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Photochemistry of *Luffa cylindrica* (L.) Roem under fungal biocontrol interaction

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Abstract

The aim of this study was to evaluate the photochemistry of *Luffa cylindrica* (L.) Roem in fungal biocontrol interacting treatments. Healthy plants were infected with *Pythium aphanidermatum* before the biocontrol application. Biocontrol agents were selected in preliminary Petri-plate experiment evaluation against causative agent *P. aphanidermatum*. Photosynthetic performance traits were studied. We found that *P. aphanidermatum* infection caused significant reduction in photosynthetic performance, pigments, and in maximum quantum yield of primary photochemistry, photochemical quenching, and electron transport rate with increase in nonphotochemical quenching as compared with non-infected control. However, application of biocontrol agents substantially improved maximum quantum yield of PSII, performance index, and total content of photosynthetic pigments in infected plants. The fluorescence intensity was used for quantifying the antagonist effect of biocontrol agents on infected plant leaves.

Additional key words: chlorophyll fluorescence transient; photochemistry; photoinhibition.

Introduction

Luffa cylindrica (L.) Roem, a member of the Cucurbitaceae family, is found in tropical and subtropical areas in Pakistan (Perveen and Qaiser 2008). Bottle gourd and sponge gourd are the popular plants of this family. Damping-off and root rot on greenhouse cucurbits are caused by a number of *Pythium* spp. (Abad *et al.* 1994, Abdelzaher *et al.* 2004). *Pythium* is the recurring problems for growers in Sindh, Pakistan. In Sindh, damping-off and root rot are during the summer generally caused by *Pythium aphanidermatum* (Edson) Fitzp (Hendrix and Campbell 1973, Lodhi *et al.* 2013). Over the last few decades, chemical herbicides and pesticides are used for crop management strategies in developing countries (Wyse 1992, Albernathy and Bridges 1994, Coombs 2012), but their residual consequence has diverted the intention of scientists toward alternate means for controlling infection.

Chemical pesticides, more stringent and costly regeneration regulations, and the necessity for alternatives in environmentally sensitive areas provide avenues for the use of plant pathogens as biocontrol agents (Larkin and Fravel 2002, Muthukumar *et al.* 2008, 2010). Treatment with biocontrol agents has been shown to be highly effective in promoting growth when dormancy is related to fungal stress (Dawar *et al*. 2008). To overcome the pathogenic effect, biological control may provide an additional method for the management of pests or pathogens. According to literature, *Trichoderma harzianum, Paecilomyces variotii,* and *Bacillus subtilis* are established against pests or pathogens (Klein 1990, Glick 1995, Postma *et al.* 2003, Khan *et al.* 2008, Agrios 2005, Perner *et al.* 2006, Jeyaseelan *et al.* 2012). However, most of the research is restricted to show control of the disease, but their physiological mechanism of controlling disease and photochemistry of infected plants are not provided. Photosynthesis contributes substantially to the plant growth and development. A number of reports showed that under fungal stress values of maximum quantum yield of

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Abbreviations: Chl – chlorophyll; C – control; F_v/F_m – maximum quantum yield of PSII; NPQ – nonphotochemical quenching; PDA – potato dextrose agar; PI – performance index; Py – *Pythium aphanidermatum*; Py+Ba – *Pythium aphanidermatum* + *Bacillus subtilis*; q_P – photochemical quenching; Py+Pa – *Pythium aphanidermatum* + *Paecilomyces variotii*; Py+T – *Pythium aphanidermatum* + *Trichoderma harzianum*; rETR – electron transport rate through PSII; Φ_{PSII} – maximum quantum yield of primary photochemistry.
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PSII (F_v/F_m), photochemcial quenching (q_p), electron transport rate through PSII (rETR) and maximum quantum yield of primary photochemistry (Φ_{PSII}) were very low due to the low content of photosynthetic pigments.

