

Plant growth-promoting fungi from desert soil

Usama A. A. Radwan*, Magdi A. El-Sayed and Sabreen Hamdy
Botany Department, Faculty of Sciences, Aswan University, Egypt

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Abstract:

Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides, but they are not yet able to replace their use. Bio-fertilizer production is cheap and does not create pollution in the natural system. Plant growth promoting fungi (PGPF) play important roles in plant nutrition, soil biology and soil chemistry, (PGPF) enhanced photosynthetic rate and other gas exchange-related traits as well as increased water uptake. PGPF are common root-associated and soil-borne fungi from diverse genera, which have the natural ability to stimulate various growth related traits of plants. Plant growth promoting fungi (PGPF) were isolated from various soils of Aswan University Campus, South–western Desert, Aswan, Egypt. The purified isolates were classified as *Trichoderma longibrachiatum* and *Fusarium solani* according to phylogenetic analysis and were used as bio fertilizers and to enhance *Vigna unguiculata* growth under irradiance stress. In current study it was found that these isolates improve *Vigna. unguiculata* growth under irradiance stress through increasing chlorophyll content compared to control and showing maximum photosynthesis rate (P_n) at high Photosynthetic Active Radiation (PAR). It was concluded that *Trichoderma longibrachiatum* and *Fusarium solani* possessed multiple beneficial effects for *Vigna. unguiculata* productivity when grown under harsh environmental conditions.

Keywords: Biofertilizer; Irradiance stress; Phylogenetic analysis and Photosynthesis rate.

Abbreviations: PGPF: plant growth promoting fungi, P_n : photosynthesis rate, E : transpiration, WUE : water use efficiency, PAR : photosynthetic active radiation and SMC : soil moisture content.

1- Introduction

At present, searching for harmless technologies that have no risks to ecosystem has received high attention from scientists by using non-pathogenic and beneficial microorganisms like bacteria, algae and fungi as bio fertilizers that are less expensive and be more environmentally–friendly than chemical fertilizers, which cause worldwide ecological problems as well as affects the human health in the long term (Tomas and Hilan, 2012).

Corresponding author*: E-mail address: radusa2009@yahoo.com, radusa@aswu.edu.eg

PGPF and PGPR are known to suppress some plant diseases. *Trichoderma* species are among the most commonly studied as bio-control microbes, also have been shown to induce local and systemic defense responses in agricultural crops. Although, it has also direct effects on plants, increasing or accelerating their growth, resistance to diseases and the tolerance to a biotic stresses, some *Trichoderma* strains are commonly used as bio-control agents in agriculture due to their abilities in antibiotics and/or hydrolytic enzymes production that directly antagonize plant-pathogenic fungi (Benítez et al., 2004). Some *Trichoderma* strains also enhance plant growth and development, and thus increase yields. *Trichoderma* spp are known to have the capability of attacking other fungi. Also they are well known as potential biological control agents and can kill plant pathogens and improve the plant growth (El Komy et al., 2015; Naher et al., 2014; Sundaramoorthy and Balabaskar, 2013; Contreras-Cornejo et al., 2015a, Contreras-Cornejo et al., 2015b; Garnica-Vergara et al., 2016). Besides, *Trichoderma* spp have been proven its ability to detoxify toxic compounds and fasten degradation of organic materials (Amira et al., 2011; Sharma et al., 2012; Vázquez et al., 2015; Zafra et al., 2015). The success of *Trichoderma* spp in the soil ecosystem and its role as natural decomposer is due to its ability to hasten the growth, by nutrient uptake and modifying the rhizosphere. It is also able to tolerate unfavourable environment and has powerful destructive capability against plant pathogenic microorganisms (Benítez et al., 2004). *Vigna* is well adapted to stress and has excellent nutritional qualities. It is a key dietary staple for the poorest sector of many developing countries and greatly improves an otherwise bland and unbalanced diet (Som and Hazra, 1993). It is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times. *Vigna* is the crop of all-round utilization that grown for dry seed, immature seed, immature green pod, green leaves, and even roots. It is one of legumes that is an important source of nutrients and provides high-quality, inexpensive protein to diets based on cereal grains or starchy food (Harman, 2006).

2- Materials and Methods

2.1. Sampling Sites

The Aswan University campus's desert was used to collect soil samples. (1- N 24°00'17.7' E 032°52'15.1', 2- N 24°00'15.1' E 032°52'05.6', 3- N 24°00'15.1' E 032°52'05.6', 4- N 24°00'03.8' E 032°52'48.3', 5- N 24°00'03.4' E 032°51'52.0', 6- N 23°59'58 in march 2017. Sterile method was developed and weighting 10 g of soil into 100 ml of distilled, sterile water created the soil suspension.

