with *Trichoderma* spp. that resulted a significant increase in whole plant area, such as leaf size, shoot length as well as progress in dry weight (Saravanakumar *et al.,* 2013; Febri *et al.,* 2014). *Trichoderma* spp. became main bio-control agents which applied on a crop variety, and can increase yield in crops and progress in vegetative growth (Vipin & Ankur, 2015).

Our present study was designed to isolate *Trichoderma* from rhizosphere soil of ornamental plant, *Salvia splendens* Ker-Gawler. A dominant *Trichoderma* strains, identified by us as *T. brevicompactum* Tb-50. We also performed antagonist assays of Tb-50 against five major soil-borne plants pathogens. Besides, Tb-50 strain was also applied to promote vegetative growth of *I. balsamina*. Meanwhile, Tb-50 was also applied against powdery mildew on *I. balsamina*. Our present study will be useful for horticultural crops and controlling their diseases.

Material and Methods

Fungal strains and Isolation: Pathogenic fungi strains *S. sclerotiorum*, *F. oxysporum*, *R. solani*, *A. alternata* and *C. chrysosperma* were kindly provided by Professor Zhihua Liu, School of Forestry, Northeast Forestry University, Harbin, China. These pathogenic fungi were cultured on PDA medium in petri dishes at 26°C in dark. The rhizospheric soil of ornamental plant, *S. splendens*, was collected from Qunli National Urban Wetland Park, Daoli District, Harbin, China. Sample of rhizospheric soils was filled in sterile sample bags that were tightly closed, put in icebox and were immediately brought to laboratory. *Trichoderma* isolates was applied through the modified soil dilution method on Rose Bengal Medium (RBM) (Elad *et al*., 1981). Collected 10 g samples of rhizospheric soils of *S. splendens* were made into 100 ml soil suspension with

double distilled water (ddH_2O). Soil suspension dilution with ratio of $1:10^3$, $1:10^4$ and $1:10^5$. 200 µl of each of diluent suspensions were evenly spread on RBM in a petri dish, and incubated at 28°C in dark. When colonies emerged, hyphae were sub-cultured on PDA medium. New hyphae were repeatedly incubated on new PDA medium until uncontaminated single colony of *Trichoderma* was obtained. Total 24 single colonies were obtained. RBM was made with ddH_2O, containing tryptone 5 g/L, glucose 10 g/L, KH_2PO_4 1 g/L, $MgSO_4$ 0.5 g/L, Rose Bengal 0.033 g/L, chloramphenicol 0.1 g/L. Agar 20 g/L medium was autoclaved at 121°C for 15 minutes.

Morphological examination of Tb-50: The isolates of *Trichoderma* were grown on PDA medium on sterilized petri dishes kept in 28°C in constant darkness. Growth rate of mycelium, both color and yield of conidia and production of secretion were monitored on daily basis. Morphological identification of these isolates was done microscopically (Leica DM2500, Germany), where hyphae septum, phialide, conidiophores and conidia were observed. Besides, all 24 colonies were evaluated online information website (http://nt.ars-grin.gov/taxadescriptions/keys/*Trichoderma* Index.cfm) for morphological identification. Finally, the dominant strain having 8 same colonies, which was higher among all 24 colonies, and named as Tb-50 for further molecular identification.

Molecular identification of Tb-50: Mycelia of Tb-50 was grown in PD liquid medium at 28°C in shaking incubator for 4 days (200 r/min). Mycelia were removed with the help of sterilized cotton gauze and forceps and then were placed in liquid nitrogen for total DNA isolation. For molecular identification of DNA isolation, E.Z.N.A DNA fungal kit (Omega) was used according to the manufacturer's instructions. ITS (internal transcribed spacer) sequence of the Tb-50 was amplified with PCR by using a pair of primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR reaction of 50 µl volume was prepared with 1×Buffer, 0.2 mM of each dNTP, 0.2 µM of each primer, 500 ng of genomic DNA of Tb-50 with 5 U/uL of TakaRa *Ex* Taq® polymerase (TakaRa Co. Dalian). Thermal-cycling conditions were set as 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min and a final extension of 10 min at 72°C. PCR products were examined at 1% of electrophoresis agarose gel, then purified with a Promega Wizard SV gel and PCR clean-up method kit (Promega) for sequencing. ITS sequence of Tb-50 was submitted on ISTH (International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy) (http://www.isth.info/) for rapid expressing of taxonomical annotation. MEGA software 6.06 and neighbor-jointing system with bootstrap re-sampling (1000 trails) were used for construction of a phylogenetic tree for phylogenetic analysis.