On the basis of these findings it is tempting to speculate that the selected biocontrol agents, *T. harzianum, P. variotii* and *B. subtilis*, might be involved in reducing infection due to fungal biocontrol interaction thereby ensure resistance against the pathogen. However, limited information is available concerning the persistence of *T. harzianum, P. variotii*, and *B. subtilis* co-applied against damping-off disease. Objectives of this research were to determine how this biocontrol agent suppresses *Pythium aphanidermatum* infection in *L. cylindrica.* In this connection, an explanatory assessment required to determine the overall photosynthetic performance through chlorophyll fluorescence parameter under fungal biocontrol interaction.

Materials and methods

Seeds of *Luffa cylindrica* (L.) Roem were obtained from the stock collection of Pathology laboratory, Department of Agriculture and Agri-business Management, University of Karachi. The seeds were sterilized in 5% sodium hypochlorite solution for 5 min before experiments. Twenty seeds per Petri plate with internal diameter of 8 cm were used for pelleting. Petri dishes were arranged in a completely randomized design with three different treatments. Each treatment was replicated thrice.

Dual culture interaction: Melted potato dextrose agar (PDA) (20 ml) was poured in each Petri plates and allowed to solidify. A disc of 9 mm with the actively growing colonies of pathogenic culture (*P. aphanidermatum,* Py) were placed near the periphery on one side of the PDA; 9 mm disc of test organisms, such as *Trichoderma harzianum* (T)*, Paecilomyces variotii* (Pa)*,* and *Bacillus subtilis* (Ba) were placed on the other side of same plate at an angle of 180 degree. The plates were incubated at 28°C for 5 d and colony growth inhibition (%) was calculated (*see* the text table below; Fig. 1).

A, B, C, D were the pure culture growing alone without interaction, whereas E, F, G, H were the dual culture interaction in which tested organism grew along with pathogen.

Selection of biocontrol agents

Fungal inoculation and experimental design: *L. cylindrica* seeds were pelleted with biocontrol agents T, Pa, and Ba grown on PDA plates by the method of Fouzia and Shahzad (2008). The number of colonies per plate was multiplied with the dilution factor and then divided by 5 to determine the spore-load per seed. The plastic pot areas of 153.2 cm^2 were filled with 300 g of sandy loam soil. The whole experiment was carried out in a greenhouse. The seedling were allowed to grow at a temperature of 30–35 °C, relative humidity of 60–65%, and light intensity at the top of plants was 42–45.5 μmol(photon) m^{-2} s^{-1}.

Test fungi	Colony diameter [mm]		Type of interaction	Growth inhibition [%]
	Pathogen (fungus)	Biocontrol agent (test microorganism)		
P. aphanidermatum + *T. harzianum*	1.8	5.6	A	77.50
P. aphanidermatum + *P. variotii*	2.95	4.67	B	36.83
P. aphanidermatum + *B. subtilis*	2.5	6.0	C	58.33

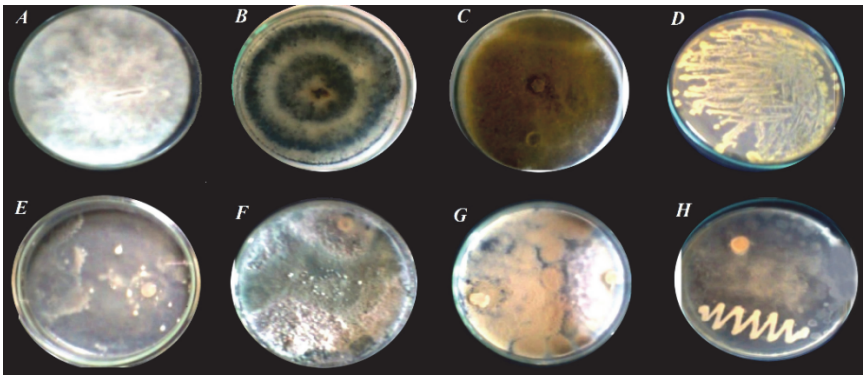

Fig. 1. Pure microbe isolates and their dual culture interaction on potato dextrose agar and nutrient agar medium. Pure culture of (*A*) *Pythium aphanidermatum*, (*B*) *Trichoderma harzianum*, (*C*) *Paecilomyces variotii*, (*D*) *Bacillus subtilis*. Dual culture interaction of (*E*) *Bacillus subtilis* against *Pythium aphanidermatum*, (*F*) *Trichoderma harzianum* against *Pythium aphanidermatum*, (*G*) *Paecilomyces variotii* against *Pythium aphanidermatum*, (*H*) *Bacillus subtilis* against *Pythium aphanidermatum*.

