

Potential of the indirect and direct beneficial effects of the use of *Trichoderma koningii*, *Aspergillus niger* and *Mucor* sp. on eggplants plants: Plant growth and systemic resistance induction

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Abstract

Several pathogens fungi responsible for total yield losses are worldwide spread notably in Iraq. The alternatives strategies to decrease disease development are those able to destruct a total or partial population density using eco-friendly approach treatments. In this investigation, we demonstrate the symbiotic interaction with *Trichoderma koningii*, *Aspergillus niger* and *Mucor* sp. on the eggplant plants growth and development, and on the defence response induction. The results revealed that the highest fungal frequency from eggplant rhizosphere was registered for *A. niger*, followed by *Mucor* sp. and *T. koningii*. Seeds treatment with *T. koningii* showed a higher value of length of shoots (2.83 cm), roots (3.00 cm), and leaves (3.50 cm). Obtained results revealed that *T. koningii* ameliorates the seedling fresh (3.91 g), dry weight (0.24 g), and accelerates plant length (48.67 cm). Obtained results revealed increasing of peroxidase activity (12.53, 12.68, and 11.28 10⁻¹ units.g.mL.min⁻¹, respectively) and chlorophyll content (2.11, 1.70, and 1.90 mg.g⁻¹ fresh weight, respectively) eggplants treated with combination *Mucor* sp. + *A. niger* + *T. koningii*, *T. koningii* + *Mucor* sp., and *T. koningii* alone. To control pathogens fungi within integrated management strategies, the biological control should be taken into consideration.

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Introduction

Several phytopathogenic fungi cause eggplants plant (*Solanum melongena* L.) damage, resulting in total crop loss. On the other hand, eggplants contribute quite a bit in the agricultural economy in Iraq. Recently, it can be produced throughout the year in greenhouses (offseason and early crops) as well as in the field (seasonal culture). The eggplant production in Iraq was reported to be around 102,452 thousand tons with an average 12.26 t.ha⁻¹

(Saeed Omar and Mohmmmed 2020). Iraqi annual economic yield losses due to several diseases have been estimated more than 70 % and about 50 – 100 % decrease in plant yield have been reportedly (Hussein 2016; 2018; El-Debaiky 2018).

Unfortunately, strategies to manage plant pathology employed by Iraqi farmers were fungicides (Matrood et al. 2020). The massive application of synthetic chemicals against fungal pathogens poses human health hazards and increases environmental pollution (Rhouma et al. 2016; 2020). Therefore, alternatives strategies are required for

phytopathogens control. Biological control is the best alternative and eco-friendly approach for such treatments, defined as a total or partial destruction of phytopathogens fungi by other organisms, which occur routinely in nature (Rhouma *et al.* 2018; Matrood *et al.* 2020).

The plants and seeds treatment with beneficial microorganisms including *Mucor* sp., *Aspergillus* sp., *Trichoderma* sp. could eliminate impacts of abiotic, biotic, and physiological stresses (Mondal *et al.* 2000; Yedidia *et al.* 2001; Mastouri *et al.* 2010).

Trichoderma species are widely used as biocontrol agents (BCA) against phytopathogens since the 1920s (Heydari and Pessaraki 2010). *Trichoderma* sp. are plant symbionts and can stimulate plant growth and development (fresh and dry weight, yields, plant length, foliar area, root volume, etc.) by increasing the macronutrients and micronutrients solubility (Altomare *et al.* 1999; Azarmi *et al.* 2011; Abd-El-Kareem *et al.* 2019). Latha *et al.* (2009), Rhouma *et al.* (2018) and Inayati *et al.* (2020) pointed out that the good colonization of *Trichoderma* spp. on the roots can lower the damages of various phytopathogens and the harmful stresses caused by environmental. It has been documented that some *Trichoderma* species can induct systemic resistance; a mechanism triggered after the colonization of *Trichoderma* spp. and regulated the plants by a cascade of specific signal transduction (Kavitha and Umesha 2008; Lorito *et al.* 2010; Kangasjärvi *et al.* 2012). This filamentous fungus rarely inducts systemic acquired resistance (Hermosa *et al.* 2012; Martínez-Medina *et al.* 2014).

