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Multifarious plant growth promotion by an entomopathogenic fungus *Lecanicillium psalliotae*

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ABSTRACT

An entomopathogenic fungus, *Lecanicillium psalliotae* strain IISR-EPF-02 previously found infectious to cardamom thrips, *Sciothrips cardamomi* promoted plant growth in cardamom, *Elettaria cardamomum*. The isolate exhibited direct plant growth promoting traits by production of indole-3-acetic acid and ammonia and by solubilizing inorganic phosphate and zinc. It also showed indirect plant growth promoting traits by producing siderophores and cell wall-degrading enzymes like, α-amylases, cellulases and proteases. In pot culture experiments, application of the fungus at the root zone of cardamom seedlings significantly increased shoot and root length, shoot and root biomass, number of secondary roots and leaves and leaf chlorophyll content compared to untreated plants. This is the first report on the plant growth promoting traits of this fungus. The entomopathogenic and multifarious growth promoting traits of *L. psalliotae* strain IISR-EPF-02 suggest that it has great potential for exploitation in sustainable agriculture.

1. Introduction

Biological control potential coupled with plant growth promoting traits of a microbe provides an alternative to synthetic pesticides and chemical fertilizers and such beneficial microbes are ideal candidates in sustainable agricultural production (Palaniyandi et al., 2013). The plant growth promoting traits of a microbe may be exhibited directly or indirectly by multiple mechanisms. In direct method, the microbe synthesizes compounds such as phytohormones, fixes atmospheric nitrogen and mobilizes minerals like phosphorus required for plant growth and development. Whereas, in indirect method, the microbe enhances plant growth by suppressing the adverse effects of phytopathogens through the production of antibiotics, siderophores, low molecular weight metabolites such as hydrogen cyanide, enzymes, depriving phytopathogens nutrients and niches, and also lowering the production of stress ethylene (Kloepper et al., 1980; Patten and Glick, 1996; Ahemad and Kibret, 2014). These microorganisms may exist as endophyte asymptomatically within the living plant tissues (Petrini, 1991) or in the plant rhizosphere mutualistically interacting with the root system (Amprayn et al., 2012). Much of the work so far carried out on beneficial aspects of microbes has been focused on bacteria (Vessey, 2003) and mycorrhizal fungi (Johansson et al., 2004). Fungi have been demonstrated to be superior to bacteria in acid tolerance (Chuang et al., 2007) and mobilizing certain bound phosphates (Wahid and Mehana, 2000). Many fungi have been reported to produce siderophore (Milagres et al., 1999), plant growth hormones such as indole-3-acetic acid (IAA) (Maor et al., 2004) and gibberellins (Tudzynski and Sharon, 2002). Besides mycorrhizial fungi, there are other groups of fungi present in the rhizosphere including non-plant pathogenic (Hermosa et al., 2012) and entomopathogenic fungi (Vega et al., 2009) having positive influence on plant growth promotion. However, entomopathogenic fungi have been largely overlooked for their plant growth promoting traits. More than 700 fungal species belonging to around 90 genera are identified as insect pathogens (Vega et al., 2009) and most of the research on these fungi is centered on their taxonomy and phylogeny, mode of action and their development as biocontrol agents (Lacey et al., 2015). Among the entomopathgenic fungi, *Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin, *Isaria* spp., and *Lecanicillium* spp. have been developed into biopesticide formulations for use against insect pests (Faria and Wraight, 2007). Some of these entomopathogenic fungi also successfully colonize the plants endophytically (Vega et al., 2009; Lopez and Sword, 2015). However, the additional roles of entomopathogenic fungi as beneficial rhizosphere colonizers, antagonists against plant pathogens and plant growth promoters have been largely not understood (Vega et al., 2009). For example, *B. bassiana* induced growth increase in tomato and cotton are assigned to its antagonistic effect to the fungal plant pathogen, *Rhizoctonia solani* Kühn., or induced systemic resistance in the host plants (Ownley et al., 2008; Griffin et al., 2005). Similarly the growth promotion in corn by *M. anisopliae* is considered through control of wireworms (Kabaluk and Ericsson,

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2007). So far, to our knowledge direct plant growth promotion traits of an entomopathgenic fungus has not been reported.

During field surveys for entomopathogens of spice crop pests in Kerala, India we have isolated an entomopathogenic fungal strain from cadavers of cardamom thrips, *Sciothrips cardamomi* Ramk. and identified as *Lecanicillium psalliotae* (Treschew) Zare & W. Gams (Ascomycota: Hypocreales), and proved its biocontrol potential in the laboratory (Senthil Kumar et al., 2015). In this study, we have evaluated this fungal strain for plant growth promoting traits such as production of IAA, ammonia, its ability to solubilize inorganic phosphate and ZnO, siderophore and cell wall degrading enzymes production. We also evaluated the influence of this fungus on the growth of cardamom seedlings. Knowledge of these properties of the fungus could help us in its promotion as a plant growth promoter in addition to its biocontrol potential against cardamom thrips, a serious insect pest of cardamom (*Elettaria cardamomum* (L.) Maton), an important spice crop in India and Sri Lanka (Gopakumar and Chandrasekar, 2002). This is the first ever report of an entomopathogenic fungus influencing plant growth promotion through multifarious traits.