Treated seed with and without pathogen: The 30 nonpelleted seeds of each control and *P. aphanidermatum* [1×10^{-3} colony forming unit (cfu) mL^{-1}] were sown in ten pots and 90 pelleted seeds with 20 mL^{-1} of *T.harzianum* (1.16×10^{-1} cfu mL^{-1}), *P. variotii* (2.5×10^{-2} cfu mL^{-1}), and *B.subtilis* (6.3×10^{-1} cfu mL^{-1}) were sown in 30 pots. The plants were subjected to same experimental conditions mentioned above. There were five replicates for each treatment. Plants were uprooted after 30 d in order to assess physiological attributes. The information regarding the applied treatments were given in the following text table:

Treament	Causative agents	Concentration [cfu mL^{-1}]	
Negative Control	*P. aphanidermatum*	1×10^{-3}	[Py]
Positive Control 1	*T. harzianum*	1.16×10^{-1}	[T]
Positive Control 2	*P. variotii*	2.5×10^{-2}	[Pa]
Positive Control 3	*B. subtilis*	6.3×10^{-1}	[Ba]
Treatment 1	*T. harzianum* + *P. aphanidermatum*	[Py+T]	
Treatment 2	*P. variotti* + *P. aphanidermatum*	[Py+Pa]	
Treatment 3	*B. subtilis* + *P. aphanidermatum*	[Py+Ba	

Photosynthetic pigments: Leaf discs of 0.5 g were extracted in 10 mL of methanol (95%), homogenized, and centrifuged at 10,000 rpm for 10 min, The absorbance of the solution was measured at 666, 653, and 470 nm by a spectrophotometer (*Shimadzu*, Japan). Total photosynthetic pigments were calculated using the equation of Lichtenthaler and Wellburn (1985) and Ritchie (2008).

$$\text{Chl } a = 15.65\ A_{666} - 7.340\ A_{653}$$
$$\text{Chl } b = 27.05\ A_{653} - 11.21\ A_{666}$$
$$\text{Carotenoids (Cx+c)} = (1{,}000\ A_{470} - 2.860\ \text{Chl } a - 129.2\ \text{Chl } b)/245$$

Chl fluorescence: Photosynthetic efficiency was measured in *L. cylindrica* by an analyzer *Os-30p (OPTI–SCIENCES,* USA). The data obtained were used to calculate various parameters that describe the photochemistry of PSII by the JIP test and F_v/F_m, Φ_{PSII}, q_P, rETR, PI, and NPQ (Strasser *et al.* 2004). The data were collected from attached leaves at ambient temperature. Samples of 48 leaves per 16 replicates were taken for the Chl fluorescence analysis (ChlF). The leaf clips were placed on the leaves 20 min to prior the measurements to provide dark adaptation. The light-pulse intensity was 3,500 μmol (photon) m^{-2} s^{-1}. F_v/F_m, performance index (PI), q_P, Φ_{PSII}, NPQ, and rETR were calculated according to the equations reviewed by Stirbet and Govindjee (2011).

Statistical analysis: The data were subjected to *Sigma Plot version 13.0* to performed analysis of variance (*ANOVA*) and *F*-test. Similar alphabets on graph represent insignificant difference at 0.05. The vertical line on bar graph showed mean ± standard error (SE).

Results

Selection and activity of biocontrol agents against pathogen Py were tested at Petri plates. The T, Pa, and Ba culture were used against Py (Fig. 1). Results showed that T exhibited the maximum (67.9%) growth inhibition as compared to other biocontrol agents. Ba showed 58.3%, and Pa exhibited 36.8% growth inhibition (Fig. 1, text table).

