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# Beneficial microbes promote plant growth and induce systemic resistance in sunflower against downy mildew disease caused by *Plasmopara halstedii*

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**Abstract**

Seven plant growth promoting fungi (PGPF) native to the rhizosphere soil were screened for their potential to promote growth and to induce resistance in sunflower against *Plasmopara halstedii,* an incitant of sunflower downy mildew disease. Effect of seed priming with each of these seven PGPF isolates on seed quality parameters and resistance induction were studied under *in vitro* and green house conditions. Root colonization ability of these PGPF was also studied under green house conditions. The results showed that seed priming with conidial suspension of PGPF at 1 x $10^8$ cfu $ml^{-1}$ significantly increased seed germination and seedling vigor compared to non primed control. Plants raised with primed seeds demonstrated a significant reduction in downy mildew disease severity and provided a maximum of 61% protection under green house conditions when compared to the untreated control. The experimental results proved that the tested PGPF, promoted growth and induced systemic resistance (ISR) in sunflower plants against the downy mildew disease caused by *P. halstedii,* due to their growth promoting and biocontrol abilities. Present study has revealed the fact that there is a strong correlation between root colonization and resistance induction in PGPF treated plants.

**Keywords:** PGPF; Rhizosphere; Sunflower; *Plasmopara halstedii*; Downy mildew; Seed priming; 1x$10^8$ cfu/ml; Root colonization; ISR.

## INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the third most important edible oil seed crop in the world. In India, sunflower is cultivated over an area of 2.4 million hectares with a production of 1.44 million tonnes and productivity of 608 kg/ha [1]. Sunflower is susceptible to many fungal, bacterial and viral diseases. Among the fungal diseases, downy mildew of sunflower caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni, is the most destructive one and its occurrence is worldwide [2]. Downy mildew of sunflower in India was first reported from Marathwada region of Maharashtra around 1986 with yield losses upto 80% [3].The disease incidence was also reported from adjoining major sunflower growing states like Andhra Pradesh and Karnataka [4]. Severe outbreaks of sunflower downy mildew with approximately 85% yield loss have also been reported from Turkey [5].

Although the use of downy mildew resistant cultivars can provide greater degree of protection against the disease, emergence of new virulent races of the pathogen that overcome host cultivar's resistance is a continuing problem. The most effective means of sunflower downy mildew disease management is by the use of fungicides like mefenoxam, metalaxyl and oxadixyl which are used as seed treatment for more than two decades [2, 6]. Occurrence of *P. halstedii* tolerant to phenyl amide compounds has been reported from Hungary [7], Turkey [8], France [9], United States [10], Spain [11] and Germany [12] and its wide spread insensitivity to these chemical fungicides have prompted researchers to think of alternative ways to overcome the disease. In addition, due to the adverse effects of chemical fungicides on human and environmental health, their usage has been termed hazardous [13]. In this context, there is an intensive search for nature's friendly strategies which can reduce pathogen population and provide effective means of disease management and crop improvement.

Enhancing a plant's own defense mechanism against a broad spectrum of phytopathogens appears to be one of the most promising and eco-friendly strategies for plant disease management and crop improvement [14]. Plants can activate a very effective arsenal of inducible defense responses, comprised of genetically programmed suicide of infected cells (the hypersensitive response, HR), as well as tissue reinforcement and antibiotic production at the site of infection [15]. These local responses can in turn, trigger a long lasting systemic response (systemic acquired resistance, SAR) that primes the plant for resistance against a broad spectrum of pathogens [16, 17].

Rhizosphere is a zone of intense microbial activity [18] as

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organic nutrients of plant roots favor the growth of microbes [19]. A good number of rhizosphere microbes present in, on and around the root system may provide a variety of benefits to plants [20]. Microbial actions like mycoparasitism, Antibiosis, and competition, production of enzymes and phytohormones and activation of a plant's defense mechanism against phytopathogens are the diverse benefits provided to host plants by these beneficial microbes [21]. These rhizosphere resident beneficial microorganisms, generally termed as plant growth promoting bacteria (PGPR) and plant growth promoting fungi (PGPF) are being used as effective biocontrol agents against a wide range of phytopathogens [22-26]. PGPF primarily include species of *Penicillium, Trichoderma, Fusarium, Phoma, Pythium, Phythophthora,* etc. The underlying mechanisms of the disease suppression engendered by PGPF have also been intensively studied and induced systemic resistance (ISR) is thought to be one of those mechanisms involved in plant disease management [27-31].