Antagonistic test: The dual culture method was carried out to assess the abilities of growth inhibition of Tb-50 to pathogenes such as *S. sclerotiorum*, *F. oxysporum*, *R. solani*, *A. alternata* and *C. chrysosperma*. These pathogenes were single inoculated separately on PDA in different petri dish at 28°C as control groups. The pathogenes were also inoculated separately with Tb-50 in opposite in plates as the treatment groups. Colony characteristics of Tb-50 and pathogenes were observed on daily basis and inhibitory rates were calculated at 6th day after the inoculation. The inhibitory rate (%) = [(the expansion radius of the colony of pathogens in control group - the expansion radius of the colony of pathogens in dual culture test) / the expansion radius of the colony of pathogenes in control group] × 100 %.

Plant materials preparation: The seeds of *I. balsamina* were purchased from local flower market of Harbin, and the seeds were grown in plastic seedling trays in field soil with vermiculite ratio 1:1 and size 5×5 cm at the research station of College of Landscape and Architecture at Northeast Forestry University. Then the seedlings of smaller height of about 10 cm were transplanted in the pots of size 21×21 cm, having cultivated soil mixed with peat soils and decayed pine needles, all soils were sterilized before planting.

Seedlings treatment: Tb-50 strain was cultured on PDA medium in petri dishes at 28°C for 6 days for harvesting adequate conidia. The concentration of Tb-50 conidia in water suspension was adjusted to 5×10^6 cfu/ml for using as inocula. For each pot, 200 ml of inoculums was poured into soil, equally around stem base of seedling. For control group, seedlings were treated with same volume of tap water. Pouring of water, weeding, aeration were done on time and green-house light was natural illumination.

Measurement of the morphological indexes of *I. balsamina*: Two groups with and without treatment by Tb-50 were measured for 10 plants with 3 replications. Pinch topping were done when the seedling height was about 20 cm. Statistical measurement began at time of the constancy of overall plants traits. Total five morphological indexes including plant height, basal diameter, crown diameter, primary branch number and leaf number were employed for evaluation of growth promoting effect.

Evaluation to flowering phenology and fructification of *I. balsamina*: Flower number of per plant was recorded on daily basis from the day of the first flowering until all flowers bloomed. Besides, the fruits and seeds yield of per plant were also counted. Flowering phenology was evaluated by initial flowering time, full flowering time, last flowering time, flowering phase, first flowering time, first fruit time, flower quantity in intraday of *I. balsamina* during flowering phase, fruit yields and weights of 100 seeds of *Impatiens*. Initial flowering time was dated as the number flowering individuals was 5% of test individuals, and full flowering stage was the period of the number flowering individuals ≥50% test individuals, then the number flowering individuals ≤10% was dated as last flowering time.

Control on powdery mildew of *I. balsamina* by Tb-50: Spore concentration of 5×10^6 cfu/ml of 200 ml Tb-50 water suspension was poured again into the soil of cultivating *I. balsamina* for 2 times, at the time of powdery mildew disease initial attack stage and at the time of poured Tb-50 for 7 days, respectively. For control group, the same

volume of tap water was used to pour in *I. balsamina*. Investigation of powdery mildew disease in *Impatiens* was done after the last treatment by Tb-50 for 7 days. The grading standards of powdery mildew disease on *I. balsamina*s shown as following symptoms were recorded at five different stages; at 0 level plants were found to be healthy; in stage 01, less than 25% all leaves showed symptoms; in stage 02, from 25% to 50% leaves were found to be covered with powdery mildew spots; in stage 03, more than 50% leaves had powdery mildew symptoms with yellow color and some leaves litter; in final stage 04, the leaves of whole plant became symptomatic with black color, showing wilt symptoms, and most of leaves were dying off. Diseased plants rate (%), including to diseases index and control disease rate (%) were counted: Diseased plant rate = (number of diseased plants /total number of tested plants) ×100%. Diseases index = [Σ (number of diseased plants at each stage × corresponding level) /(total number of tested plants × highest disease level)]× 100%. Control disease rate (%) = [(disease index of control group - disease index of treatment group) / disease index of control group] × 100%.

Data processing: We used Microsoft Office Excel 2007 Software Package, Minitab16 statistical software package for statistical analysis. Difference between treatments and control at same time point were performed by ANOVA analysis (p=0.05).

Results

Tb-50 identified as *T. brevicompactum*: Both macroscopic and microscopic morphological traits of Tb-50 were observed. Mycelium, which inoculated on PDA medium at 26°C in dark, was found to develop rapidly, radiating to the peripheral in initial stage of inoculation; pale green and white conidiation started at the 4th day. The conidia turned dark green gradually at 6th day. In addition, the initial growth position of colonies on the medium was infertile (Fig. 1 a and b). The back of the colony was pale green (Fig. 1c). The hyphae of Tb-50 were surface-smooth, transparent under microscope (Fig. 1d). Conidia were sub-spherical or oval and smooth, conidiophores was 1.8-2.9×0.9-2.1 µm. Conidiophore was produced in clusters of spores, initially with a long sterile section, or along itself scattered branches. Sterile section of conidiophore at later stage was not obvious. Conidiophores were branched and symmetrically distributed. Branches were shorter rarely the secondary branches were found. 2-4 phialides, having ampoule shape and middle of the obvious expansion, were present at nearly right angles on top of the conidiophore. The observed Tb-50 characteristics matched well with the descriptions of *T. brevicompactum* on ISTH.