Plants naturally have dormant defense genes in healthy plants which are activated by biotic or abiotic inducers. *Trichoderma* spp. induced systemic resistance by stimulating these genes. Induced resistance is persistent and generally non-specific to biotic and abiotic constraints (Safin *et al.* 2020; Matrood *et al.* 2021). Phoka *et al.* (2020) and Safin *et al.* (2020) revealed that the treatment with *Trichoderma* spp. increased catalase and peroxidase activities and that this increase could be related to lignification. Al-Askar *et al.* (2016) and Ghoniem Abeer *et al.* (2021) noted that the total chlorophyll content in plant leaves was

significantly increased in response to foliar spraying and root inoculation of *Trichoderma* spp. Plant peroxidases, which play an important role in the growth and differentiation of plants, are widely distributed in higher plants. In addition, they are also enzymes responsible for the lignification of the cell wall and destructive process such as aging and senescence (Velazhahan and Vidhyasekaran 1994; Deborah *et al.* 2001; Nath *et al.* 2015).

In addition to systemic resistance induction, *Trichoderma* species are known to have beneficial physiological/growth-promoting effects on plants, including delaying of leaf senescence, greater stress tolerance, exudation of plant growth regulators, solubilization of phosphates, micronutrient and minerals (Fe, Mn and Mg), and secretion of exogenous enzymes, siderophores and vitamins. These physiological effects may contribute to greater yield and plant vigor to overcome biotic and/or abiotic stresses (Azarmi *et al.* 2011; Abd-El-Kareem *et al.* 2019; Matrood and Rhouma 2021).

The aim of this investigation was to i) evaluate the substantial differences in the response to the symbiotic interaction with *T. koningii*, *A. niger* and *Mucor* sp. on the eggplant plants growth and development, and ii) examine the defence response induction from treated eggplant plants separately or simultaneously with *T. koningii*, *A. niger* and *Mucor* sp.

Experimental

Fungal community

Eggplant rhizosphere was collected in 2020 from four greenhouses (9 m x 60 m) located in Basra Iraq (Chatt-el-Arab, Abu Al-Khaseeb, Hartha and Az Zubayr) at 10 – 20 cm depth. For each greenhouse, soil samples were mixed into a single one. Nine soil samples (100 g) per replicate (3 replicates) were collected in sterile polythene bags from each greenhouse (Rhouma *et al.* 2019; 2020). The soil-borne fungi number was determined by the method of dilution-plate according to Boughalleb-M'Hamdi *et al.* (2017). The fungal species identification was carried by observing the macroscopic (growth, colour, aspect of the colony) and microscopic characterization (mycelium, conidiophore, conidia, resistance structures, sexual

form), after a series of sub-culturing until purification. The fungal species were identified by using blue cotton as a mounting liquid and with reference to different identification keys.

Promoting growth and development of eggplant seeds and seedlings by using Trichoderma koningii, Aspergillus niger, and Mucor sp.

Three fungal species namely; *A. niger*, *Mucor* sp., and *T. koningii* (highest fungal frequency) were used for this assay. Fungal cultures were developed on PDA medium at 25 °C for 4 days. Four discs (1 mm) of each species were transferred to Erlenmeyer flasks containing 50 ml of PDB (Potato Dextrose Broth). Flasks were incubated at 25 °C for 7 days (for *A. niger* and *Mucor* sp.) and 4 days (for *T. koningii*) in an orbital shaker. The conidial suspensions were filtered through Whatman's filter paper and were adjusted 10^8 CFU.mL⁻¹ using a haemocytometer. Eggplant (cv. Barcelona) seeds were sterilized by soaking in 3 % solution of NaOCl for 3 min and washed with sterilized distilled water three times. The seed eggplants were treated by dipping into the flask containing a conidial suspension of the different antagonists for 30 min. One control was performed by inoculating the seeds with sterilized distilled water (negative control). The treated eggplant seeds were transferred on the surface of Petri dishes containing cotton balls soaked in sterilized distilled water. In each Petri dish, 10 seeds were placed (with a total of 10 Petri dishes for each replicate (three replicate)). The plates were incubated in the dark at 25 ± 2 °C for 10 days, and then examined the length of the shoot (LS) (cm), root (LR) (cm) and leaf (LL) (cm). LS, LR and LL were assessed on 100 eggplant seedlings per treatment and per replicate (Rhouma *et al.* 2018; Matrood *et al.* 2020).