Oomycete fungal diseases are commonly controlled by microorganisms belonging to the genera *Bacillus*, *Pseudomonas*, *Streptomyces* and fungi in the genera *Coniothyrium*, *Gliocladium* and *Trichoderma* [32]. Several microorganisms sush as *Bacillus cereus* and *B. subtilis, Enterobacter cloacae, Pseudomonas fluorescens* and *Trichoderma harzianum* have been used as biocontrol agents. *Trichoderma* spp. have been the most widely commercialized and efficacious inoculants used for the control of oomycete and other fungal diseases [33]. Sunflower downy mildew disease in recent times has been managed by the use of resistant breeds and cultural practices. An experimental result [34] reports, the enhanced expression of defense related enzymes against *P. halstedii* attack in sunflower treated with PGPR. Also there is a report on the activation of defense mechanism in sunflower against *P. halstedii* attack by an essential oil of a plant species *Bupluerum gibraltarium* [35].

Although significant success has been achieved with the use of PGPF as biocontrol agents, no reports of PGPF working against sunflower downy mildew disease are available. Accordingly, in this investigation we have chosen to isolate, identify and evaluate the effectiveness of rhizosphere PGPF, on growth promotion and resistance induction in sunflower against downy mildew disease caused by *P. halstedii.*

## MATERIAL AND METHOD

### Seed material

Sunflower seeds, highly susceptible (Morden) to downy mildew disease *Plasmopara halstedii* (Farl.) Berl. and de Toni were collected from Karnataka State Seeds Corporation Ltd; Bijapur, India, and were used throughout the study.

### Collection of sporangia and preparation of inoculum

*P. halstedii* was isolated from the heavily infested fields of sunflower downy mildew disease. Infected leaves of sunflower showing profuse sporulation of *P. halstedii* on their abaxial side were collected in the evening hours and washed thoroughly under running tap water to remove the existing sporangia. The leaves were then blot dried, cut into small pieces and placed in humidity chamber prepared by lining the interiors of Petri dishes 50 cm x 30 cm x 12 cm sizes with a wet double layer of blotting paper. These chambers were kept at 20° C and >95% RH in an incubator for overnight. After incubation, fresh sporangia produced on the leaves were harvested into Sterile distilled water (SDW); the spore load was adjusted to 4 x $10^4$ zoospores/ ml using Haemocytometer and used as a source of inoculum for greenhouse studies [36].

### Collection of rhizosphere soil samples, isolation, identification and maintenance of plant growth promoting fungi

Rhizosphere soil samples were collected from vegetable crop plants like Cabbage, Tomato, Brinjal, Chilli, Beans and Sunflower in sterile plastic covers, from different agro-climatic regions of Karnataka, Kerala, Tamilnadu, Andhra Pradesh and Goa States of India. Collected soil samples were brought to the laboratory and subjected to serial dilutions. An aliquot of 0.1 ml of $10^{-3}$ to $10^{-5}$ dilutions from each of the sample was spread uniformly over the potato dextrose agar (PDA) medium supplemented with chloramphenicol (100 mg $L^{-1}$) in Petri plates. Inoculated PDA plates were incubated at 25±2°C for 72 hours. After 72 hours of incubation, individual fungal colonies with different morphology were picked from the edge with a sterile fine tipped needle subcultured on to PDA plates supplemented with chloramphenicol and were incubated at 25±2°C for 7 days. After 7 days of incubation pure cultures of isolated rhizosphere fungi were enumerated and identified individually on the basis of microscopic (conidia, fruiting body, mycelia) and macroscopic (culture morphology, color and appearance) characteristics. All the fungal isolates obtained were labeled and maintained on slants and Petri plates of PDA media and used for further studies.