DNA barcoding method was employed to further confirm that Tb-50 was *T. brevicompactum*. Previously reported primers were selected to amplify ITS segments of Tb-50 and was successfully cloned and then sequenced, the sequence were deposited in GenBank with accession number of KY408028. Thereafter the Tb-50 ITS sequence was submitted to *Trich*OKEY and *Tricho*BLAST on ISTH for identification. Results showed that ITS sequence of Tb-50 contained five anchors located at 74 bp, 96 bp, 258 bp, 416 bp and 508 bp, respectively, suggesting with high reliability that Tb-50 was a strain of *T. brevicompactum*. According to both of morphological and molecular characteristics, Tb-50 strain was identified as *T. brevicompactum.*

Phylogenetic analysis for Tb-50: A phylogenetic tree based on BLAST (Expected= 0.0) results of the sequences of ITS comprising 6 *Trichoderma* strains (Tb-50 and another 5 strains from ISTH database that had a higher identity) was constructed (Fig. 2). The phylogenetic tree showed that 6 strains formed two clades, cluster I and II. Tb-50 was clustered with *T. brevicompactum* (n/a Cornell 9, AF400267), and *H. melanomagna* (GJS99-153, AY737770) (cluster I). *T. brevicompactum* (n/aCornell 9) from ISTH database (cluster I) was the closest genetic relative of Tb-50 with highest identity (96%). Contrary, *T. fertile* (DAOM167161) was not closely related to Tb-50 and the identity was only 94% with the sequence length of 422 bp. The phylogenetic tree based on ITS sequences further verified that Tb-50 was *T. brevicompactum.*

***In vitro* antagonistic capacity of Tb-50:** The results of dual-culture showed that Tb-50 had inhibition effects to different extents on five common soil-borne pathogenic fungi, and final antifungal effects were *S. sclerotiorum* > *R. solani* > *C. chrysosperma* > *A. alternata* > *F. oxysporum* (Fig. 3A, B and C). We found that mycelia of pathogenes had touched Tb-50 at the 4th days-time (Fig. 3A). However, the inhibition effects of Tb-50 against pathogenes were significant excluding *F. oxysporum* strain. *R. solani* grew faster in control colonies where they occupied entire plate. It also occupied over 57% nutrition space on petri dish when in dual culture with Tb-50 sustained for 4th days. Growth of Tb-50 showed much rapid, its sporulation was increased significantly at the location touching *R. solani* at the 6th days-time. Meanwhile, it revealed bacteriolysis of *Trichoderma* by the phenomenon that an inhibition zone appeared at the juncture of Tb-50 and *R. solani* strain. In addition, Tb-50 was a strong competitor because it grew faster than pathogens (except *R. solani*), could occupy the space quickly and ingest nutrition, which revealed the mechanisms of nutritional competition of *Trichoderma*. In dual test, with the passage of time mycelia of five pathogenes hardly grew and/or the growth was slower than that of control group (Fig. 3B). Tb-50 continued to grow towards the colonies of pathogenes and had lived on them consuming their mycelia as nutrients for Tb-50 growth and reproduction. The inhibitory rates of Tb-50 to the five pathogenes were detected on the 6th day after completed dual culture. It revealed that the inhibitory rate of Tb-50 to different pathogenes was significant differences (p< 0.05). Inhibitory effect of Tb-50 to *S. sclerotiorum* was the best with inhibitory rate of 80.6% and inhibitory effect was the least to *F. oxysporum* with the inhibitory rate of 46.97% (Fig. 3C). Moreover, medium inhibitory rate to *A. alternate*, *C. chrysosperma* and *R. solani* were found as 51.6%, 67.5% and 73.5%, respectively showing a positive result that Tb-50 controled these soil-borne plant pathogenes.

Effects on growth of *I. balsamina* treated by Tb-50: The results showed many positive effects on growth and development of *I. balsamina* treated by Tb-50 Table 1. Under the treatment of Tb-50, compared with control group, plant height and basal diameter of *I. balsamina* were increased substantially of 1.25 and 1.21 times, respectively. Meanwhile, the crown diameter, primary branch number and number of leaves of *I. balsamina* were also increased by 1.10, 1.21 and 1.55 times compared with control. All these improvements promoted *I. balsamina* growth.