2.2. Media Employed to Isolate Fungi

Trichoderma sp. isolation and other important prospective fungi candidates: Tsm, or the *Trichoderma* Selective Media Bengal agar-based Martin's rose media (MRB), The following ingredients were included in the basal medium (g/l distilled water): 0.1 g chloramphenicol, 0.15 g rose bengal (BDH Chemicals), 0.15 g KCl, 1.0 g N H₄NO₃, 3.0 g glucose, 0.22 g MgSO₄ (7H₂O), and 20 g agar. 950 ml of distilled water with these ingredients was autoclaved at 121 °C for 15 minutes. The biocidal components were 0.2 g each of captan and quinterozone. According to Elad and Chet (1983), these were combined with 50 ml of sterilized (autoclaved at 121 °C for 15 min) distilled water and put to the autoclaved basal medium.

In order to make potato dextrose agar medium (PDA), 200 grammes of potato infusion, 20 grammes of dextrose, and 20 grammes of agar were combined with 1000 ml of distilled water and autoclaved at 121 degrees celsius for 15 minutes.

2.3. Identification of the Isolated Fungi by Morphology

On the basis of their culture properties (colour, texture, and pigmentation), spores, and spore-bearing structure, all the isolated fungal colonies were morphologically identified. The fungi's genus and species were identified using the sources below:

1. Barron, (1968) for the genera of Hyphomycetes.
2. Domsch & Gams, (1972) for fungi in general.
3. Ellis (1971 and 1976) for Dematiaceous Hyphomycetes.
4. Gilman, (1957) for soil fungi in general.
5. Klich, (2002) for *Aspergillus* Species.
6. Moubasher, (1993) for fungi in general.
7. Raper & Fennell, (1965) for *Aspergillus* species.
8. Raper & Thom, (1949) for *Penicillium* species and related genera.

2.4. Genotypic characterization of isolated fungi

Genotypic characterization was carried out using a small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence (Wolf et al., 2004).

2.5. An Infection Test

During the experiment, pots holding 4 kg of sterile sand-clay soil were prepared for planting in the greenhouse (1:2). Healthy *V. unguiculata* seeds, meantime, were sterilized in 70% ethanol for 2 minutes before being thoroughly cleaned with sterile distilled water and prepared for planting. Each pot was planted with two sterilized seeds, and our experiment was divided into eight groups, each with ten pots. The first group served as the control and received no inoculation; the second group received an inoculation with calcium (egg shells) and phosphate, and the third group

received *Trichoderma* spore suspension. *Fusarium* spore suspension was used in the fourth group's inoculation, *Trichoderma* spore suspension was used in the fifth group's inoculation, along with Phosphate, *Fusarium* spore suspension was used and Calcium in the sixth group's inoculation, and a mix of *Trichoderma* and *Fusarium* spore suspension was used in the seventh group's inoculation. The last group was inoculated with mix of *Trichoderma*, *Fusarium* spore suspension in addition to Phosphate and Calcium. For 73 days, all of the experiment-specific pots received frequent irrigation to maintain the soil's 9% moisture content.

2.6. Analysis of Photosynthesis (P_n), Water Use Efficiency (WUE) and Transpiration Rates (E) in Plants

According to readings from the Research Unit for Study Plants of Arid Lands (RUSPAL)'s meteorological station, photosynthesis (P_n) and

transpiration rate (E) were measured for plants grown under irradiance stress at photosynthetic active radiation (PAR) ranges from 0 to 2500 mol m⁻²s⁻¹ at the 9th week prior to plants growing (Ali et al., 2018). The following formula was used to calculate water use efficiency (WUE), as described by Silva et al. (2013), Ali et al. (2018) and, Radwan and Saleh (2022).

WUE was calculated from the ratio: $WUE = Pn/E$

2.7. Statistical analysis

Statistics were used to analyze each parameter that was measured. MINITAB12 statistical software, developed in the USA (Min-itab Inc., 1998), was used to do a two-way analysis of variance. Data are displayed as mean SE.

3- Results and Discussion

3.1. 16S rDNA Gene Sequence of Isolated Strains

Two fungal strains that were isolated from our study were identified as *Trichoderma longibrachiatum* T1 and *Fusarium solani* F2 (Figure 3) with accession numbers: (OQ615796), (OQ615913), respectively, as deposited in the Gene bank (Figure 2a, b).