### Mass multiplication of Plant Growth Promoting Fungi

PGPF isolates were mass multiplied on PDA plates and incubated at 25±2° C under 12/12 h alternate cycles of near ultraviolet (NUV) light and darkness for 10-12 days. After incubation, an aliquot of 10 ml of SDW was added to each of the culture plates and gently shaken to dislodge conidia from the culture surface. Conidial suspension of each fungus was collected separately in 100 ml conical flask and passed through four layers of cheesecloth, centrifuged at 2500 rpm for 10 min and the pellet was resuspended in SDW. Conidial concentration was adjusted to 1 x $10^8$ cfu/ml and 2.1 × $10^8$ cfu/ml using Haemocytometer [37]. Conidial suspension of 100 ml containing 2.1 × $10^8$ cfu/ml was added to 1 kg of sterile talc mixture (1:10 v/w) and mixed thoroughly to prepare talc formulation of 2.1 × $10^7$ cfu/gm, packed in polythene bags and stored under ambient conditions of 25±2°C before being used for formulation treatments in the present study.

### Seed treatment with Plant Growth Promoting Fungi

Seeds of sunflower highly susceptible (cv. Morden) to downy mildew disease were surface sterilized with 0.02% sodium hypochlorite (NaOCl) by shaking for 3 min, followed by three washes with sterile distilled water. Sterilized seed samples were treated separately with, both conidial suspension (5 ml/ 400 seeds at 1 x $10^8$ cfu/ml) and talc formulations (8 g $kg^{-1}$ and 10 g/kg seeds at 2.1x$10^7$ cfu/g), of each of the PGPF . Treated seeds were kept at 25±2°C in a rotary shaker for 6 h to facilitate proper and uniform seed coating with the inducer. SDW treated seeds served as control. Each treatment consisted of four replicates of 100 seeds each and repeated twice.

**Effect of seed coating with PGPF on seed germination and seedling vigor under *In vitro* conditions**

PGPF treated and control seeds (four replicates of 100 seeds each) were plated equidistantly on three layers of moistened blotter discs placed in Perspex plates to evaluate percent germination and another set of treated seeds were subjected to between paper method to record seedling vigor [38]. The experiment consisted of four replications of 100 seeds each (50 seeds each in eight towels). The paper towels were rolled with polythene wrapping to prevent drying of the towels, and incubated in an incubation chamber at 25 ± 2° C for 7 days. After seven days of incubation, percent germination, root length and shoot length were recorded and vigor index was calculated as follows.

Vigor index = Seed Germination (%) × [Mean Root Length + Mean Shoot Length]

**Effect of seed treatment with PGPF on downy mildew disease incidence upon challenge inoculation with *Plasmopara halstedii* under Green house conditions**

Sunflower seeds treated with different rhizosphere fungal isolates namely PGPFYCMTv; PGPFYCMTh; PGPFYCMAf; PGPFYCMPo; PGPFYCMPc; PGPFYCMFs and PGPFYCMPh were sown separately in earthen pots (9 inch diameter) containing 2:1:1 red soil, sand and farmyard manure (FYM). Pots used for sowing were autoclaved at 121° C for 1 h on the previous day and were arranged in a randomized complete block design and maintained under greenhouse conditions (25±2° C, 95% relative humidity). Four-day-old-seedlings were whorl inoculated with *P. halstedii* zoospore suspension ($4x10^4$ zoospores $ml^{-1}$) early in the morning for three consecutive days. In the whorl inoculation method, droplets of *P. halstedii* zoospores were dropped onto the cotyledonary leaves formed in the emerging seedlings and allowed to flow down to the base. Distilled water treated seeds served as control [36]. Each treatment consisted of 50 plants in four replications and repeated twice. Plants were observed daily and the disease progression was recorded. Plants were rated diseased when they showed any one of the typical symptoms of downy mildew, i.e. chlorosis, sporulation on the abaxial surface of the leaf and stunted growth. Disease incidence was recorded after 30 days after sowing (DAS) and per cent downy mildew disease protection was calculated using the formula;