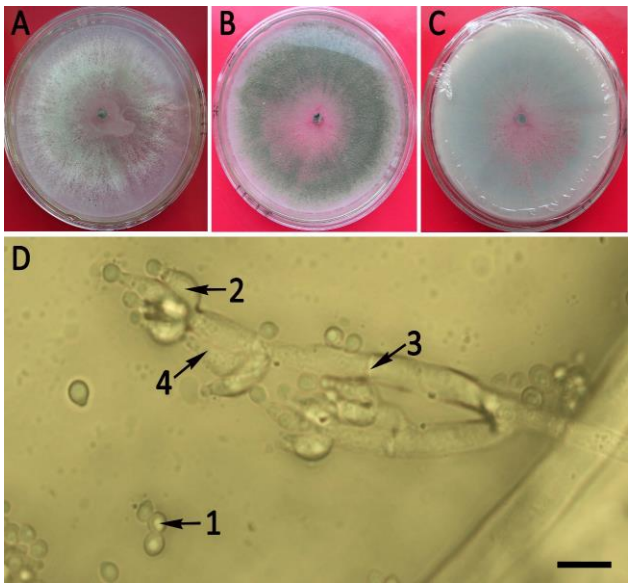

Fig. 1. Colony morphology of Tb-50 cultured on PDA medium at 26°C. A: front side incubated for 4 days; B front side and C back side incubated for 6 days, respectively. D: Microscopic morphology of Tb-50; Bar=4 μm. 1. Conidia; 2. Phialide; 3. Mycelium septum; 4. Conidiophore.

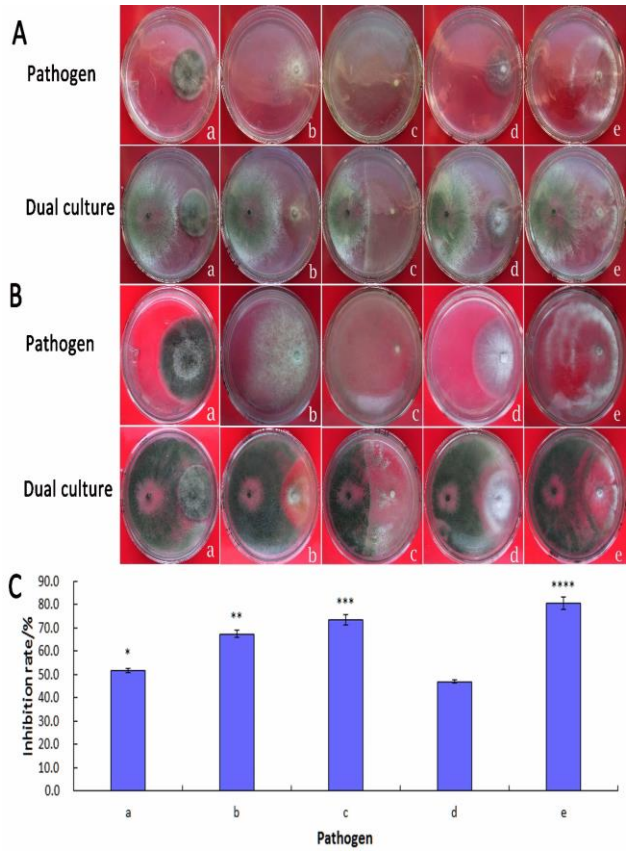

Fig. 3. Pathogen and dual-culture results of Tb-50 with each of the five pathogenes, respectively. In A-C: a. *A. alternata*; b. *C. chrysosperma*; c. *R. solani*; d. *F. oxysporum*; e. *S. sclerotiorum.* A: incubated at 26°C in dark for 4th days. B: incubated at 26°C in dark for 6th days. C. Inhibition rates of Tb-50 against each of the five pathogenes after 6th days of dual-culture (Data= mean ± SD; n=3; * represented the significance of difference at the level of $p<0.05$).