Germinated eggplant seeds were placed in a pot containing a mixture of peat (50 %) and vermiculite (50 %) with 3 seedlings per pot. The pots were placed in a greenhouse for 21 days. For each treatment, eggplant plants were randomly distributed with 60 plants per replicate (3 replicates), and the entire experiment was repeated twice. The evaluation parameters were measured within 21 days following inoculation. After determination of the fresh weight (FW) (g), eggplant plants were placed in an oven at 60 °C for

48 h to determine the dry weight (g) (DW). The plant length (PL) (cm) was measured using a flat rule. FW, DW and PL were assessed on 45 eggplant plants per treatment and per replicate (Boughalleb-M'Hamdi *et al.* 2018; Rhouma *et al.* 2018).

Eggplant leaves were collected at different sampling moments (5, 10, 15 and 21 days after inoculation) to isolate airborne pathogens. The fragments leave of the eggplant (0.5 – 1 cm) were sterilized by soaking in 3 % solution of NaOCl for 2 min and washed with sterilized distilled water 3 times. The samples were dried and inserted on the surface of Petri dishes (9 cm) containing PDA medium amended with streptomycin (60 µg.mL⁻¹). In each Petri dish, seven fragments were placed (with total of 30 Petri dishes of each treatment). The plates were incubated in the dark at 25 ± 2 °C for 5 – 7 days, and then examined for fungal analysis. The fungal species identification was carried by observing the macroscopic and microscopic characterization after a series of sub-culturing until purification. The airborne pathogens isolation was assessed on 15 eggplant plants per treatment and per replicate (Rhouma *et al.* 2016; Matrood *et al.* 2020).

Peroxidase activity and chlorophyll content in eggplant plant treated separately or simultaneously with A. niger, Mucor sp., and T. koningii

Sterilized (soaking in 3 % solution of NaOCl for 2 min and washing with sterilized distilled water 3 times) eggplants seeds (cv. Barcelona) were placed in a pot (50 cm diameter) containing a mixture of peat and vermiculite (1 : 1) at the rate of one seedling in each pot. The experimental design was a randomized complete block and arranged in three blocks each of 10 pots per treatment, and the entire experiment was repeated twice. The treatment applications were occurred after 30 days of the eggplant plant growing by inoculated the roots with conidial suspension (10 mL) of the different antagonists (10^8 CFU.mL⁻¹). Treatments were applied separately or simultaneously as follows: *A. niger* alone, *Mucor* sp. alone, *T. koningii* alone, *A. niger* and *Mucor* sp. inoculated simultaneously, *A. niger* and *T. koningii* inoculated simultaneously, *T. koningii* and *Mucor* sp. inoculated simultaneously,

Mucor sp., *A. niger*, and *T. koningii* inoculated simultaneously and control (inoculated with distilled water). The treated plants were placed in a greenhouse for 21 days (Rhouma *et al.* 2018; Matrood *et al.* 2020).

Peroxidase activity and chlorophyll content were conducted 10 days after inoculation and were evaluated on five eggplant leaves per treatment and per replicate (three replicates).

Plant tissue extraction for enzyme activities were prepared by freezing a 0.1 g of leaf samples in liquid nitrogen to stop the activity of proteolytic, followed by homogenizing with extraction buffer (1 : 5) (0.1 M phosphate buffer + 0.5 mM EDTA, pH = 7.5), and by centrifugation at 15,000 × g for 20 min at 4 °C. Peroxidase (POX) activity was assayed according to Castillo *et al.* (1984). 3 ml of reaction mixture composed of 0.5 mL guaiacol, 1 mL phosphate buffer, 0.5 mL H₂O₂, 0.1 mL enzyme extract, and 0.9 mL water. The absorbance was examined at 470 nm.