Per cent protection=

$$\frac{\text{Percent downy mildew in control plants - Percent downy mildew in treated plants}}{\text{Percent downy mildew in control plants}} \times 100$$

***In-planta* colonization**

PGPF isolates namely PGPFYCMTv; PGPFYCMTh; PGPFYCMAf; PGPFYCMPo; PGPFYCMPc; PGPFYCMFs and PGPFYCMPh were treated to susceptible sunflower seeds and sown in earthen pots as mentioned above and maintained under greenhouse conditions. Colonization frequency of each of the PGPF was recorded with the presence or absence of the fungus in the rhizosphere region of the treated plant. 15 day-old seedlings of sunflower were uprooted and root segments up to 6 cm measured from the radical were collected separately for each treatment and were cut into 3 pieces (2 cm length) and named as upper, middle and lower regions. One gram soil adhered to each corresponding root region of every treatment was also collected. The collected soil samples were subjected to serial dilutions ($10^{-3}$ to $10^{-5}$), from $10^{-4}$ dilution 0.1 ml aliquot was inoculated onto PDA plates. Each root bit was washed with running tap water and rinsed in 0.02% sodium hypochlorite for 1 min followed by 3 washes in SDW aseptically and placed on PDA agar plates. All PDA plates were incubated for 7 days at 23±2 °C and colonization frequency of each PGPF isolate was assessed from each of the root bit and corresponding soil sample [31]. The experiment consisted of 100 root bits for each fungus/ treatment and repeated twice.

**Statistical analysis**

*In vitro* experiment consisted of four replications of 100 seeds each (400 seeds / treatment). Greenhouse experiments were also conducted in four replicates of 10 pots in each replication, with 10 plants in each pot and were arranged in a randomized complete block design and were analyzed separately. Each experimental data was subjected to analysis of variance (ANOVA) using SPSS Inc. 16.0. Significant effects of treatments were determined by the magnitude of the F value ($P \leq 0.05$). Treatment means were separated by Tukey's HSD test.

## RESULTS

**Isolation and identification of rhizosphere fungi**

A total of fifty nine fungal isolates belonging to the genera *Trichoderma*, *Aspergillus*, *Penicillium*, *Phoma* and *Fusarium* were isolated from the rhizosphere soils collected from different agro-climatic regions of Karnataka, Kerala, Tamilnadu, Andhra Pradesh and Goa States, India, as mentioned above (Table 1).

**Effect of seed treatment with PGPF on seed germination and seedling vigor**

Among fifty nine rhizosphere fungal isolates screened, only seven isolates belonged to the genera *Trichoderma, Aspergillus, Penicillium, Phoma* and *Fusarium* revealed significantly ($P \leq 0.05$) enhanced seed germination and seedling vigor to varying degrees (Table 2). Among the PGPF treatments, PGPFYCMTh recorded highest seed germination when treated with conidial suspension ($1\times10^8$ cfu $ml^{-1}$) of 92% and 1871 seedling vigor, followed by PGPFYCMPo and PGPFYCMTv which recorded 90% and 88% seed germination and 1826 and 1787 seedling vigor respectively. The PGPF formulation treatments (8 g $kg^{-1}$ and 10 g $kg^{-1}$) enhanced seed germination and seedling vigor at varied degrees when compared to control but were not up to the extent of conidial suspension treatments. The distilled water treated control seeds recorded 76% seed germination and 1205 seedling vigor (Table 2).

***In-planta* colonization**

*In-planta* colonization of the treated PGPF were noticed in the tested plant roots both in serial dilution and in root segments placed on PDA plates. The colonization of PGPF both in rhizosphere soil and root segments were observed in the upper as well as in middle roots segments. While least or no colonization of the PGPF was observed in lower root segments (Table 3). The untreated root

segments didn't show any colonization of the PGPF.