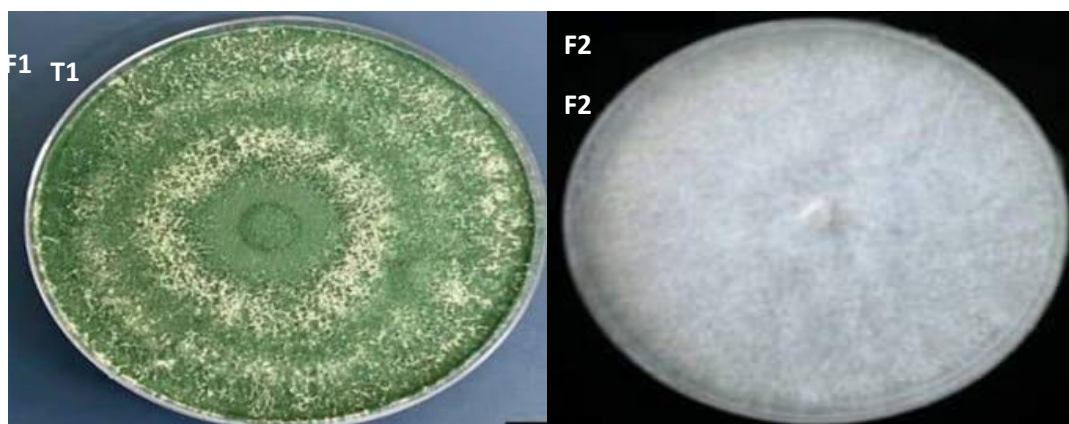


Fig. 1. Showed T1 and F2 strains on petri dishes

1. Strain T1 (Nucleotide Sequence of 16s rRNA of Strain T1)

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1 taggtgaaac tgcggaggga tcattaccga gtttacaact cccaaacccc aatgtgaacg
61 ttaccaatct gttgcctcgg cgggattctc ttgccccggg cgcgtcgcag ccccggatcc
121 catggcgccc gccggaggac caactccaaa ctctttttc tctcgtcgc ggctcccgtc
181 gggctctgt tttattttg ctctgagcct ttctcggcga ccctagcggg cgtctcgaaa
241 atgaatcaaa acttcaaca acggatctct tggttctggtc atgatgaag aacgcagcga
301 aatgcgataa gtaatgtgaa ttgcagaatt cagtgaatca tgaatcttt gaacgcacat
361 tgcgcccgcc agtattctgg cgggcatgcc tgtccgagcg tcatttcaac cctcgaacce
421 ctccgggggg tcggcgttgg ggatcggccc ctaccgggc cgccccgaa atacagtggc
481 ggtctcgccg cagcctctcc tgcgcagtag ttgcacact cgcaccggga gcgcggcgcg
541 gccacagccg taaaacacc caaactctg aatgttgac ctccgatcag gtaggaatac
601 ccgctgaact taagcatatc aa
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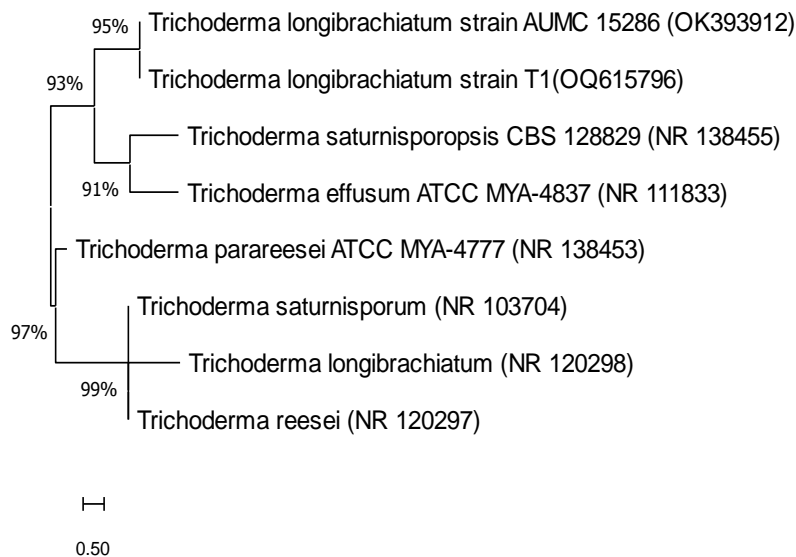


Fig. 2a. Phylogram generated from maximum likelihood analysis based on ITS sequence data of *T. longibrachiatum* T1, and related species.

2. Strain F2 (Nucleotide Sequence of 16s rRNA of Strain F2)

1 gacataccta taacgttgc tcggcgggaa cagacggccc cgtaacacgg gccgccccg
 61 ccagaagacc cctaactct gttctatrat tgtttctct gagtaaaca gcaataaat
 121 taaacttc aacaacggat ttcttgctc tggcatgat gaagaacgca gcgaaatgcg
 181 ataagtaatg tgaattgaat aattcagtga atcatcgaat cttgaaacgc acattgcgcc
 241 cgccagtatt ctggcgggca tgctgttcs agcgtcatta caaccctcag gccccggggc
 301 ctggcgttgg ggatcggcgg aagccccctg cgggcacaac gccgtcccc aaatacagt
 361 gcggtcccc gcgagcttc attcgtagt agctaacacc tcgcaactgg agagcggcgc
 421 ggccccgccg taaaacacc aacttctgaa tgttgacctc aatcaggtg ggaataccg
 481 ctgaactaa gcatatcaat aagcggagga