2. Materials and methods

2.1. Fungus

The fungal strain was isolated from cadavers of cardamom thrips during a survey in Wayanad District of Kerala, India in 2012 (Senthil Kumar et al., 2015). Single spore culture of the fungus available in the Entomopathogenic Fungal Repository of Indian Institute of Spices Research (IISR) with accession number IISR-EPF-02 was used for the study. The sequence data of the conserved regions (partial internal transcribed spacer) (ITS), beta tubulin (TUB) and translation elongation factor (TEF) of this fungus are available in NCBI database with accession numbers KF358373–KF358375 (Senthil Kumar et al., 2015).

2.2. In vitro plant growth promoting traits of Lecanicillium psalliotae strain IISR-EPF-02

The fungus was evaluated for production of various direct and indirect plant growth promoting traits, including IAA, siderophore, ammonia, α-amylase, protease and also for its ability to solubilize inorganic phosphate and zinc oxide (ZnO). All the assays, otherwise mentioned were carried out in triplicate and the experiments repeated three times.

2.2.1. Indole-3-acetic acid production

The production of IAA by the fungus was quantitatively estimated following the protocol of Patten and Glick (1996) with slight modifications. 20 mL of Potato Dextrose Broth (PDB) amended with 0.1% L-tryptophan (w/v) was inoculated with an agar plug (6 mm) obtained from 15 days old culture of the fungus grown on potato dextrose agar (PDA) medium and incubated for 72 h at 26 ± 1 °C. Five mL of the culture filtrate was centrifuged at 10,000*g* for 5 min and 1 mL of the clear supernatant taken into test tubes in triplicate was allowed to react with 2 mL of Salkowski reagent (Gordon and Weber, 1951) in the dark for 30 min. PDB without L-tryptophan was used as a control. Development of pink red color in the medium indicated IAA production by the fungus. The IAA was quantified by reading the color intensity at 530 nm in a UV–vis spectrophotometer (Shimadzu) and the amount of IAA released was calculated from a standard graph prepared using known quantities of pure IAA.

2.2.2. Phosphate and zinc oxide solubilization

Phosphate solubilizing capability of the fungus was tested using Pikovskaya's agar medium (Zaidi et al., 2006). Zn solubilization efficiency of the fungus was determined in mineral salts agar medium (Rokhbakhsh-Zamin et al., 2011) amended with 0.1% of insoluble ZnO. Agar plugs (6 mm) obtained from 15 days old culture of the fungus was placed onto the middle of the plate and incubated at 26 ± 2° C. Formation of zone of clearance around the fungal colony post 96 h indicated solubilization of P. The Zn solubilization efficiency was measured 15 days after inoculation (DAI) by measuring the clearing zone around the fungal colony. The phosphate and ZnO solubilization efficiency (SE) was calculated and expressed as a ratio between the diameter of the halo zone and the diameter of the fungal colony.

2.2.3. Ammonia (NH_3) production

An agar plug (6 mm) obtained from 15 days old culture of the fungus grown on potato dextrose agar (PDA) medium was inoculated into 5 mL yeast peptone dextrose (YPD) broth and incubated on a rotary shaker at 26 ± 2 °C for 5 d. The ability of the fungus to produce NH_3 was detected by adding Nessler's reagent. The development of a yellow-to-brown colour indicated a positive result for ammonia production (Cappuccino and Sherman, 1992).

2.2.4. Siderophore production

Agar plugs (6 mm) obtained from 15 days old culture of the fungus grown on potato dextrose agar (PDA) medium was inoculated onto chrome azurol S (CAS) agar (Schwyn and Neilands, 1987). The cultures were incubated at 26 ± 2 °C up to 15 days. Development of an orange zone surrounding the fungal colony was considered as siderophore production by the fungus and expressed as activity units (AU). An AU is defined as the diameter of the orange zone divided by the diameter of the fungal colony.