Chlorophyll content (C_{chl}) was estimated by rinsed the eggplants leaves in 85 % acetone solution according to Mackinney's method and assessing its absorbance by Spectrophotometer at λ = 663 nm and λ = 645 nm (Mackinney 1941). Arnon (1949) formulated the work done by Mackinney's to get chlorophyll concentration shown in equation (Eq. 1):

$$C_{chl} = 20.21 A_{645} + 8.02 A_{663} \quad (1)$$

Statistical analysis

The data were analyzed by ANOVA using the SPSS version 20.0 statistical software (SPSS, SAS Institute, USA), to evaluate parameter values differences. Differences between treatments were determined by least significant difference (LSD) test at 5 % of significance level.

Results and Discussion

Fungal community

The results of fungal species isolated from eggplant rhizosphere of four experimental greenhouses are presented in Table 1. The list includes 15 species belonging to 13 genera. Obtained data showed that

Trichoderma koningii, *Aspergillus niger*, and *Mucor* sp. were recovered from all sampling greenhouses at 10 – 20 cm depth. The highest fungal frequency was registered for *A. niger*, followed by *Mucor* sp., and *T. koningii*. Another antagonistic fungus may be used in biological control was recovered from the four sites (*A. flavus*, *Penicillium* sp., *Purpureocillium* sp., *Chaetomium* sp., *Trichoderma* sp., and *Paecilomyces* sp.) as well as pathogenic fungus may be caused plant disease (*Sclerotinia* sp., *Fusarium* sp., *Macrophomina* sp., *Rhizoctonia solani*, *Cladosporium* sp., and *Alternaria* sp.).

Table 1. Fungal community isolated from eggplant rhizosphere.

Locations	Fungal isolates
Chatt-el-Arab	<i>Trichoderma koningii</i>
	<i>Aspergillus niger</i>
	<i>A. flavus</i>
	<i>Mucor</i> sp.
	<i>Fusarium</i> sp.
	<i>Penicillium</i> sp.
	<i>Purpureocillium</i> sp.
	<i>Trichoderma koningii</i>
	<i>A. niger</i>
	<i>A. flavus</i>
Abu Al-Khaseeb	<i>Mucor</i> sp.
	<i>Chaetomium</i> sp.
	<i>Trichoderma</i> sp.
	<i>Trichoderma koningii</i>
	<i>Penicillium</i> sp.
	<i>A. niger</i>
Hartha	<i>Mucor</i> sp.
	<i>Macrophomina</i> sp.
	<i>Sclerotinia</i> sp.
	<i>Trichoderma koningii</i>
	<i>Penicillium</i> sp.
	<i>A. niger</i>
Az Zubayr	<i>Alternaria</i> sp.
	<i>Mucor</i> sp.
	<i>Rhizoctonia solani</i>
	<i>Cladosporium</i> sp.
	<i>Paecilomyces</i> sp.
	<i>Fusarium</i> sp.

This prevalence in plants rhizosphere is supported by previous investigations undertaken by Cwalina-Ambroziak and Wierzbowska (2011), Gaddeyya *et al.* (2012), Onyimba *et al.* (2014), and Boughalleb-M'Hamdi *et al.* (2017). Sangeetha *et al.* (2020) studied fungal diversity in cultivable fields. These authors noted that 15 species belonging to more than 6 genera were recorded at 10 – 20 cm with the

highest species density for *Aspergillus* spp. and *Penicillium* spp. These findings are in concordance with Ratna Kumar *et al.* (2015) showing that *Aspergillus* and *Penicillium* species were dominant in all agricultural fields due to high sporulation capacity. Rosas-Medina *et al.* (2020) and Sangeetha *et al.* (2020) pointed out that the species fungal diversity and conidia dispersion has been varied according to the specific conditions, the ecological factors of each soil, the geographical area, the climatic conditions, the host physiology and the specificity of the colonized plant tissue.

Promoting growth and development of eggplant seeds and seedlings by using Trichoderma koningii, Aspergillus niger, and Mucor sp.

Table 2. Shoot, root, and leaf length of eggplant seedlings treated separately with *Trichoderma koningii*, *Mucor* sp., and *Aspergillus niger*.