**Greenhouse conditions**

**Effect of seed treatment with PGPF on downy mildew disease incidence upon inoculation with *P. halstedii***

PGPF treated plants showed significant ($P \leq 0.05$) resistance to downy mildew disease of sunflower caused by *P. halstedii*, but the degree of disease resistance varied according to the type and nature of treatments. Among the PGPF tested PGPFYCMTh recorded a maximum protection of 63% when the susceptible sunflower seeds were treated with conidial suspension at $1x10^8$ cfu/ml followed by PGPFYCMPo and PGPFYCMTv which showed 60% and 55% respectively (Table 4). The untreated control plants recorded 95% disease incidence.

Table 1. List of fungal isolates of rhizosphere soil samples

| Fungi/ Place | Andhra Pradesh | Goa | Karnataka | Kerala | Tamilnadu | Total |
|---|---|---|---|---|---|---|
| *Trichoderma* sp. | 02 | 01 | 03 | 01 | 02 | 09 |
| *Aspergillus* sp. | 04 | 03 | 04 | 03 | 04 | 18 |
| *Penicillium* sp. | 03 | 03 | 04 | 03 | 04 | 17 |
| *Fusarium* sp. | 03 | 01 | 02 | 02 | 03 | 11 |
| Phoma sp. | 01 | - | 02 | - | 01 | 04 |
| Total | 11 | 08 | 17 | 10 | 13 | 59 |

Table 2. Effect of seed treatment with PGPF isolates on seed germination and seedling vigor of sunflower.

| Treatment | Concentration | Seed germination (%) ± SE | Seedling vigour ± SE |
|---|---|---|---|
| PGPFYCMTv | 1 x $10^8$ cfu/ml | 88±0.6$^{b}$ | 1787±10.8$^{ab}$ |
| | 8 g $kg^{-1}$ | 82±0.4$^{c}$ | 1688±6.4$^{bc}$ |
| | 10 g $kg^{-1}$ | 80±0.5$^{cd}$ | 1642±8.2$^{c}$ |
| PGPFYCMTh | 1 x $10^8$ cfu/ml | 92±0.7$^{a}$ | 1871±6.7$^{a}$ |
| | 8 g $kg^{-1}$ | 88±0.4$^{b}$ | 1727±6.1$^{b}$ |
| | 10 g $kg^{-1}$ | 88±0.7$^{b}$ | 1695±3.8$^{bc}$ |
| PGPFYCMAf | 1 x $10^8$ cfu/ml | 83±0.2$^{c}$ | 1632±4.2$^{c}$ |
| | 8 g $kg^{-1}$ | 76±0.5$^{ef}$ | 1590±9.8$^{d}$ |
| | 10 g $kg^{-1}$ | 76±0.8$^{ef}$ | 1523±5.7$^{de}$ |
| PGPFYCMPo | 1 x $10^8$ cfu/ml | 90±0.2$^{ab}$ | 1826±5.6$^{a}$ |
| | 8 g $kg^{-1}$ | 85±0.4$^{c}$ | 1674±6.9$^{bc}$ |
| | 10 g $kg^{-1}$ | 87±0.5$^{b}$ | 1734±11.0$^{b}$ |
| PGPFYCMPc | 1 x $10^8$ cfu/ml | 85±0.9$^{c}$ | 1696±7.2$^{bc}$ |
| | 8 g $kg^{-1}$ | 78±0.4$^{e}$ | 1588±8.8$^{d}$ |
| | 10 g $kg^{-1}$ | 76±0.5$^{ef}$ | 1524±10.2$^{de}$ |
| PGPFYCMPh | 1 x $10^8$ cfu/ml | 78±0.5$^{e}$ | 1590±5.9$^{d}$ |
| | 8 g $kg^{-1}$ | 72±0.6$^{g}$ | 1480±7.5$^{f}$ |
| | 10 g $kg^{-1}$ | 74±0.2$^{f}$ | 1525±8.2$^{de}$ |
| PGPFYCMFs | 1 x $10^8$ cfu/ml | 88±0.6$^{b}$ | 1726±4.7$^{b}$ |
| | 8 g $kg^{-1}$ | 80±0.5$^{cd}$ | 1658±9.5$^{bc}$ |
| | 10 g $kg^{-1}$ | 80±0.8$^{cd}$ | 1688±7.8$^{bc}$ |
| Control | Distilled water | 76±0.2$^{ef}$ | 1205±11.7$^{g}$ |