2.2.5. Production of cell wall-degrading enzymes

The ability of the fungus to produce cell wall degrading enzymes like proteases and α-amylases was determined by inoculating agar plug (6 mm) of the fungus grown on PDA onto the surface of skimmed milk agar (Cattelan et al., 1999) and starch agar (Dinesh et al., 2015) plates as done for yeast and bacteria. The presence of a clear zone around the fungal colony indicated the ability of the fungus to produce these enzymes. Cellulolytic activity of the fungus was determined by inoculating the mycelial disc (6 mm) on the surface of quarter strength Saboraud's dextrose agar with yeast (SDAY/4) extract amended with 1% carboxymethyl cellulose (CMC) and incubated at 26 ± 2 °C. At 10 DAI, the medium was flooded with 0.01% congo red solution for 15 min and the plates were destained using 1% NaCl solution for 5 min. A clear zone against the red background indicated the cellulase production ability (Dinesh et al., 2015) of the fungus. The enzyme production is expressed as AU and an AU of production is defined as the diameter of the entire clearing zone divided by the diameter of the fungal colony.

2.3. Evaluation of Lecanicillium psalliotae strain IISR-EPF-02 for plant growth promotion in cardamom seedlings

Three leaf stage cardamom seedlings (variety: Njallani Green Gold) were utilized for the study. The seedlings were raised following the package of practices for cardamom of ICAR-IISR (Ankegowda et al., 2015) with modifications. Briefly, the seeds were extracted by gently pressing the capsules and then demucilaged by washing four times in tap water. The seeds were subjected to acid scarification by treating them with nitric acid to increase the germination percentage. For this, the extracted seeds were wrapped in a loosely tied nylon net and immersed in 25% nitric acid for 10 min. After treatment, the seeds were removed and washed repeatedly four times in sterile distilled water to remove traces of acid. The seeds (50 seeds/tray) after surface-disinfection with sodium hypochlorite (0.1% v/v) for 10 min and subsequent washing in sterile distilled water four times were sown in four round plastic trays (25 cm) half filled with sterilized potting mixture (autoclaved twice for 45 min) containing equal volumes of soil, farm yard manure (FYM) and composted coir pith in the ratio of 1: 1: 1 v/v. The experimental setup was maintained in a ventilated greenhouse under

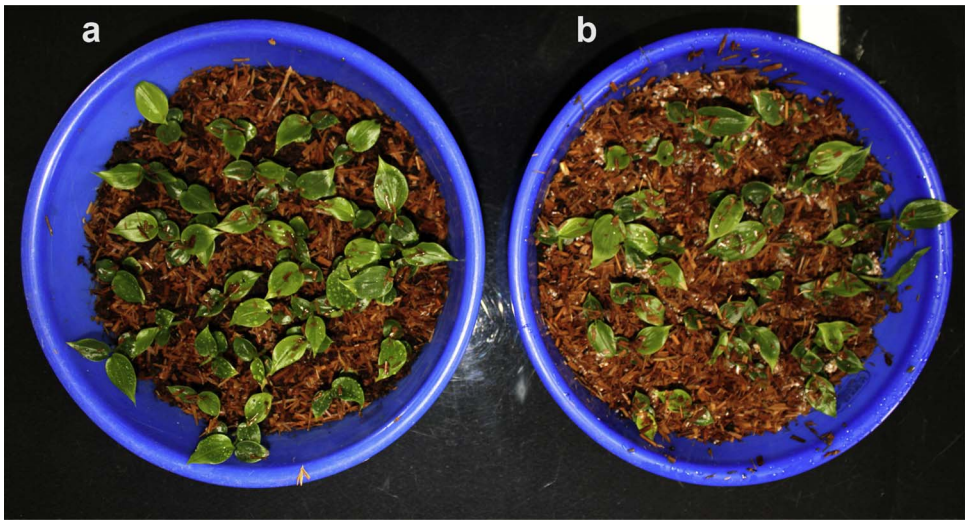

Fig. 1. Experimental setup for assessing the effect of *Lecanicillium psalliotae* strain IISR-EPF-02 on the growth and development of cardamom seedlings (a) 3 leaf stage seedlings planted in plastic trays treated with broken paddy seeds and mulched with paddy straw (control) (b) 3 leaf stage seedlings treated with *Lecanicillium psalliotae* strain IISR-EPF-02 grown on broken paddy seeds and mulched with paddy straw (treatment).

ambient natural conditions (28–32 ± 2 °C, 70–80% RH and natural photophase). No other fertilizer inputs were given except for spraying with sterile distilled water till the end of the experiment.

On germination, ~30 healthy, equal sized seedlings were transplanted in three plastic trays each for the experiment and control containing medium as described above providing equal spacing between the seedlings. After attaining 3 leaf stage, the plants in experimental trays received a uniform broadcast of 25 g of *L. psalliotae* strain IISR-EPF-02 grown on broken paddy seeds (10^8 cfu/g) over the potting mixture and the control trays were similarly treated with broken paddy seeds without the fungus. A thin layer of dried, chopped and sterilized paddy straw was spread between the plants on the surface of the potting mixture to maintain the moisture and to avoid exposure of the fungus (Fig. 1). All the trays were maintained under net house conditions under ambient natural conditions (28–32 ± 2 °C, 70–80% RH and natural photophase). Observations on plant growth parameters like shoot length, number of leaves, width and length of the terminal leaf, root length, dry weight of shoot and root biomass were recorded 40 days after fungal treatment on 20 plants randomly selected from each tray.