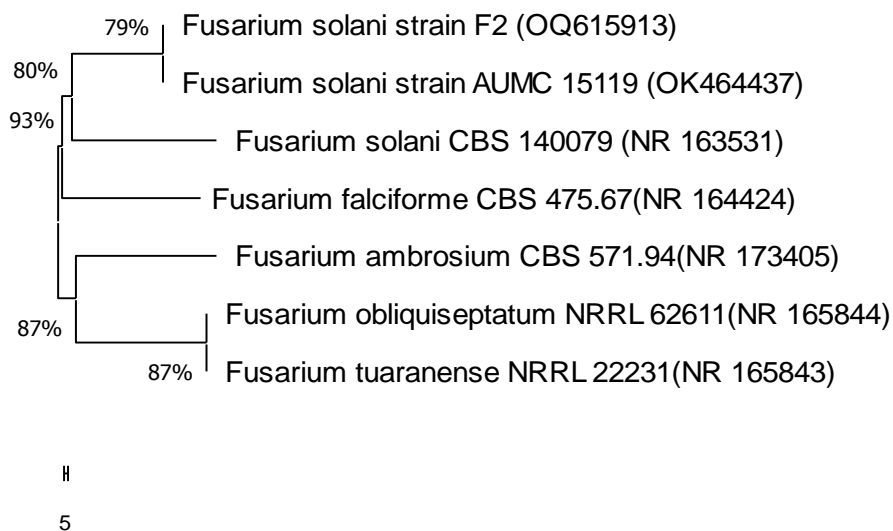


Fig.2b. Phylogram generated from maximum likelihood analysis based on ITS sequence data of *F. solani* F2 and related species.

3.2. Photosynthesis (P_n) rate of *Vigna unguiculata*

P_n rate recorded maximum values (3.78, 6.86, 8.06, 3.52, 3.25 and 3.12 $\mu\text{molm}^{-2}\text{s}^{-1}$) at the following treatment: *Trichoderma*, *Fusarium*, *Trichoderma* + P^{+++} , *Fusarium* + Ca^{++} , *Trichoderma* + *Fusarium* *Trichoderma*+ *Fusarium*+ P^{+++} + Ca^{++} . The lowest values of (P_n) (1.10 and 1.06 $\text{molm}^{-2}\text{s}^{-1}$) were at high (PAR) levels (1500 - 2500 $\text{molm}^{-2}\text{s}^{-1}$) during control and P^{+++} & Ca^{++} treatments, respectively, were recorded in *V. unguiculata*. Highest (P_n) value (8.06 $\mu\text{molm}^{-2}\text{s}^{-1}$) where observed in *V. unguiculata* plants treated with (*Trichoderma* + P^{+++}) at high PAR (1500 - 2500 $\mu\text{molm}^{-2}\text{s}^{-1}$).

Significant changes in (P_n) were attributed via a two-way analysis of variance to (PAR), therapy, and their interaction, where $F = 51.21$, $P \leq 0.0001$, $F = 33.46$, $P \leq 0.0001$, and $F = 3.05$, $P \leq 0.0001$, respectively under PAR ranges (0–2500 $\mu\text{molm}^{-2}\text{s}^{-1}$) (Fig. 3).