Treatments	Length of shoot [cm]	Length of root [cm]	Length of leaf [cm]
<i>T. koningii</i>	2.83	3.00	3.50
<i>A. niger</i>	2.56	2.83	3.14
<i>Mucor</i> sp.	2.83	2.67	3.32
Control	2.10	2.50	2.00
LSD^a	<0.05	<0.05	<0.05

^a Probabilities associated with individual F tests.

Data are the average of 100 eggplant seedlings per treatment and per replicate (3 blocks).

Data presented in Table 2 indicated clearly that the three treatments exerted a significant increasing (< 0.05) on shoot (LS), root (LR), and leaf (LL) length of eggplant seedlings after 10 days of incubation. Seeds treatment with *T. koningii* showed a higher value of LS (2.83 cm), LR (3.00 cm) and LL (3.50 cm) compared to the negative control (2.10, 2.50, and 2.00 cm, respectively).

The effect of three treatments on the fresh (FW) and dry (DW) weight and plant length (PL) is shown in Table 3. All treatments increased significantly (<0.01) the FW, DW, and PL as compared with the negative control (2.22 g, 0.13 g, and 35.23 cm, respectively). Results showed that *T. koningii* were found effective to increase the FW (3.91 g), DW (0.24 g), and PL (48.67 cm).

Several *Trichoderma* species had beneficial effects on plant growth (crop yield increasing, seedling fresh weight and foliar area increasing, root volume development, secondary roots proliferation, etc.)

and induced resistance to both biotic and abiotic stresses. Lindsey and Baker (1967) reported that the treated tomato plants by *Trichoderma* spp. revealed a significant augmentation of fresh weight (8 %) and height (28 %) plants in sterile conditions.

Table 3. Fresh and dry weight and plant length of eggplant treated separately with *Trichoderma koningii*, *Mucor* sp. and *Aspergillus niger*.

Treatments	Fresh weight [g]	Dry weight [g]	Plant length [cm]
<i>T. koningii</i>	3.91	0.24	48.67
<i>A. niger</i>	2.28	0.16	43.00
<i>Mucor</i> sp.	2.83	0.21	41.67
Control	2.22	0.13	35.23
LSD^a	<0.05	<0.01	<0.05

^a Probabilities associated with individual F tests.

Data are the average of 45 eggplant plants per treatment and per replicate (3 replicates).

Several *Trichoderma* species had beneficial effects on plant growth (crop yield increasing, seedling fresh weight and foliar area increasing, root volume development, secondary roots proliferation, etc.) and induced resistance to both biotic and abiotic stresses. Lindsey and Baker (1967) reported that the treated tomato plants by *Trichoderma* spp. revealed a significant augmentation of fresh weight (8 %) and height (28 %) plants in sterile conditions. Windham *et al.* (1986) revealed that the amended cucumber by the propagules of *Trichoderma* spp. ameliorated the seedling emergence (30 %) comparing to controls. Lynch *et al.* (1991) explained the beneficial effect of *T. harzianum* on lettuce plants, which increased the emergence rate and enhanced the dry weights. Rabeendran *et al.* (2000) showed that the treated cabbage seedlings by *Trichoderma* spp. are enhanced shoot (91 – 102 %) and root (100 – 158 %) dry weight, and leaf area (58 – 71 %) under glasshouse assays. Yedidia *et al.* (2001) depicted that *Trichoderma* spp. increased shoot length (45 %), cumulative root length (75 %), root (95 %) and leaf (80 %) area, and dry weight (80 %). Mastouri *et al.* (2010) demonstrated that the tomato seeds treatment with *T. harzianum* increased the vigour of seedling and accelerated the germination of seeds by triggering the plant physiological protection. Lorito *et al.* (2010) noted that *Trichoderma* spp. ameliorated the plant growth through the phytohormones and several secondary metabolites production.