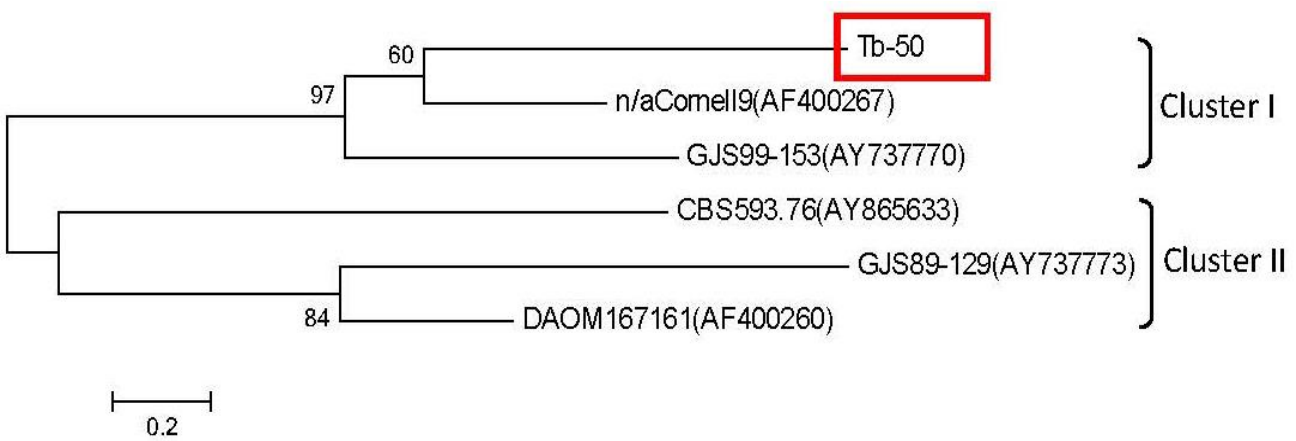

Fig. 2. Phylogenetic tree based on ITS sequences. Adjacency Neighbor Joining (NJ) method was used to construct phylogenetic tree (Bootstrap=1000). I and II: clusters. Th62 is marked with red frame.

Table 1. Effects of Tb-50 on the growth of *I. balsamina.*

Group	Plant height/cm	Basal diameter/mm	Crown diameter/cm	Primary branch number	Leaf number
CK	38.93 ± 1.30b	13.37 ± 0.13b	34.57 ± 0.94b	8.53 ± 0.25b	37.83 ± 1.31b
T	48.53 ± 1.05a	16.24 ± 0.27a	37.90 ± 0.69a	10.33 ± 0.33a	58.80 ± 1.27a

Significant level at $p<0.05$

Table 2. Effects of Tb-50 on flowering phenology of *I. balsamina.*

Group	Initial flowering time	Fully flowering time	Last flowering time	Flowering phase /days	First flowering time	First fruit time
CK	30th Aug.	9th Sep.	1st Nov.	74	30th Aug.	4th Sep.
T	25th Aug.	7th Sep.	7th Nov.	80	24th Aug.	27th Aug.

Table 3. Flowering number in intraday of *I. balsamina* during flowering phase.

Group	Initial flowering phase	Fully flowering phase	Last flowering phase
CK	2	133	2
T	2	192	2

Table 4. Effect on the yields of the seeds of *I. balsamina* treated by Tb-50.

Group	Average number of fruits/per plant	Fruit rate/%	100-seed weight/g
control	79.13 ± 1.49b	54.3 ± 1.7b	1.220 ± 0.20a
T	92.37 ± 1.05a	69.8 ± 1.6a	1.293 ± 0.16a

Table 5. Effect on powdery mildew in *I. balsamina* treated by Tb-50.

Group	diseased plants rate/%	Disease index	Control effect/%
Control	54.44 ± 1.57a	17.78 ±0.19a	-
T2	32.22 ± 4.16b	8.06 ± 1.04b	54.82

Effect on floral phenology and fructification of *I. balsamina* and improve seed setting treated by Tb-50: It showed clearly that the floral phenology of *I. balsamina* was changed by Tb-50 treatment. Both initial flowering time and full flowering time of *I. balsamina* were moved up 5 days and 2 days, respectively, and the final flowering time was delayed 6 days. In addition, the present fruit time was also delayed by 7 days (Table 2). On the other hand, the survey during flowering phase showed that the number of flowers was the same in intraday of *I. balsamina* during the initial flowering phase and the last flowering phase. While, it was 1.44 times during the fully flowering phase (Table 3). It revealed the significant effect on the flowering number at full flowering phase, but showed little influence on the flowering number at initial and last flowering phase. Meanwhile, fructification of *I. balsamina* was influenced by Tb-50. The average number of fruits of a plant and the fruit rate from flowers of *I. balsamina* were enhanced significantly by 16.7% and 28.48%. 100-seed weight was higher than control with 0.073 g (Table 4). The results showed that seeds yield of *I. balsamina* was increased by Tb-50 treatment.

Control of powdery mildew in *I. balsamina* by Tb-50: Our results showed positive effect to controlling the powdery mildew in *I. balsamina* by Tb-50. After poured Tb-50 in the soil, both the rate of the diseased plant and disease indexes were decreased significantly to 32.22% and 8.06, respectively. Meanwhile the control effect was 54.82% (Table 5).