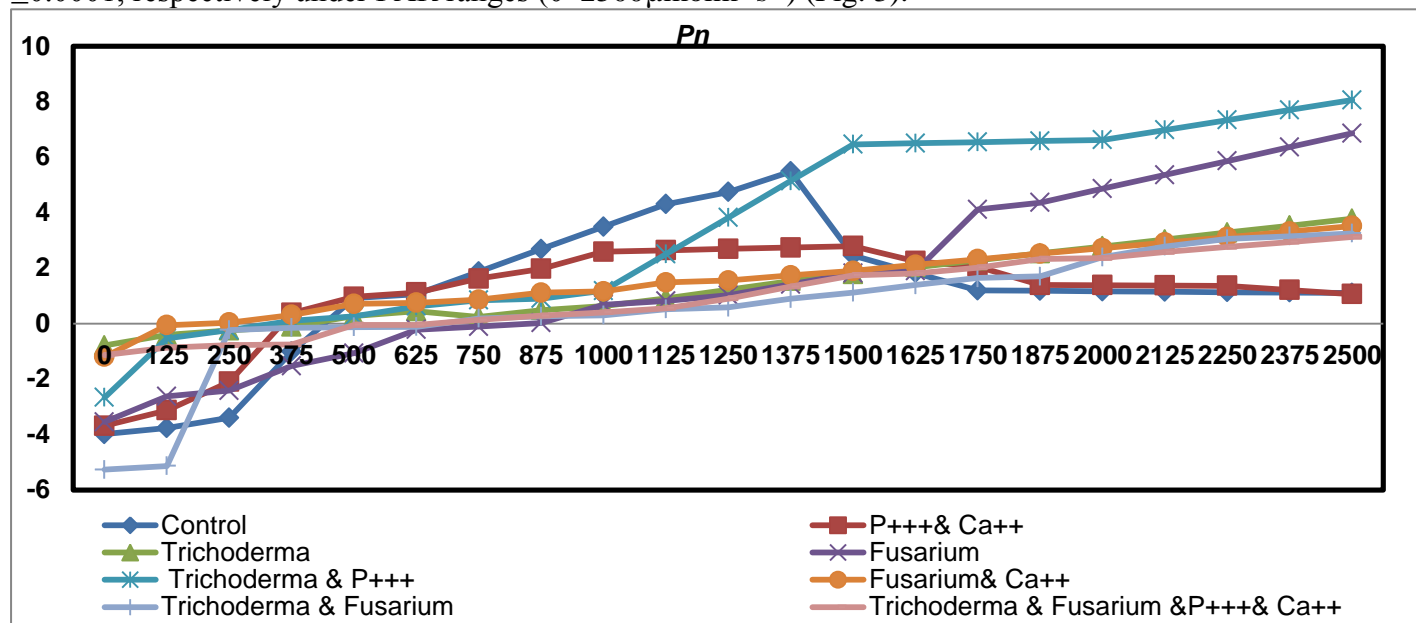


Fig 3. Showed (P_n) ($\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$) photosynthesis rate of light response curve of *Vigna unguiculata* under PAR (photosynthetic active radiation rang 0-2500 $\mu\text{molm}^{-2}\text{s}^{-1}$) and treated with (control, $P^{+++}+\text{Ca}^{++}$, *Trichoderma* , *Fusarium* , *Trichoderma* + P^{+++} , *Fusarium* + Ca^{++} , *Trichoderma* + *Fusarium* , *Trichoderma* + *Fusarium* + P^{+++} + Ca^{++})

3.3. Transpiration rate (E) of *Vigna unguiculata* inoculated with different treatment of (PGPF) under irradiance stress

Figure (4) depicts the light response curves of the *V. unguiculata* at (PAR) range (0 - 25000 $\text{molm}^{-2}\text{s}^{-1}$). Minimum (E) values from *V. unguiculata* were (0.19, 0.27, 0.33, 0.36, and 0.39 $\text{mmolm}^{-2}\text{s}^{-1}$) was achieved at (*Trichoderma* + P^{+++} , *Trichoderma*, P^{+++} + Ca^{++} , control, and *Trichoderma* + *Fusarium* + P^{+++} + Ca^{++}), respectively, at high (PAR) level (1500 - 2500 $\text{molm}^{-2}\text{s}^{-1}$). The lowest (E) value (0.19 $\text{mmolm}^{-2}\text{s}^{-1}$) where observed in *V. unguiculata* plants treated (*Trichoderma* + P^{+++}) at high PAR (1500 - 2500 $\mu\text{molm}^{-2}\text{s}^{-1}$).

From two - way analysis of variance significant (E) changes of *V. unguiculata* attributed to both (PAR) and treatments were where: $F = 20.83$; $P \leq 0.0001$, $F = 30.75$; $P \leq 0.0001$ and $F = 0.70$; $P \leq 0.992$, respectively under PAR ranges (0–2500 $\mu\text{molm}^{-2}\text{s}^{-1}$).