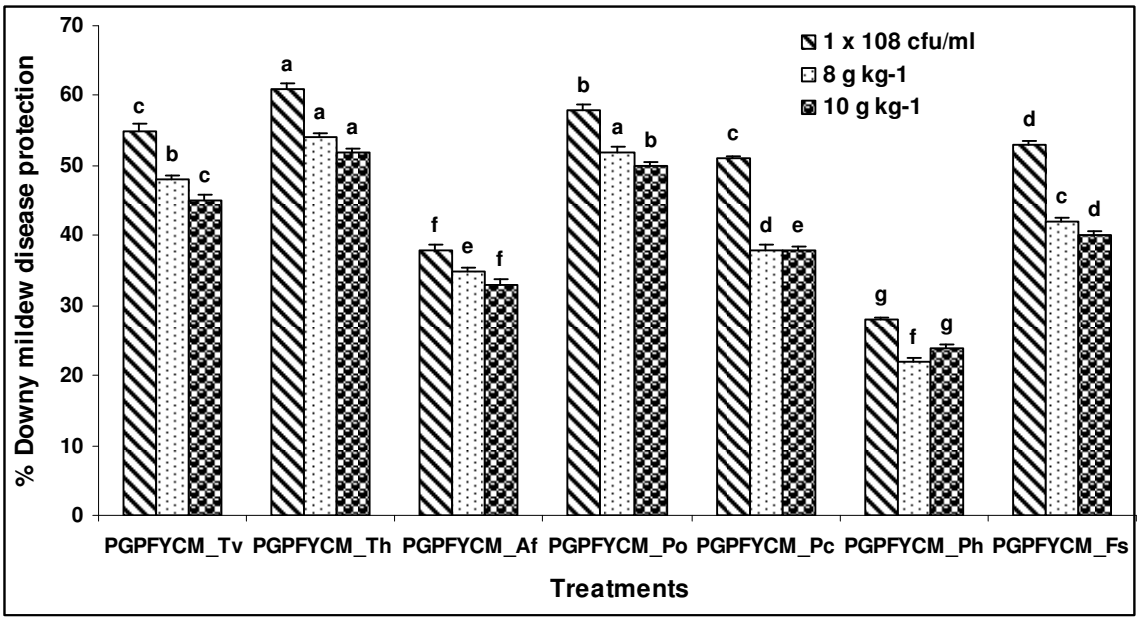
[a] Values are means of four independent replicates with ± SE.

[b] Means followed by the same superscript (s) within the same column are not significantly different according to Tukey's HSD at $P \leq 0.05$.

Table 3. *In-planta* colonization of PGPF treated (1x108 cfu/ ml) sunflower seedlings

| Treatment | Root segments | | | Rhizosphere soil | | |
|---|---|---|---|---|---|---|
| | Upper | Middle | Lower | Upper | Middle | Lower |
| PGPFYCMTv | ++ | ++ | ++ | ++ | ++ | ++ |
| PGPFYCMTh | ++ | ++ | ++ | ++ | ++ | ++ |
| PGPFYCMAf | ++ | ++ | -- | ++ | ++ | -- |
| PGPFYCMPo | ++ | ++ | ++ | ++ | ++ | ++ |
| PGPFYCMPc | ++ | -- | -- | ++ | -- | -- |
| PGPFYCMPh | ++ | ++ | -- | ++ | ++ | -- |
| PGPFYCMFs | ++ | -- | -- | ++ | ++ | -- |
| Control (SDW) | -- | -- | -- | -- | -- | - |

++: Present; -- : Absent.




[a] Values are means of four independent replicates with ± SE.
[b] Means followed by the same superscript (s) within the same column are not significantly different according to Tukey's HSD at $P \leq 0.05$.