In pot experiments, two-week old seedlings were inoculated by the spore suspensions of Py (1×10^{-3} cfu mL^{-1}). During the disease development, visible discoloration effect was observed in the form of yellowish green leaves. Py inoculation significantly reduced a growth and biomass allocation of treated plants as compared with untreated control (C) (Fig. 2). However, the application of biocontrol agents enhanced plant growth and biomass in the infected plants. All the applied biocontrol agents were able to increase seedling growth including root and shoot length as compared with their negative control (infected plants only). Maximum increase of the whole plant biomass was recorded when infected plants were subjected to *T. harzianum* (Py+T). Photosynthetic attributes were examined in the infected plant after their biocontrol treatments. The infected plants subjected to biocontrol treatments displayed significant increase in the F_v/F_m ratio. Biocontrol treatment Py+T caused 64% enhancement, while treatment Py+Pa and treatment Py+Ba showed 61–62% increase compared to Py-infected plants. In infected plants, PI was substantially reduced at the average rate against untreated control (Fig. 3*B*). However, application of biocontrol agents improved the PI in Py-infected plants. Thus, Py+Ba treatment exhibited 98% enhancement, while Py+T and Py+Pa showed 96%, respectively.

Our results revealed that Φ_{PSII} decreased under fungal stress of Py. Application of biocontrol treatment *T. harzianum* and *B. subtilis* exhibited higher Φ_{PSII} (63%) in infected plant as compared with their negative control (Fig. 3*E*).

Py infection exhibited 41% decrease in q_P, which indicated closure of reaction centers. However, the application of biocontrol agents suppressed the effect of pathogen up to 27–46% (Fig. 2*C*).

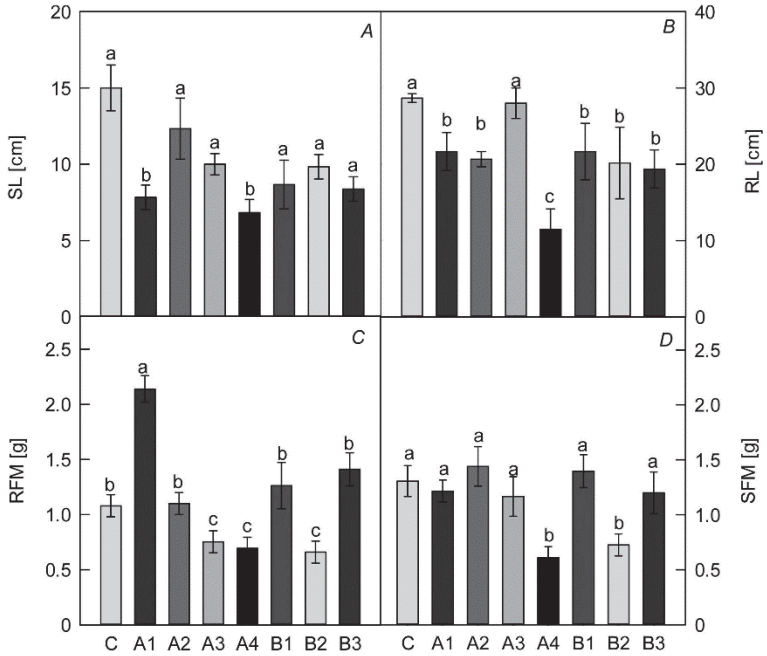

Fig. 2. Effect of biocontrol treatments on the shoot length (SL), root length (RL), root fresh mass (RFM), and shoot fresh mass (SFM) of *Luffa cylindrica*. C – Control, A1 – *Trichoderma harzianum*, A2 – *Paecilomyces variotii*, A3 – *Bacillus subtilis*, A4 – *Pythium aphanidermatum*. B1 – Py+T, B2 – Py+Pa, B3 – Py+Ba. Vertical lines on bar graphs represent mean ± SE. *The same letters* show insignificant difference at $p<0.05$ level according to one-way *ANOVA* and *F*- test.