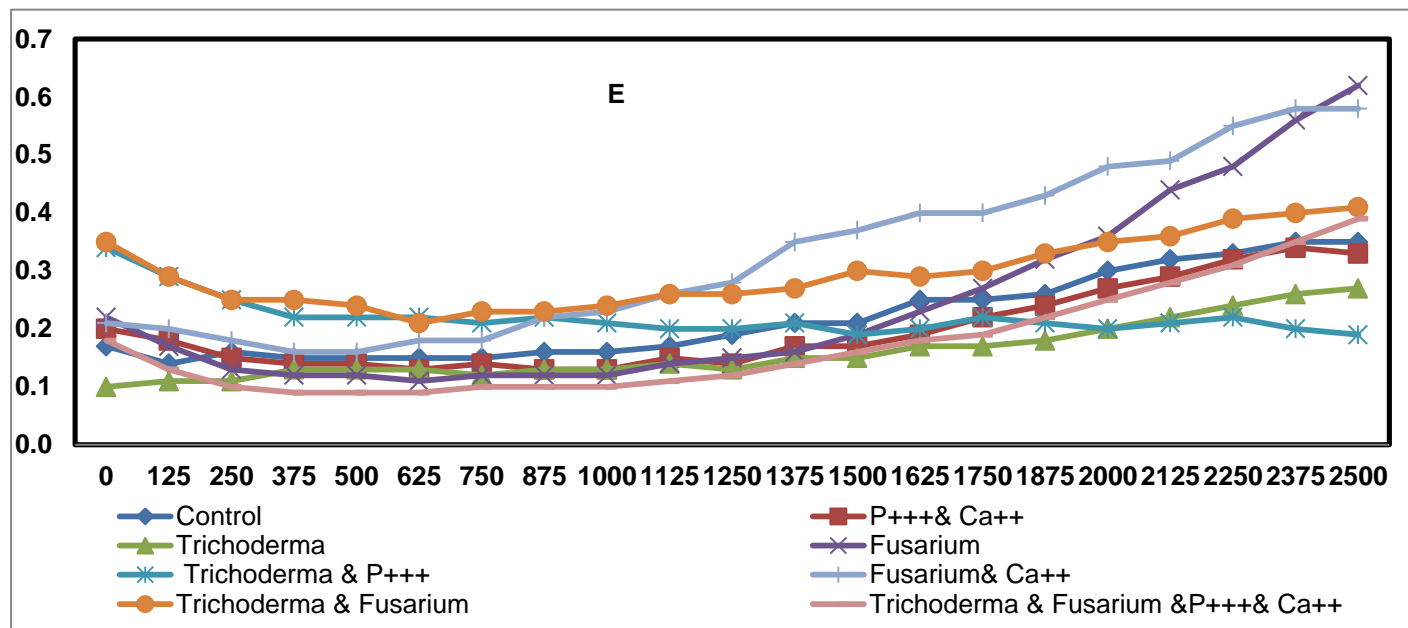


Fig 4. Showed (E) ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) transpiration rate of curve of *Vigna unguiculata* under PAR (photosynthetic active radiation rang 0-2500 $\mu\text{mol m}^{-2}\text{s}^{-1}$) and treated with (control, $\text{P}^{+++} + \text{Ca}^{++}$, *Trichoderma*, *Fusarium*, *Trichoderma* + P^{+++} , *Fusarium* + Ca^{++} , *Trichoderma* + *Fusarium*, *Trichoderma* + *Fusarium* + P^{+++} + Ca^{++})

3.4. Water Use Efficiency (WUE) rate of *Vigna unguiculata* inoculated with different (PGPF) under irradiance stress

When treated with *Trichoderma* + P^{+++} , *Trichoderma*, *Fusarium*, *Trichoderma* + *Fusarium* & P^{+++} + Ca^{++} , *Trichoderma* + *Fusarium*, and *Fusarium*, respectively, *V. unguiculata* inoculated with different treatments with (PGPF) under irradiance stress (WUE) of *V. unguiculata* gave maximum values (42.24, 14, 11.06, 8, 7 $\mu\text{mol m}^{-2}\text{s}^{-1} / \text{mmol m}^{-2}\text{s}^{-1}$), and the lowest values of (WUE), which are 3.15 and 3.21 $\mu\text{mol m}^{-2}\text{s}^{-1} / \text{mmol m}^{-2}\text{s}^{-1}$).

The highest value (42.24 $\mu\text{mol m}^{-2}\text{s}^{-1} / \text{mmol m}^{-2}\text{s}^{-1}$) where observed in *V. unguiculata* plants treated with *Trichoderma* + P^{+++} at high PAR (1500 - 2500 $\mu\text{mol m}^{-2}\text{s}^{-1}$) (Fig. 5).

From a two-way analysis of variance, substantial (WUE) variations in the *V. unguiculata* were attributed to the treatments and PAR, with $F = 26.55$; $P \leq 0.0001$, $F = 38.68$; $P \leq 0.0001$, and $F = 2.89$; $P \leq 0.0001$, respectively, under the PAR ranges (0-2500 $\text{mol m}^{-2}\text{s}^{-1}$).