Fig 1. Effect of seed treatment with PGPF isolates on disease resistance

## DISCUSSION

Biological control is considered as an alternative or supplemental method for reducing the use of chemicals in agriculture [39]. PGPF is reported to increase plant growth through nutrient mineralisation and suppression of deleterious micro-organisms. Moreover, PGPF is also reported to stimulate plant growth through interaction with hormone synthesis or transport in plants [30]. The present study reveals the dual role of PGPF in inducing resistance against downy mildew disease and enhancement of plant growth in sunflower plants. Among the fungi isolated from different agro-climatic conditions only seven PGPF isolates significantly enhanced seed quality parameters and induced resistance against downy mildew disease of sunflower.

The seed quality parameter studies revealed that out of 59 non-pathogenic fungal isolates, only seven fungal isolates enhanced seed germination and seedling vigour significantly ($P \leq 0.05$), when compared to untreated control. Among the seven PGPF isolates, highest seed germination (92%) and seedling vigor (1871) was observed with PGPFYCMTh treated (1 x $10^8$ cfu/ml) sunflower seeds when compared to other treatments and control. Similar reports on seed treatment with some *Trichoderma* spp. have been shown to increase seed germination, plant length and weight of sunflower [40]. There are also reports on some rhizosphere competent *Trichoderma* strains where they have shown direct effects on plants by increasing seed germination, nutrient uptake, plant growth and stimulation of plant defenses against biotic and abiotic stresses [41]. Treatment of tomato seeds with *T. harzianum* accelerates seed germination and increases seedling vigor [42], while [43] reported that tomato seeds treated with *T. harzianum* treated tomato seedlings did not affect seed germination but significantly increased shoot height, shoot diameter, shoot fresh and dry weight and root fresh and dry weight of tomato seedlings. There are also reports on enhancement of root growth and development, crop productivity, resistance to abiotic stresses and nutrient uptake with root colonization by *Trichoderma* spp. [44].

Root colonization by PGPF is considered as one of the most important characteristic features as it helps them to interact with plants to enhance growth and protection [45]. All the tested PGPF isolates established varied levels of root colonization with respect to different root segments of sunflower seedlings. A significant correlations between root colonization and protection and the prolonged presence of PGPF GS8-3 in soil and particularly on/in roots might have triggered the plants to produce defense alarms continuously thus inducing a continuous protection [27].

All the tested PGPF treatments enhanced disease resistance induction in sunflower against downy mildew disease but their ability to initiate resistance varied with the treatment. Sunflower seeds treated with conidial suspension of PGPF significantly induced resistance in sunflower plants against challenge-inoculation with *P. halstedii*. The result was an evident of the act of systemic expression of enhanced defense mechanism against the pathogen invasion. Disease resistance capacity expressed by above ground parts in response to seed treatment against the invading pathogen was attributed due to root colonization effect of PGPF isolates. Disease suppression effect was systemic, as inducer (PGPF) treated seeds expressed resistance in above ground parts inoculated with the pathogen, thereby separating the two spatially. Among the treatments, conidial suspension offered a better control when compared to the formulation treatments. The present study has revealed that PGPFYCMTh treated seeds (1x$10^8$ cfu/ml) showed maximum disease protection of 61% followed by 58% in PGPFYCMPo with same concentration. The plant growth promoting activities and disease resistance by PGPF have also been reported in many crops [25, 31, 46]. Earlier reports have shown that *Trichoderma* isolates, known to act directly on pathogens as biocontrol agents and have been found capable of inducing systemic resistance [47]. These observations, corroborates with that of [30] wherein cucumber plants were protected from anthracnose and damping off diseases by induction with PGPF isolates.

In the present study, PGPF isolates showed enhanced seed germination and seedling vigor and induced systemic resistance in sunflower against downy mildew disease. The study will help to improve knowledge on these PGPF to use them as plant growth promoters as well as biocontrol agents. Further, a detailed investigation is under way to understand the exact mechanism of growth promotion and systemic resistance in sunflower using plant growth promoting isolates against downy mildew disease of sunflower.

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