2.4. Chlorophyll content

One hundred mg tissue from the terminal opened tender leaf of the fungus treated and control plants were separately cut into small pieces and treated with 7 mL of dimethyl sulphoxide (DMSO) in test tubes at 65° C for 3 h. The volume was made up to 10 mL with DMSO and the absorbance of the clear extract was measured using UV–vis Spectrophotometer (Shimadzu) at 645 and 663 nm using pure DMSO as blank (Sharma et al., 2003). The estimations were carried out in triplicate and repeated three times with samples collected from three individual plants. Total chlorophyll, chlorophyll a and chlorphyll b were expressed as mg/g of fresh leaf tissue.

2.5. Statistical analysis

All data are expressed as mean ± standard error (SE). Significance among treatments was determined using Student *t*-tests and analysis of variance at 0.05% probability level.

3. Results

3.1. In vitro plant growth promoting traits of Lecanicillium psalliotae strain IISR-EPF-02

The fungus was able to produce IAA in the medium supplemented with 0.1% L-tryptophan as indicated by the change in color of the medium to pink red by the addition of Salkowski reagent. The IAA in the medium reached a concentration of 6.9 ± 0.01 µg/mL, 3 DAI (Table 1).

The ability of the fungus to solubilize insoluble phosphate was proved by the formation of clear halo zone around the fungal colony in Pikovskaya's agar medium 96 h post inoculation (Fig. 2a). The fungus was able to solubilize Zn as indicated by the formation of halo zone 15 DAI (Fig. 2b). The solubilizing efficiency (SE) of the fungus was strong for both the minerals as revealed by high SE values of 1.3 ± 0.05 and 2.0 ± 0.02 for phosphate and ZnO respectively (Table 1). The fungus showed positive results for NH_3 and siderophore production, as indicated by the development of brown colour in the YPD medium by adding Nessler's reagent and change in color of the CAS-blue medium from blue to orange around the fungal colony respectively (Fig. 2c, d). The siderophore producing capacity of the fungus was determined as 1.3 ± 0.03 AU (Table 1). Extracellular protease activity of *L. psalliotae* strain IISR-EPF-02 determined by casein degradation indicated clearing zones on skim milk agar (Fig. 2e) with an AU of 1.7 ± 0.02 (Table 1). The ability of the fungus to produce α-amylase was evident by the production of clearing zone on starch media (Fig. 2f) with an AU of 1.2 ± 0.03 (Table 1). Cellulase production by the fungus was revealed

Table 1
Plant growth promoting (PGP) traits of *Lecanicillium psalliotae*.

PGP traits	Response units[a]
Direct PGP traits	
Indole acetic acid production (µg/mL)	6.9 ± 0.01
Phosphate solubilization (SE)	1.3 ± 0.05
Zinc oxide solubilization (SE)	2.0 ± 0.02
Ammonia production	+
Indirect PGP traits	
Siderophore production (AU)	1.3 ± 0.03
Protease activity (AU)	1.7 ± 0.02
α-Amylase activity (AU)	1.2 ± 0.03
Cellulase activity (AU)	2.1 ± 0.30

Solubilization efficiency (SE) = Halo zone ÷ Diameter of colonies; Activity unit (AU) = Diameter of reaction zone ÷ Diameter of colony.
'+' = positive result.
[a] Values are means of three replicates ± standard error (SE).