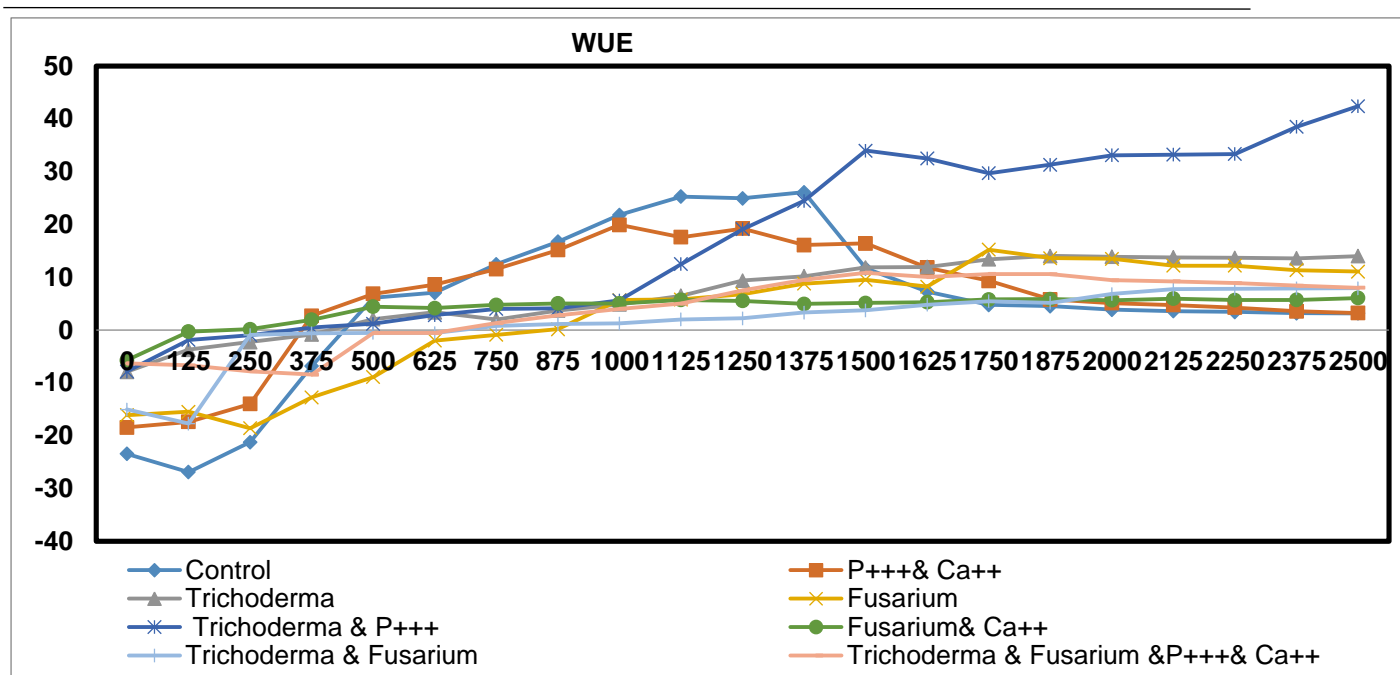


Fig 5. Showed (WUE) ($\mu\text{molCO}_2\text{m}^{-2}\text{s}^{-1}/ \text{mmol H}_2\text{Om}^{-2}\text{s}^{-1}$) water use efficiency rate of curve of *Vigna unguiculata* under PAR (photosynthetic active radiation rang 0-2500 $\mu\text{molm}^{-2}\text{s}^{-1}$ and treated with (control, $\text{P}^{+++} + \text{Ca}^{++}$, *Trichoderma* , *Fusarium* , *Trichoderma* + P^{+++} , *Fusarium* + Ca^{++} , *Trichoderma* + *Fusarium* , *Trichoderma* + *Fusarium* + P^{+++} + Ca^{++}).

3.5. The ratio of PSII photochemistry of *Vigna unguiculata* inoculated with different (PGPF) under irradiance stress

In *V. unguiculata* plants at high levels of (PAR) ($1500\text{-}2500 \text{molm}^{-2}\text{s}^{-1}$), when treated with (*Trichoderma* + P^{+++}), (*Trichoderma* + *Fusarium* + P^{+++} + Ca^{++}), (*Trichoderma* + *Fusarium* and *Fusarium* + Ca^{++}), respectively, the ratio of PSII photochemistry maximum quantum efficiency to quantum yield showed significant declines along with progressing phenological stages (Fig. 6). The highest value (2.056) was observed in *V. unguiculata* plants treated with (*Trichoderma* + P^{+++}) at high PAR ($1500 - 2500 \mu\text{molm}^{-2}\text{s}^{-1}$).

According to two-way analysis of variance, substantial ratio changes were linked to several phonological phases, with Fv/Fm versus treatment being associated with $F = 2310.65$ and $P \leq 0.0001$, respectively, under the PAR ranges ($0\text{-}2500 \text{molm}^{-2}\text{s}^{-1}$).

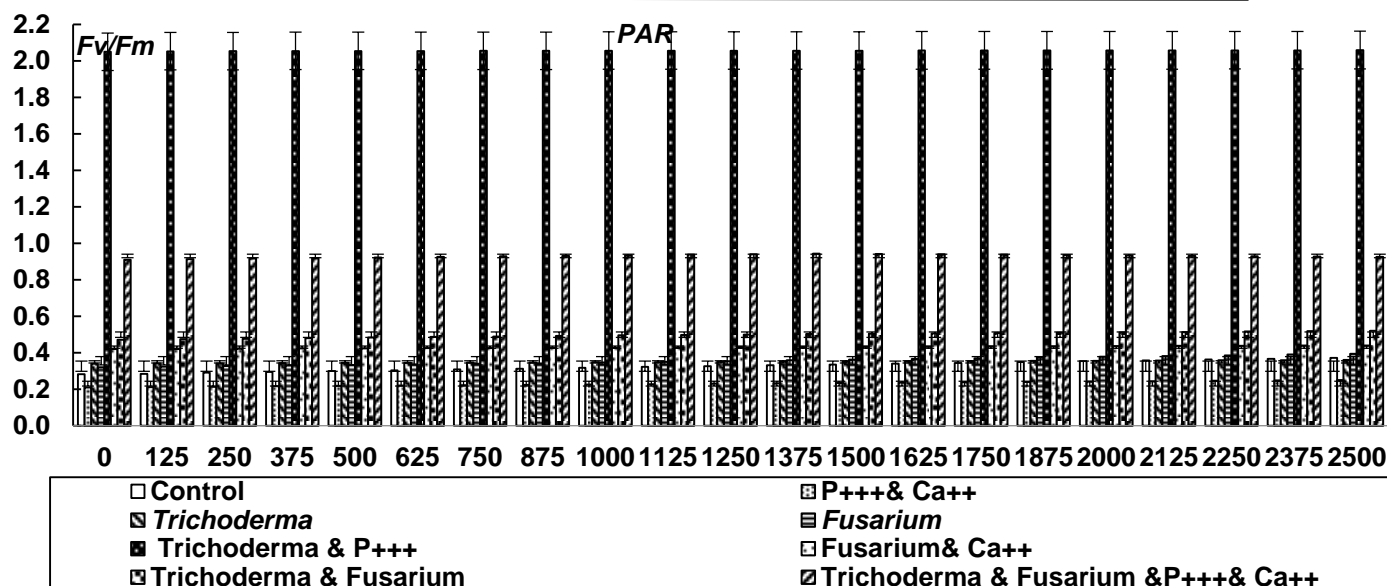


Fig 6. Showed the ratio of PSII photochemistry maximum quantum efficiency measured in *Vigna unguiculata* under PAR (photosynthetic active radiation) range 0-2500 $\mu\text{molm}^{-2}\text{s}^{-1}$ and treated with (control, P^{+++} & Ca^{++} , *Trichoderma*, *Fusarium*, *Trichoderma* & P^{+++} , *Fusarium* & Ca^{++} , *Trichoderma* & *Fusarium*, *Trichoderma* & *Fusarium* & P^{+++} & Ca^{++}).