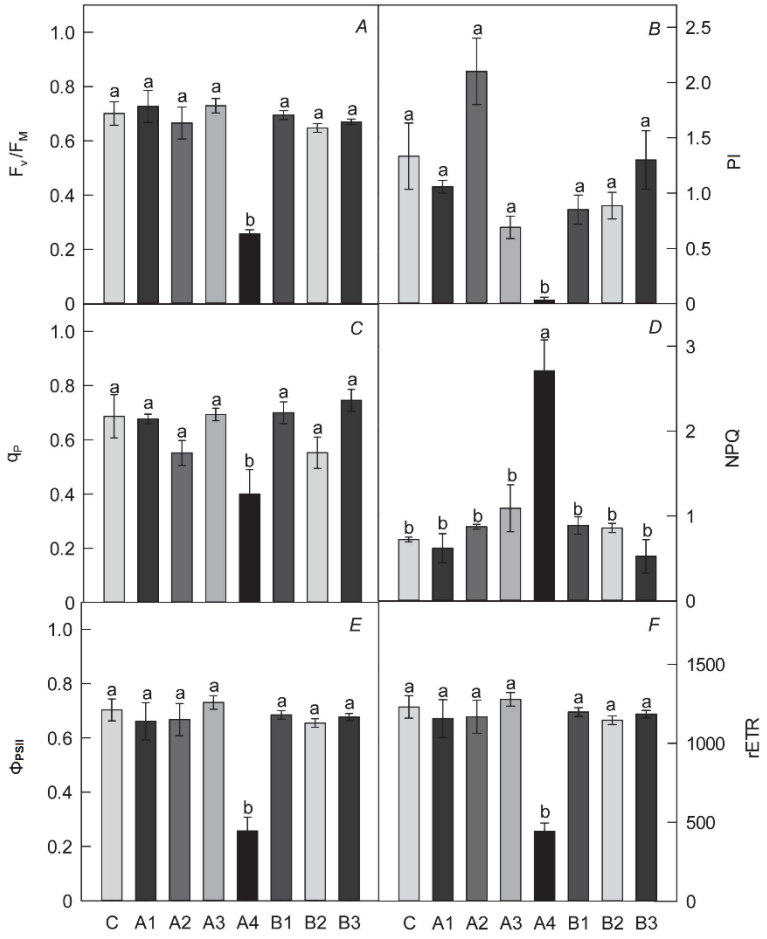

Fig. 3. Effect of fungal biocontrol treatment on maximum quantum yield of PSII (F_v/F_m), performance index (PI), photochemical quenching (q_P), nonphotochemical quenching (NPQ), maximum quantum yield of primary photochemistry (Φ_{PSII}), electron transport rate through PSII (rETR) of *Luffa cylindrica* leaf. C – control, A1 – *Trichoderma harzianum*, A2 – *Paecilomyces variotii*, A3 – *Bacillus subtilis*, A4 – *Pythium aphanidermatum*. B1 – Py+T, B2 – Py+Pa, B3 – Py+Ba. Vertical lines on bar graphs represent mean ± SE *The same letters* show insignificant difference at $p<0.05$ level, According to one-way *ANOVA* and *F*- test.

In Py-infected plants, NPQ drastically increased by 90% as compared with C and biocontrol treatments (Fig. 3*D*). The drop in NPQ up to 33–68% was more accentuated by biocontrol treatments as compared to the infected plants. In the infected plants (infected plants represented as A4 on bar graph), the greater increase in