Discussion

Trichoderma is widely distributed fungus in nature and is an important part of soil microbial community (Zhang *et al.,* 2018). At present, *Trichoderma* was widely used in industrial and agricultural production, therefore its identification was quite important (Rosemary & Maarten, 2005). With the development of biotechnology, methods of morphological and molecular biology identification have been used to classify and identify species of *Trichoderma*. Thus, in this study, the Tb-50 from rhizophere soil of *S. splendens* was identified by the method combining the morphological traits and DNA oligonucleotide molecular marker. First, its spores, hyphae and colony morphology characteristics of growth and distribution as well as microstructure of conidia, bottle stalk, diaphragm and conidia were observed. Preliminarily, Tb-50 was consistent with the morphology of *T. brevicompactum*. ITS sequence of Tb-50 was submitted to ISTH website with ITS database, more consistent of ITS sequence for phylogenetic analysis, and the identification of Tb-50 was confirmed as *T. brevicompactum*. *Trichoderma* often exists in plant rhizosphere where it performs dual functions *i.e.,* promotes plant growth and controls plant diseases (Rai, 2016). The interaction mechanism between *Trichoderma* and plants is quite complex. It has been shown that *Trichoderma* were colonized in plant roots affecting the plant metabolism, inducing plant resistance, increasing nutrient utilization, thereby boosting plant growth and increasing crop yields (Brotman *et al.,* 2008; Doni *et al.,* 2013; Hermosa *et al.,* 2013). However, it was found that the interactive effects of plant promotion growth were different when *Trichoderma* were colonized in the roots of different plant species. At present, the mechanism of plant promotion growth of *Trichoderma* spp. was constantly observed. *Impatiens* are annual herbaceous plants that are common ornamental flowers and have important medicinal value (Ahmed & Koperuncholan, 2012; Baskar *et al.,* 2012). Our results showed that Tb-50 had important role on the flowering phenology of *impatiens*, i.e., advance and extending the flowering time, and increasing flower number, thus enhancing their ornamental value. Simultaneously, Tb-50 can also increase the yields of the seeds and branches of *Impatiens*, which contributes in the increasing of medicinal value. Based on these results, it was necessary to reveal further the mechanism of increasing yields and changing flowering phenphase of *Impatiens* induced by Tb-50.

Previous research suggested that *Trichoderma* had strong competitiveness and hyperparastic action on pathogenic fungi, where it produced antibiotics and/or various cell wall degrading enzymes such as chitinase,

cellulase, xylanase, glucanase, protease and so on, to break and dissolve mycelia of pathogen, then inhibited or killed pathogenic fungi (Howell, 1993). Because the mechanism of *Trichoderma* spp. inhibited or killed plant pathogens was different, the biological control effects on different plant pathogenes were also different. Therefore, it was beneficial for improving *Trichoderma'*s biocontrol effects to confirm strains function and reveal the mechanism of *Trichoderma* and pathogen interaction. Tijerino *et al.,* (2011) thought that the trichodermin produced by *T. brevicompactum* had higher bacteriostatic activity against a variety of pathogenes. In our study, we found that Tb-50 presented positive antagonistic effects, which affected the growth on five soil borne plant pathogenes by the dual culture method with the inhibitory results of *S. sclerotiorum* > *R. solani* > *C. chrysosperma* > *A. alternate* > *F. oxysporum.* It was also shown that a competitive role appeared of Tb-50 to *A. alternata, C. chrysosperma. F. oxysporum* and *S. sclerotiorum* in the dual culture, respectively. Tb-50 displayed fast growth and also quickly seizing nutrients and space in a short time. Therefore, the growth rate of these pathogenes slowed down, and the colonies were smaller compared with control. In addition, Ngueko & Tong (2002) found that *T. harzianum* C184 produced antibiotics and cell wall degrading enzymes to kill *F. oxysporum* fungi where the both strains grown on dual culture; and other study also found that *T. viride* grew normally on the colonies of *Coleosperium solidaginis* of Pine needle rust, where it destroyed the spore to control the rust (Zhou *et al.,* 2017). Our results are consistent with this, we found brown ribbon at the junction between Tb-50 and *R. solani*, which might be the lytic effect of Tb-50; however, further detailed studies should be done to find the mechanism behind this phenomenon. Besides, on dual culture, hyphae and conidia of Tb-50 had parasitized on *F. oxysporum* and *S. sclerotiorum* (Fig. 3). In brief, the dual culture results suggested that Tb-50 could inhibit the growth of these five pathogenic fungi by strong competitiveness and hyperparasitism.