Similarly, Yedidia *et al.* (2001) pointed out that the roots treatment by *Trichoderma* spp. enhanced the phosphorus and iron availability to plants. These authors observed a significant augmentation in shoot length, dry weight and leaf area. Altomare *et al.* (1999) showed that *Trichoderma* spp. can solubilize many plant nutrients. The uptake of nutrient may stimulate by *Trichoderma* spp. in two ways by changing the anchorage of root system or by the substances exudation increasing the availability of nutrient (nitrogen, phosphorus, iron, potassium, etc.) to plants (Mastouri *et al.* 2010; Azarmi *et al.* 2011). In the same sense, Altomare *et*

al. (1999), Benítez *et al.* (2004), Rudresh *et al.* (2005), Molla *et al.* (2012) confirmed that *Trichoderma* spp. augments the macronutrients and micronutrients solubility.

Different airborne pathogens at different sampling moments (5, 10, 15, and 21 days after inoculation) are presented in Table 4. A total of 8 species belonging to 4 genera were identified from eggplant leaves. The genera with the highest species number were *Alternaria* (4) and *Cladosporium* (2). Present results are in analogy with Boughalleb-M'Hamdi *et al.* (2017), Sangeetha *et al.* (2020).

Table 4. Airborne pathogens contribution at different sampling moments (5, 10, 15, and 21 days after inoculation).

Treatments	Sampling moments			
	5 DAI	10 DAI	15 DAI	21 DAI
<i>T. koningii</i>	-	-	-	<i>Alternaria alternata</i>
<i>A. niger</i>	-	<i>Cladosporium</i> sp.	<i>Cladosporium</i> sp. <i>Alternaria</i> sp. <i>A. alternata</i>	<i>Cladosporium</i> sp. <i>A. solani</i>
<i>Mucor</i> sp.	-	<i>A. alternata</i>	<i>A. alternata</i>	<i>A. alternata</i> <i>Alternaria</i> sp.
Control	-	<i>Cercospora</i> sp. <i>Cladosporium</i> sp.	<i>Cercospora</i> sp. <i>Cladosporium</i> sp. <i>A. alternata</i> <i>Alternaria</i> sp.	<i>Cercospora</i> sp. <i>Botrytis cinerea</i> <i>A. alternata</i> <i>A. solani</i> <i>Alternaria</i> sp.

Data are the average of 15 eggplant plants per treatment and per replicate (3 replicates),

DAI: days after inoculation,

- Absence of airborne pathogens.

Peroxidase activity and chlorophyll content in eggplant plant treated separately or simultaneously with A. niger, Mucor sp., and T. koningii

The change of peroxidase activity and chlorophyll content from eggplants treated separately or simultaneously with *T. koningii*, *Mucor* sp. and *A. niger* were increased significantly comparing to the negative control ($5.81 \cdot 10^{-1}$ units.g⁻¹.mL⁻¹.min⁻¹ and 0.70 mg.g⁻¹ fw) (Table 5). There was increasing in peroxidase activity in treated plants with *Mucor* sp. + *A. niger* + *T. koningii* ($12.53 \cdot 10^{-1}$ units.g⁻¹.mL⁻¹.min⁻¹), *T. koningii* + *Mucor* sp. ($12.68 \cdot 10^{-1}$ units.g⁻¹.mL⁻¹.min⁻¹), and *T. koningii* ($11.28 \cdot 10^{-1}$ units.g⁻¹.mL⁻¹.min⁻¹). However, the combination of *A. niger* and *Mucor* sp. ($8.06 \cdot 10^{-1}$ units.g⁻¹.mL⁻¹.min⁻¹) cause peroxidase activity decrease indicating that there was *T. koningii*-specific response in peroxidase activity changes (Table 5). Chlorophyll content was higher in eggplants leaves

when combined with microorganisms non-pathogenic (*Mucor* sp. + *A. niger* + *T. koningii*) with 2.11 mg.g⁻¹ fw. *T. koningii* (1.90 mg.g⁻¹ fw) inoculation increases the chlorophyll content as well as *A. niger* + *T. koningii* (1.73 mg.g⁻¹ fw) treatment (Table 5).