3.6. The role of PGPF in alleviating irradiance stress:

The survival, toleration, competence, colonization, and expression of desired features in the plant-soil system are all influenced by both biotic and abiotic variables. The ability of plants to respond to abiotic conditions such salt, drought, heavy metal toxicity, severe temperatures, and oxidative stress is strongly influenced by their microbial communities. According to recent studies, the fitness advantages provided by some PGPF help plants adapt to stresses (Khan et al., 2011). Even in less-than-ideal settings, there have been reports of increased plant growth due to the connection of PGPF with plants (Bae et al., 2009).

Trichoderma spp., which are free-living fungi that are widespread in soil and root ecosystems and have been known for many years to create a variety of antibiotic substances as well as parasitize other fungi (Denis and Webster, 1971), are known to produce a wide range of antibiotic substances. According to recent research, they are virulent, opportunistic plant symbionts that cause systemic resistance in plants similar to rhizo-bacteria (Harman et al., 2004). These symbionts are well known for their amazing interactions with host plants and their capacity to provide broad-spectrum resistance to plant diseases (Harman et al. 2004).

Decreases in Fv/Fm are typically noticed when plants are subjected to abiotic and biotic stresses (such drought, heat, salinity, cold, or pathogen infection) in the sun. Because it is such a common occurrence, Fv/Fm measurements offer a quick and easy method of tracking stress. Unfortunately, the causes of reductions in Fv/Fm brought on by stress are sometimes complicated. Increases in non-photochemical quenching processes can lead to a drop in Fm when light-stressed photosynthetic tissues. Such quenching causes drops in Fv/Fm and may not recover over a brief time of dark adaption, or even overnight (Adams and Adams, 2006; 2004).

Increases in non-photochemical quenching are frequently accompanied by photo inactivation of the PSII reaction centre under stress, which causes excitation energy to be released

as heat rather than through photochemistry (Melis, 1999). According to Aro et al. (1994), photo in activation can result in oxidative damage and the loss of the PSII reaction centre, both of which are linked to an increase in F0 (Bradbury and Baker, 1986; Osmond, 1994).

However, it can frequently be challenging to pinpoint the underlying causes of such declines. Since water loss was accompanied by strong irradiance at all stages, the parameters of chlorophyll fluorescence were primarily affected. In the assimilatory organs of *H. muticus*, chlorophyll fluorescence parameter values showed a statistically highly significant negative that was related to water deficiency stress (Mihaljevi et al., 2021).

In the current study, *Trichoderma* and P+++ -treated *Vigna* plants had the highest Pn values ($8.06 \text{ molm}^{-2}\text{s}^{-1}$), while *Vigna unguiculata* had the lowest (E) values (0.19, 0.27, 0.33, 0.36, and $0.39 \text{ mmolm}^{-2}\text{s}^{-1}$). was achieved at (*Trichoderma* &P+++ , *Trichoderma*, P+++& Ca++, control, and *Trichoderma* &*Fusarium* &P+++ &Ca++), respectively at high (PAR) level ($1500\text{-}2500 \text{ molm}^{-2}\text{s}^{-1}$). When treated with (*Trichoderma*&P+++ , *Trichoderma*, *Fusarium*, *Trichoderma* &*Fusarium* &P+++ &C and the lowest values of (WUE), which are 3.15 and $3.21 \text{ molm}^{-2}\text{s}^{-1}$.

4- Conclusion

When exposed to irradiance stress, *Trichoderma longibrachiatum* and *Fusarium solani* strains produced well in *Vigna unguiculata* and reaped numerous benefits. When treated with fungal strains (T1 and F2) under irradiance stress, *V. unguiculata* showed enhanced rates of photosynthesis (Pn), transpiration (E), and water use efficiency (WUE). Highest Pn value where observed in *V. unguiculata* plants treated with (*Trichoderma* + P+++) at high PAR ($1500 - 2500\mu\text{molm}^{-2}\text{s}^{-1}$). The lowest (E) value where observed in *V. unguiculata* plants treated (*Trichoderma* + P+++) at high PAR ($1500 - 2500\mu\text{molm}^{-2}\text{s}^{-1}$). The highest (WUE) value where observed in *V. unguiculata* plants treated with *Trichoderma* + P+++ at high PAR ($1500 - 2500\mu\text{molm}^{-2}\text{s}^{-1}$). the ratio of PSII photochemistry maximum quantum efficiency to quantum yield showed significant declines along with progressing phenological stages . The highest value was observed in *V. unguiculata* plants treated with (*Trichoderma* + P+++) at high PAR ($1500 - 2500\mu\text{molm}^{-2}\text{s}^{-1}$).

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