Powdery mildew is a kind of widely spread plant disease by pathogenic fungi. It causes harmful effects to certain plant species (Lind & Shishkoff, 2003; Helgard *et al.,* 2012). It had been found that powdery mildew of *Impatiens* was caused by white powder pathogens *viz., Podosphaera* spp., or *Erysiphe cichoracearum* (Garibaldi *et al.,* 2012). At initial stage, it causes the white spots on the leaves of *Impatiens*, and even severe phenomenon of these white powdery expanding into the whole leaf. More than this, the stem, flowers and fruits are also infected and ultimately causing whole plant to die, that not only affect the ornamental value of *Impatiens*, but also causes severe economic losses. It had been verified that *Trichoderma* could promote plant growth by inducing plant local and system resistance (Howell, 2006). Experimental results showed that Tb-50 could reduce the incidence of the powdery mildew of *Impatiens* by promoting growth and enhancing the resistance against plant diseases. Our present results had provided a new method to control powdery mildew. In summary, Tb-50 present inhibitory effects against a variety of pathogenic fungi, promoting growth of *Impatiens*, improving the resistance to powdery mildew; however, its mechanism to inhibit powdery mildew pathogenes needs to be further revealed.

Conclusions

A novel native bio-control agent Tb-50 was identified as *T. brevicompactum via* combination of macroscopic and microscopic morphological features in the study. The phylogenetic analysis according to ITS sequences also confirmed Tb-50 as *T. brevicompactum*. In addition, it verified that Tb-50 controlled positively plant pathogenes such as *F. oxysporum, A. alternate, C. chrysosperma, R. solani* and *S. sclerotiourm* in the antagonist test. Besides, Tb-50 showed positive effect, promoted vegetative growth and flower production of *I. balsamina* e.g. plant height, number of leaves, number of flowers from initial to final, and increased seeds yields in the greenhouse. Increased flowers and seeds of *I. balsamina* not only added beauty to the urban landscape, but also were used for Chinese traditional medicinal purpose to obtain economic benefits.

Acknowledgment

This work was supported by grant from the National Natural Science Foundation of China (NSFC: 31370642), the Natural Science foundation of Heilongjiang Province of China (C201216) and the financial support of postdoctoral science-research developmental foundation of Heilongjiang Province (LBH-Q11176).

References

Ahmed, S. and M. Koperuncholan. 2012. Antibacterial activities of various solvent extracts from impatiens balsamina. *Int. J. Pharma Bio. Sci.*, 3(2): 401-406.

Baskar, N., B.D. Parimala and B. Jayakar. 2012. Anticancer studies ethanol extract of *Impatiens balsamina. Int. J. Res. Pharm. Pharmacotherapeutics*, 3(4): 631-633.

Brotman, Y., E. Briff, A. Viterbo and I. Chet. 2008. Role of swollenin, an expansin-like protein from *Trichoderma*, in plant root colonization. *Plant Physiol.*, 147(2): 779-89.

Doni, F., N.K.N Al-Shorgani, E.M.M. Tibin, N.N. Abuelhassan, I. Anizan, C.M.Z. Che Radziah and W.Y.W. Mohtar. 2013. Microbial involvement in growth of paddy. *Curr. Res. J. Biol. Sci.*, 5(6): 285-290.

Elad, Y., I. Chet and Y. Henis. 1981. Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma harzianum*. *Plant Soil*, 60: 245-254.

Febri, D.I., C.M.Z. Aniza, R. Che, H.S. Ahmad, H.R. Muhammad and M.W.Y. Wan. 2014. Enhancement of rice seed germination and vigour by *Trichoderma spp*. *Res. J. Applied Sci. Engin. Tech.*, 7(2): 4547-4552.

Garibaldi, G., A.P. Gilardi and M.L. Gullin. 2012. First report of powdery mildew of impatiens new guinea, caused by podosphaera sp., in Italy. *J. Plant Patho*., 94(4): 85-105.

Gerhardson, B. 2002. Biological substitutes for pesticides. *Trends Biotech.*, 20: 338-343.

Goudjal, Y., O. Toumatiaa, A. Yekkoura, N. Sabaoua, F. Mathieuc and A. Zitouni. 2014. Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara. *Microbiol. Res*., 169: 59-65.

Helgard, K., Q. Xianqin, W. Juliane and D. Thomas. 2012. Isolation, molecular characterization, and mapping of four rose MLO orthologs. *Frontiers in Plant Sci.*, 3: 1-13.

Hermosa, R., R.M. Belén, R.E. Cardoza, C. Nicolás, E. Monte and S. Gutiérrez. 2013. The contribution of *Trichoderma* to balancing the costs of plant growth and defense. *Int. Microbiol.*, 16(2): 69- 80.

Howell, C.R. 2006. Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. *Phytopathol*., 96: 178-180.

Howell, C.R., R. Stipanovic and R. Lumsden. 1993. Antibiotic production by strains of *Gliocladium virens* and its relation to biocontrol of cotton seedling diseases. *Biocontrol. Sci. Technol*., 3: 435-441.

Jaklitsch, W.M. 2009. European species of Hypocrea. Part I. The green-spored species. *Stud. Mycol.*, 6: 1-91.