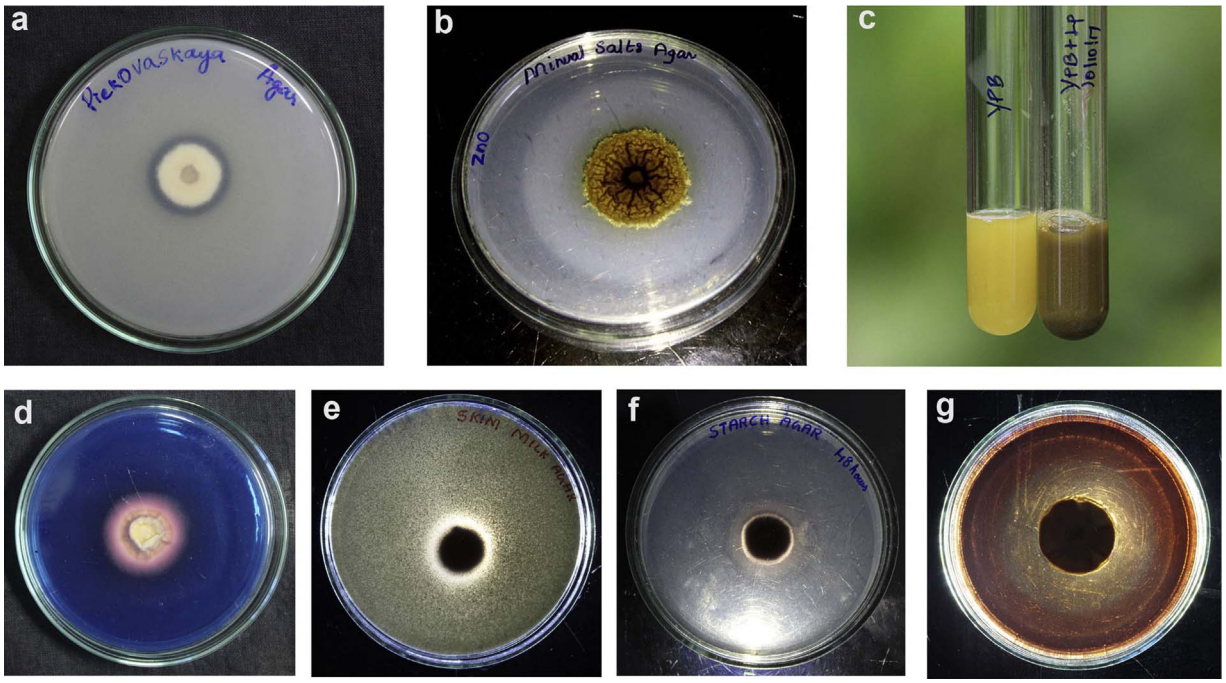

Fig. 2. *In vitro* plant growth promoting traits of *Lecanicillium psalliotae* strain IISR-EPF-02 revealed by (a) phosphate-solubilizing activity on Pikovskaya's agar medium 96 h post inoculation by production of clear zone (b) Zinc solubilizing activity by formation of clearing zone on mineral salts agar medium amended with 0.1% of insoluble ZnO 15 days after inoculation (DAI) (c) production of NH_3 in YPD broth (d) Siderophore production on CAS-blue agar by development of orange colour around the fungal colony 15 DAI (e) protease activity by formation of clear zone on skimmed milk agar 48 h post inoculation (f) α-amylase activity by formation of clear zone on starch agar 48 h post inoculation (g) cellulase activity by formation of clearing zone on the surface of quarter strength sabouraud's dextrose agar with yeast (SDAY/4) extract amended with 1% carboxymethyl cellulose (CMC) 10 DAI stained with 0.01% Congo red solution and de-stained using 1% NaCl solution. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by the formation of clear zone after staining (Fig. 2g) as determined by measuring the carboxymethylcellulase (CMCase) activity with an AU of 2.1 ± 0.3 (Table 1).

3.2. Plant growth promotion and chlorophyll content in cardamom seedlings

Application of *L. psalliotae* strain IISR-EPF-02 significantly influenced the growth of cardamom plants as evidenced by different growth parameters (Fig. 3). The average shoot, root length, terminal leaf length and width in treated plants was 9.6 ± 0.27, 10.9 ± 0.48, 8.4 ± 0.29 and 2.8 ± 0.09 cm, respectively, whereas it was 5.8 ± 0.20, 5.1 ± 0.26, 5.9 ± 0.20 and 2.1 ± 0.08 respectively for untreated plants 40 DAI. The average number of leaves and roots in treated plants (6.1 ± 0.12, 6.6 ± 0.17) was also more when compared to untreated plants (4.9 ± 0.16, 3.6 ± 0.21) (Fig. 4). The fungal application significantly increased shoot and root biomass of cardamom plants (average: 132.8 ± 8.48, 26.8 ± 2.44) when compared to untreated plants (average: 66.4 ± 3.89, 10.3 ± 0.90) (Fig. 5).The treated plants showed a significant increase in chlorophyll a, b, and total chlorophyll when compared to untreated plants 40 DAI. The mean chlorophyll a, b

Fig. 3. Comparison of growth of cardamom seedlings in untreated control and plants applied with *Lecanicillium psalliotae* strain IISR-EPF-02 (40 DAI).