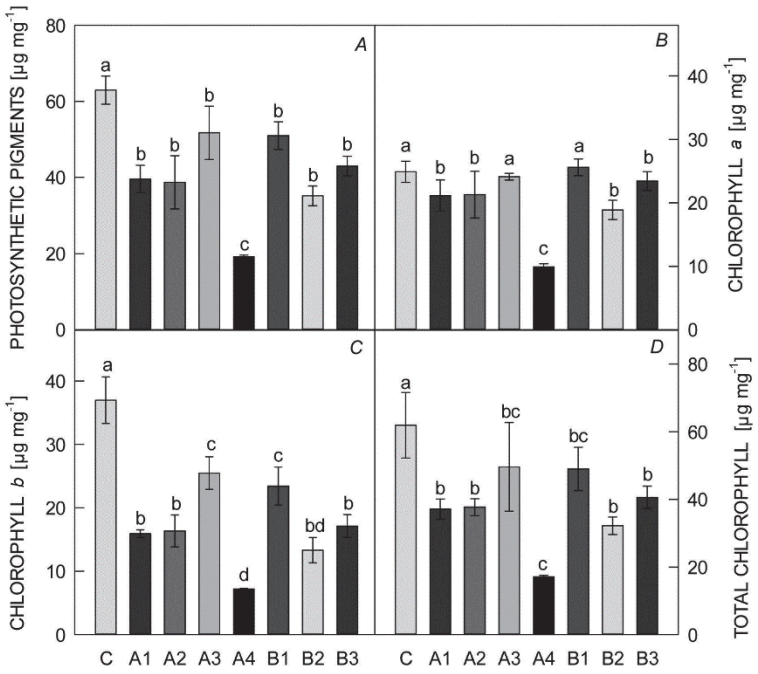

Fig. 4. Effect of fungal biocontrol interaction on photosynthetic pigments µg mg^{-1} (Chl *a*, Chl *b*, total Chl, and Car) of *L. cylindrica*. C – control, A1 – *Trichoderma harzianum*, A2 – *Paecilomyces variotii*, A3 – *Bacillus subtilis*, A4 – *Pythium aphanidermatum* and B1 – Py+T, B2 – Py+Pa, B3 – Py+Ba. Vertical lines on bar graphs represent mean ± SE. *The same letters* show insignificant difference at $p<0.05$ level according to one-way *ANOVA* and *F*- test.

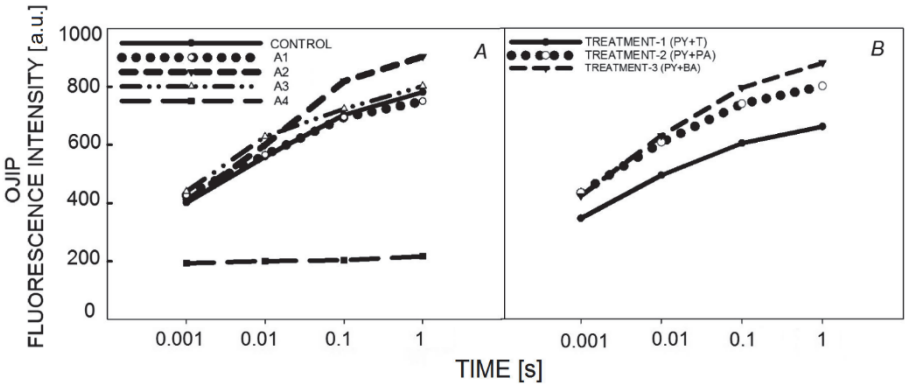

Fig. 5. Fluorescence induction curve (OJIP) estimating *Luffa cylindrica* leaves under stress and treatment. C – control, A1 – *Trichoderma harzianum*, A2 – *Paecilomyces variotii*, A3 – *Bacillus subtilis*, A4 – *Pythium aphanidermatum* and B1 – Py+T, B2 – Py+Pa, B3 – Py+Ba.

NPQ was accompanied by a substantial decrease in Φ_{PSII} up to 64% during the course of experiment. As rETR is derived from Φ_{PSII} value indicated (Fig. 2*E,F*) that the application of biocontrol treatments enhanced of rETR and Φ_{PSII} activity up to 61–63% as compared to infected plants.

In the infected plants, photosynthetic pigments decreased by 65% as compared with untreated control (Fig. 4). However, application of biocontrol agents, such as treatment by Py+T and Ba+T, showed significant increase in Chl *a* and *b* and total Chl up to 58–65%. Maximum increase in photosynthetic pigments was found in the infected plant exposed to *T. harzianum*. In infected plants the photosynthetic pigment contents including Chl *a, b* and total Chl was relatively lowered up to 45–48% as compared to biocontrol treatments (Fig. 4).

Our results further confirmed by the OJIP test showed that in infected plants the fluorescence intensity at 3,500 µmol(photon) m^{-2} s^{-1} PAR substantially declined up to the injurious level at 200 µmol(photon) m^{-2} s^{-1} PAR (Fig. 5*A*). Biocontrol treatments caused that fluorescence intensities were enhanced from 400 to 900 µmol(photon) m^{-2} s^{-1} which showed effective enhancement of the photosynthetic rate leading to better yield of the plant (Fig. 5*B*).