Kredics, L., Z. Antal, I. Doczi, L. Manczinger, F. Kevei and E. Nagy. 2003. Clinical importance of the genus *Trichoderma*. A review. *Acta Microbiologica et Immunologia Hungarium*, 50: 105-117.

Linde, M. and N. Shishkoff. 2003. Fungi powdery mildew in Ency- clopedia of Rose Science, (Eds.): Roberts, A.V., T. Debener and S. Gudin *Oxford: Elsevier*, 158-165.

Mamta, S.G. Raju and P. Suresh. 2013. Occurrence of *Alternaria alternata* causing *Alternaria blight* in pigeonpea in India. *Adva. Bio. Biotech.*, 4: 702-705.

Meenu, B., E.D. Neeraja, R. Greeshma and V. Alexeyena. 2015. *Impatiens balsamina*. An overview. *J. Chem. Pharm. Res.*, 7: 16-21.

Midhun, B. and S. Sobita. 2017. Efficacy of *Trichoderma spp.* against *Fusarium oxysporum* f. sp. lycopersici. (FOL) infecting pre-and post-seedling of tomato. *J. Pharm. Phyto.*, 6(4): 616-619.

Ngueko, R.B. and X. Tong. 2002. Antagonism *in vitro* of *Trichoderma harzianum* C184 against the root pathogens of banana and plantain in Cameroon. *J. Zhejiang Agri. Univ.*, 28: 407-410.

Pereira, J.L., R.M.L. Queiroz, S. Charneau, C.R. Felix, C.A. Ricart, Lopes, F. da Silva. A. Stecca, C. Steindorff, C.J. Ulhoa and E.F. Noronha. 2014. Analysis of *Phaseolus vulgaris* response to its association with *Trichoderma harzianum* (ALL-42) in the presence or absence of the phytopathogenic fungi *Rhizoctonia solani* and *Fusarium solani*. *PLoS ONE*, 9(5): 1-23.

Rai, S., L.P. Kashyap, K.A. Sudheer, Srivastav and R.V. Pramod. 2016. Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. *Springer Plus*, 5: 2-16.

Rajendran, C., Manikandan, A. Hemalatha, K. Mary, M. Sweety and P. Prabhavathi. 2014. Antimicrobial activity of *Impatiens balsamina* plant extract. *World J. Pharm. Pharm. Sci.*, 3(7): 1280-1286.

Rosemary, A. and M.H. Maarten. 2005. Preventive and therapeutic effects of *Trichoderma* on radiation of grains. *J. Shandong agri. Sci.*, 18(3): 107-112.

Saravanakumar, K., V.S. Arasu and K. Kathiresan. 2013. Effect of *Trichoderma* on soil phosphate solubilization and growth improvement of *Avicennia marina*. *Aquat. Bot.*, 104:101-105.

Shafique, H.A., R. Noreen, V. Sultana, J. Ara and S. Ehteshamul-Haque. 2015. Effect of endophytic *Pseudomonas aeruginosa* and *Trichoderma harzianum* on soil-borne diseases, mycorrhizae and induction of systemic resistance in okra grown in soil amended with *Vernonia anthelmintica* (L.) seed's powder. *Pak. J. Bot.*, 47(6): 2421-2426.

Suk-Nam, K., G. Young-Min, Y. Mi-Ra, I.H.I. Rashid, C. Jae-Hyeon, K. II-Suk and L. Ok-Hwan. 2013. Antioxidant and antimicrobial activities of ethanol extract from the stem and leaf of *Impatiens balsamina* L. (*Balsaminaceae*) at different harvest times. *Molecules*, 18: 6856-65.

Tijerino, A., R.E. Cardoza and J. Moraga. 2011. Overexpression of the trichodiene synthase gene tri5, increases trichodermin production and antimicrobial activity in *Trichoderma brevicompactum*. *J. Fungal Genetics Bio*., 48(3): 285.

Vipin, P. and J.S. Ankur. 2015. Production and multiplication of native compost fungal activator by using different substrates and its influence on growth and development of capsicum chinensis Jacq. Bhut Jolokia. *Biotech. Res. Int.*, 1-7.

Zhang R.S., A.M. Baloch, Z.H. Liu, C.Y. Jiang, Y. Chang, H. Wang, A.W. Baloch and G.P. Diao. 2018. Improvement in biomass, IAA levels and auxin signaling-related gene expression in Shanxin poplar seedlings (*Populus davidiana* × *P. alba* var.. *pyramidalis*) induced by *Trichoderma asperellum*. *Pak. J. Bot.*, 50(4):

Zhou, H., Z.R. Bao and Y.I. Shi. 2017. Effect of four species of *Trichoderma* on cell wall of pine blister rust. *J. Southwest Fores. Univ.*, 37(4): 120-125.

(Received for publication 17 October 2017)