BCAs induce the activity of peroxidase to protect the plants from biotic and/or abiotic damage (Gusain *et al.* 2014; Ahmad *et al.* 2015). The peroxidase activity was implicated in the self and growth regulation (respiration, photosynthesis, etc.) (Kavitha and Umesha 2008). This activity was increased in plants treated separately or simultaneously with by *Trichoderma* spp. comparing to plants treated only with water as reported by Latha *et al.* (2009), Saksirirat *et al.* (2009), and Heydari and Pessarakli (2010). Yedidia *et al.* (2000) reported a higher level of peroxidase, β -1,3-glucanases, and chitinase when cucumber plants were treated with *Trichoderma* spp.

compared to controls. Abd-El-Kareem *et al.* (2019) revealed that all tested *Trichoderma* species increased significantly the peroxidase activity. These authors' also pointed out that the mixture of *T. harzianum*, *T. viride*, and *T. koningii* attained the highest increase of activity of peroxidase with 150 %. Mastouri *et al.* (2012), Zehra *et al.* (2017), Herrera-Téllez *et al.* (2019) and Phoka *et al.* (2020) documented that the pretreatment of tomato plants with *Trichoderma* suppressed Reactive Oxygen Species (ROS) by enhancing mechanisms antioxidant defense and increased catalase and peroxidase activities. Peroxidase helps in conversion of H₂O₂ to water and oxygen (Gusain *et al.* 2014; Ahmad *et al.* 2015).

Table 5. Comparison of peroxidase activity and chlorophyll content of eggplant leaves recorded by eggplant plants treated separately or simultaneously with *Trichoderma koningii*, *Mucor* sp., and *Aspergillus niger*.

Treatment	Peroxidase activity [10 ⁻¹ units.g ⁻¹ . mL ⁻¹ .min ⁻¹]	Chlorophyll content [mg.g ⁻¹ fw]
<i>T. koningii</i>	11.28	1.90
<i>A. niger</i>	10.78	1.43
<i>Mucor</i> sp.	9.14	1.66
<i>A. niger</i> + <i>Mucor</i> sp.	8.06	1.67
<i>A. niger</i> + <i>T. koningii</i>	9.74	1.73
<i>T. koningii</i> + <i>Mucor</i> sp.	12.68	1.70
<i>Mucor</i> sp. + <i>A. niger</i> + <i>T. koningii</i>	12.53	2.11
Control	5.81	0.70
LSD^a	< 0.05	< 0.01

^a Probabilities associated with individual F tests.

Data are the average of 5 eggplant leaves per treatment and per replicate (3 replicates).

The chlorophyll content related to biophysical conditions indicating the plants health and plays critical role in photosynthesis (Moharan and Dutta 2016; Inayati *et al.* 2020). Photosynthesis plays an important role in the physiology of plant and a critical process in the regulation of plant defense (Kangasjärvi *et al.* 2012; Pérez-Bueno *et al.* 2019). Durairaj *et al.* (2018) pointed out that the increase chlorophyll content in plant influence photosynthesis and enchanted the yield. Harman (1992), Rawat *et al.* (2012), Vitti *et al.* (2016), Doni *et al.* (2017), Fu *et al.* (2018) and Zhao-Ying *et al.* (2018) demonstrated an increase in chlorophyll content in many plant species treated

with *Trichoderma* sp.. Azarmi *et al.* (2011) showed that the chlorophyll content augmented when plants were inoculated with *Trichoderma* spp. Chlorophyll content was increased in *Trichoderma*-treated plants as was observed in melon and cacao plants inoculated. This result suggests an optimal physiological status of plants (Martínez-Medina *et al.* 2009; 2013; Tchameni *et al.* 2017). Inayati *et al.* (2020) demonstrated that the changes of chlorophyll content from treated *Vigna radiata* leaves by *Trichoderma* spp. could be defense mechanisms part to limit the availability of nutrients to the *R. solani*.

Conclusions

Based on the current results, it was deduced that *T. koningii* (alone or with other antagonistic fungi) could be employed in leaves treatments as BCA's to induce *S. melongena* of systemic resistance, through a specific signal transduction cascade. This antagonistic fungus allowed not only the induction systemic resistance but also the good ability to stimulate plant growth and development by increasing macronutrients and micronutrients solubility. The systemic resistance induction of *S. melongena* by *T. koningii* against soilborne and airborne pathogens represents a subject of future research.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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