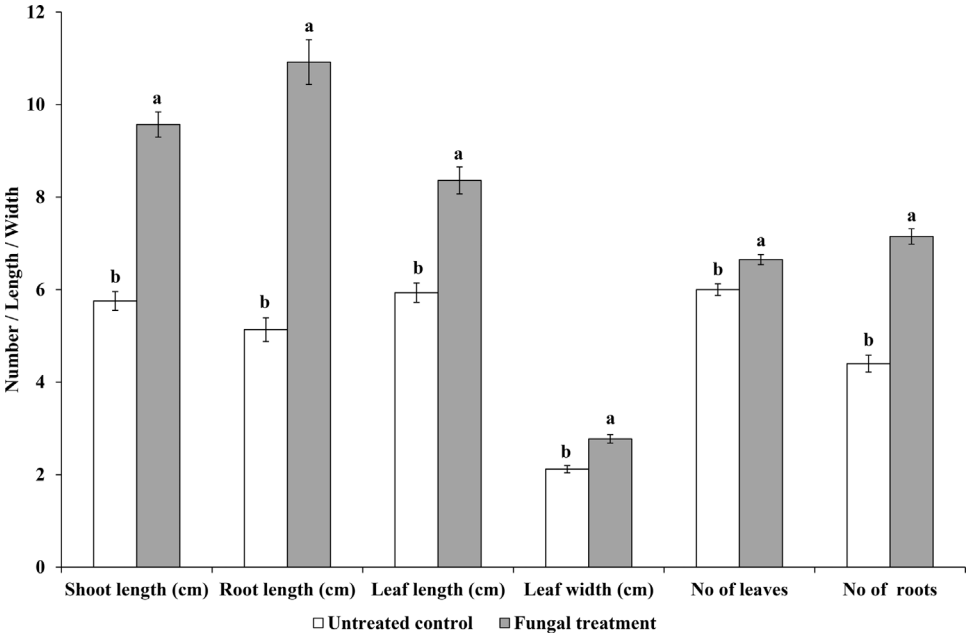

Fig. 4. Effect of *Lecanicillium psalliotae* strain IISR-EPF-02 treatment on shoot and root length, length and width of leaves and number of leaves and roots of cardamom seedlings 40 DAI. Error bar represents mean of 20 seedlings. Bars represented by different letters are significantly different by Student *t*-test ($P < 0.05$).

and total chlorophyll content in treated plants was 1.3 ± 0.09, 0.4 ± 0.03 and 1.7 ± 0.12 mg/g of leaf tissue, respectively whereas it was 0.7 ± 0.09, 0.22 ± 0.02 and 0.9 ± 0.11 mg/g of leaf tissue, respectively in untreated plants (Fig. 6).

4. Discussion

One of the foremost important growth promoting traits of plant growth promoting (PGP) microorganisms is production of IAA and such organisms are receiving attention as good sources of biofertilizers (Sasikala and Ramana, 1998) because IAA-producing microorganisms are believed to enhance root growth and increase the root length, resulting in an increased root surface area, thus enabling increased access to soil-based nutrients (Fu et al., 2016). In our study, the fungus was able to synthesize IAA in the medium, which is comparable to many reported plant growth promoting yeasts (Nutaratat et al., 2014; Fu et al., 2016) demonstrating its capability to promote root and shoot growth in plants. Earlier, it was reported that isolates of *Pochonia chlamydosporia,* which produced IAA in medium, in turn promoted growth and development of tomato seedlings (Zavala-Gonzalez et al., 2015).

Our studies established the ability of *L. psalliotae* strain IISR-EPF-02 to liberate phosphate and Zn from their fixed forms for direct utilization by plants. Phosphorus is a key element for plants because it is the least available of all essential nutrients in soil and is vital for plant growth and productivity (Fu et al., 2016). The release of insoluble and fixed forms of phosphorus is an important aspect of PGP microorganisms increasing soil phosphorus availability (Ahemad and Kibret, 2014). *L. psalliotae* strain IISR-EPF-02 was able to solubilize mineral phosphate, with good solubilizing efficiency, a commonly found trait in plant growth promoting microorganisms (Vassilev et al., 2006) indicating its ability to increase plant growth as proved in *Metarhizium robertsii* J.F. Bisch., Rehner & Humber (O'Brien, 2009) and *P. chlamydosporia* (Zavala-Gonzalez et al., 2015). Zinc, though required in extremely