Discussion

Present study revealed that *T. harzianum* as a biocontrol treatment was highly effective in inhibiting the mycelial growth of *P. aphanidermatum* (the damping-off disease) in *L. cylindrica*. We observed that *P. aphanidermatum*-infected plants demonstrated the significant reduction in photosynthetic pigments (65%), F_v/F_m, Φ_{PSII}, rETR (64%), q_P (41%), PI (98%), and NPQ, which presented 90% inhibition, but treatment by *T. harzianum* showed

stimulating effect compared to other treatments. It was observed that *T. harzianum* enhanced photosynthetic performance up to 60%, F_v/F_m (64%), Φ_{PSII}, and rETR (64%), PI (96%), q_P (52%), and NPQ (32%) in infected plants as compared to negative control (infected plants only)

It was reported that increase in NPQ is based on downregulation of PSII function under fungal infection (Singh *et al* 2013). On the other hand, similar result were found in our experiment; NPQ attained the highest level up to 90% under fungal stress. The F_v/F_m ratio is an important parameter, which determines the maximum quantum efficiency of PSII. It provides a measure of the rate of linear electron transport, hence, an indication of overall photosynthetic capacity (Jamil *et al.* 2007, Tang *et al.* 2007, Balouchi 2010). Similarly, a decline in F_v/F_m value below 0.7 occurs due to photoinhibition caused by the pathogen *P. aphanidermatum* subsequently decreased q_P value to 0.4. It was observed that reaction centers activity was reduced due to the reduction of photochemistry and their F_v/F_m, Φ_{PSII}, rETR and q_P values. It showed the possibility of energy transfer between the two photosystem were disconnected due to the infection. It was evident from our research that microbial biocontrol agents not only inhibited plant infection but also promoted photosynthetic efficiency of the plants.

The application of biocontrol treatments showed an effective response in the infected plants with respect to growth and photosynthetic efficiency. The spore of *P. aphanidermatum* induced substantial reduction in seedling growth and biomass allocation. However, application of *T. harzianum* to infected plants increased seedling growth and biomass up to 45–71%.

Some positive correlation was found under the Py+Ba treatment, which was linked to the photosynthetic performance. Increased NPQ up to 68% due to the induction of pathogen was significant at low light level (Schroth and Becker 1990, van Peer *et al.* 1991, Maxwell and Johnson 2000, Yuan 2016). The decrease of PI, F_v/F_m, Φ_{PSII}, and rETR supported marked physiological effects on the infected plants showing sensitivity to pathogen. The selected biocontrol agents showed their efficacy individually or interacting with the pathogen which effectively enhanced the photosynthetic rate; similar results were also reported by Vargas *et al*. (2009).

In infected plants, total photosynthetic pigments decreased after the induction of fungal pathogen, while applying biocontrol agents improved the Chl concentration. The Py+T treatment showed maximum enhancement in the total Chl content of infected plants up to 65% as comapred to negative control (infected plant only) indicating the suppressing effect against infection. It was reported that infection in plants causes reduction in photosynthetic pigment which can inhibit plant growth but also induce photoinhibition (Baker and Rosenqvist 2004, Zlatev 2009, Vaz and Sharma 2011). Similarly high activities of electron transport chain were observed after the Py+T and Py+Ba treatments ameliorating 63% photoinhibition of PSII. It is assumed that linear correlation between Φ_{PSII} and ETR values due to biocontrol treatments in infected plants indicated sufficient electron movement in the photosynthetic pathways which not only demonstrated enhancement in photosynthetic ability but also reflected better plant growth.

The OJIP transients were also analyzed and correlated with the internal leaf quality and they positively coincided with photosynthetic performance. The JIP test showed that fungal infection in plants significantly decreased the OJIP values representing the inhibition in Chl excitation activity as compared with untreated control (Fig. 5). However, the application of biocontrol agents revealed enhancement in OJIP parameters as compared to the infected plant which indicated improvement in electron flow under infection; the Py+Ba treatment showed the highest OJIP parameters as compared to other treatments.

Based on the present study, we can conclude that the antagonistic effects of biocontrol treatments, such as *T. harzianum*, *P. variotti,* and *B.subtilis* enhanced the photosynthetic activity of *L. cylindrica* which not only suppressed plant infection but also improved plant growth.

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