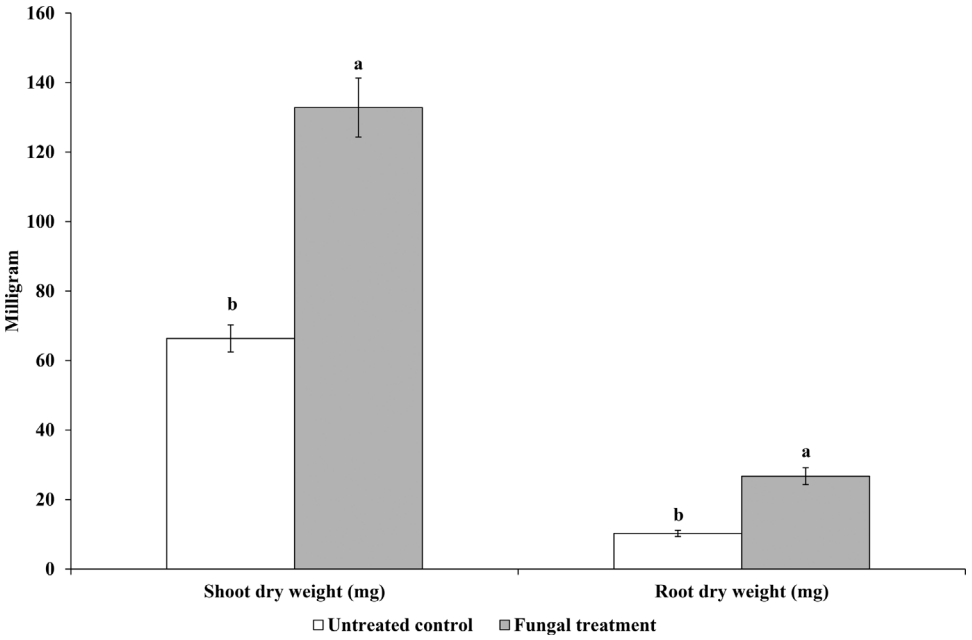

Fig. 5. Effect of *Lecanicillium psalliotae* strain IISR-EPF-02 treatment on shoot and root dry weight of cardamom seedlings 40 DAI. Error bar represents mean of 20 seedlings. Bars represented by different letters are significantly different by Student *t*-test ($P < 0.05$).

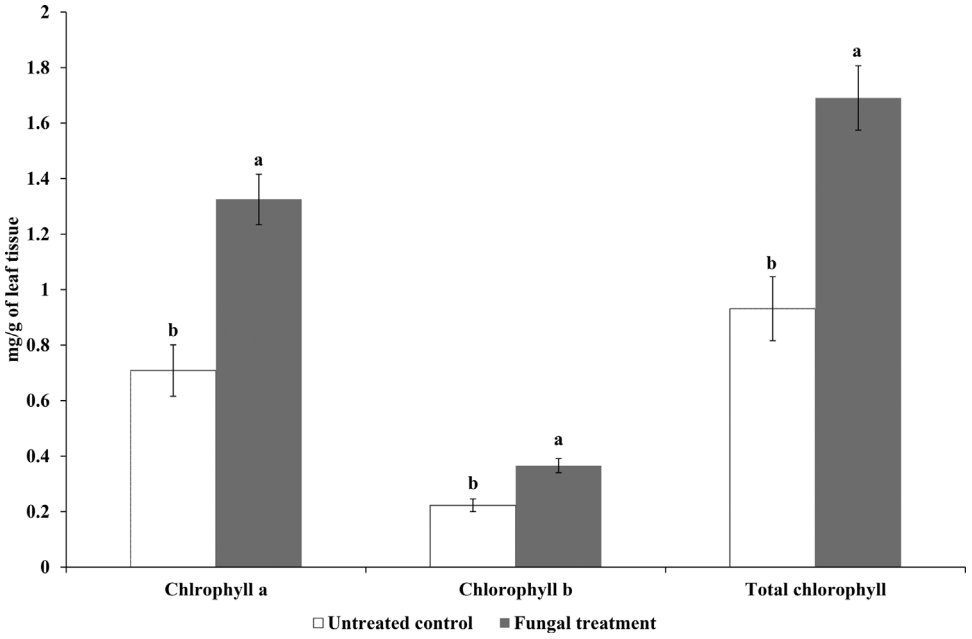

Fig. 6. Effect of *Lecanicillium psalliotae* strain IISR-EPF-02 treatment on chlorophyll a, b and total chlorophyll content of cardamom seedlings 40 DAI. Error bar represents mean of three plants. Bars represented by different letters are significantly different by Student *t*-test ($P < 0.05$).

small amounts for plants, is a critical component of various enzymes responsible for driving several plant metabolic reactions. PGP microorganisms solubilize zinc salts, thus providing the micronutrient zinc to the plant (Fu et al., 2016). An entomopathogenic fungus with Zinc solubilization efficiency has not so far been reported. *Lecanicillium psalliotae* IISR-EPF-02 is an unique entomopathogen with both P and Zn solubilization abilities.

Ammonia (NH_3) production by plant associated microorganisms is advantageous to plants because NH_3 provides available nitrogen that supports plant growth (Nutaratat et al., 2014). Among all essential nutrients, nitrogen is the most essential nutrient required in high amounts and is most frequently the limiting factor in crop yield. Thus, microorganisms possessing nitrogen fixing or NH_3 releasing ability are beneficial to plants (Fu et al., 2016). Plant growth promoting bacteria (Ahemad and Kibret, 2014) and several strains of yeast have been demonstrated as producers of NH_3 (Nutaratat et al., 2014). There are no reports of NH_3 production by entomopathogenic fungi. Our studies revealed that *L. psalliotae* strain IISR-EPF-02 possesses the potential to produce NH_3 indicating its secondary role as a biofertilizer in the ecosystem.

Siderophores are vital for promoting plant growth and suppressing the effect of plant pathogens because of their iron-transporting abilities (Kloepper et al., 1980). PGP microorganisms support plant growth directly by producing siderophores that trap iron. Further, these microorganisms indirectly promote plant growth by negatively affecting the growth of plant pathogenic fungi by depriving them of iron (Calvente et al., 1999). Many strains of fungi and bacteria produce siderophores (Milagres et al., 1999) and their production influence the uptake of various metals such as Zn and Cu by plants (Carrillo-Castaneda et al., 2005; Gururani et al., 2013). Siderophore production by entomopathogenic fungi is not so far reported. Our studies revealed the siderophore producing ability of *L. psalliotae* strain IISR-EPF-02 with strong SE value comparable to that produced by isolates of plant growth promoting yeasts (Fu et al., 2016) supporting its ability to produce antimicrobial compounds (Nagaoka et al., 2004) and thereby enhancing plant growth.

The production of nematode cuticle degrading serine proteases (Yang et al., 2005) and chitinase (Gan et al., 2007) by *L. psalliotae* has been reported earlier. In the present investigation, we have proved the production of cellulases and proteases by *L. psalliotae* strain IISR-EPF-02 and the quantity produced are comparable to that of many yeast strains (Mangunwardoyo et al., 2011; Fu et al., 2016). The fungus also tested positive to α-amylase production. These hydrolytic enzymes play important roles in the degradation of cell walls of oomycetes such as *Phytophthora* spp. and *Pythium* spp. (Lima et al., 1998) and may indirectly promote plant growth.

In the present study, there was significant increase in plant growth parameters in seedlings applied with *L. psalliotae* strain IISR-EPF-02. There were 66% and 112% increase in shoot and root length, 40% and 31% increase in leaf length and width, and 11% and 63% increase in number of leaves and roots, respectively, in plants treated with the fungus, when compared to untreated plants. Our results also demonstrated that there was increase in shoot and root biomass of fungus treated seedlings by 100% and 160%, respectively, when compared to untreated plants providing proof for direct plant growth promotion by the fungus. A similar increase in plant growth parameters in crops such as barley, wheat (Monfort et al., 2005) and tomato (Zavala-Gonzalez et al., 2015) were reported for *P. chlamydosporia* isolates, which could be due to the production of plant hormones or due to the nutrient solulbilizing efficiency of the fungus (Zavala-Gonzalez et al., 2015). Plant growth promotion by other entomopathogenic fungi such as *B. bassiana* and *Purpureocillium lilacinum* (Thom) Luangsa-ard, Hou- braken, Hywel-Jones & Samson has been assigned to their negative effect on insect pests (Lopez and Sword, 2015). *Metarhizium* strains are reported to produce auxins (Liao et al., 2014) and *M. robertsii* could solubilize phosphate (O'Brien, 2009). In the present study, since the plants were free from biotic and abiotic stresses, rather than the hydrolytic enzymes produced by the fungus, other factors such as production of IAA, siderophore and ammonia and mineral solubilization could be considered as directly enhancing growth in cardamom seedlings.

Lecanicillium psalliotae strain IISR-EPF-02 treated plants significantly enhanced total chlorophyll content of leaves by 82% (chlorophyll a by 87% and chlorophyll *b* by 64%) over untreated control plants. Chlorophyll biosynthesis has been considered as an indicator of net physiologically available iron to the plant (Lopez-Millan et al., 2001). Higher absorption of iron is correlated with higher contents of chlorophyll a, chlorophyll *b* and total chlorophyll (Katyal and Sharma, 1980). The increase in chlorophyll content could be due to the utilization of microbial siderophore by the plants. Siderophore production by growth promoting microbes increases the soil availability of iron to the plants (Sharma et al., 2003). Iron deficiency results in chlorosis and 30% of the crops suffer from this disorder worldwide (Imsande, 1998).

Hence this strain of fungus will be of interest to combat iron deficiency in plants grown in problematic soils and can increase crop yields.

5. Conclusion

Traditionally entomopathogenic fungi have been regarded exclusively as pathogens of arthropods. The mechanisms underlying plant growth enhancement by entomopathogenic fungi is poorly understood. We report for the first time the existence of both direct and indirect plant growth promoting traits by an entomopathogenic fungus. The plant growth promoting properties of this fungus are insect-independent and the mechanisms are multifarious like that of plant growth promoting rhizobacteria and hence hold promise as a biofertilizer. Further studies are aimed to establish this fungus as an endophyte in cardamom plants and other crops to induce systemic resistance in plants and also for improving crop yield.

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