

# ***Piriformospora indica* Enhances Germination, Growth and Yield in Bitter Gourd (*Momordica charantia* L.): A Sustainable Approach**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author DRC carried out the experiments and wrote the manuscript. Author JMJ conceived and supervised the experiments, and wrote the manuscript. Authors RNS, HG, SKB, SS and RNV provided critical comments. All authors read and approved the final manuscript.*

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## ABSTRACT

*Piriformospora indica*, a beneficial fungal root endophyte, has shown promising effects on growth promotion in addition to biotic and abiotic stress tolerance in several crops. The present study investigates the influence of *P. indica* on germination of seeds, seedling vigour, growth and yield in bitter gourd plants. Bitter gourd seeds and seedlings were subjected to colonization by *P. indica*, and grown in controlled and insect proof conditions, and different biometric and yield parameters were compared with the non-colonized control plants. In the presence of *P. indica*, the days taken for seed germination have been reduced to 3.6 days in contrast to 10.8 days in the control. It was also found that the seeds raised in *P. indica*-multiplied medium produced more vigorous seedlings of seedling vigour index 3061.81 compared to 1840.38 in the control seedlings at 15 days after germination (DAG). The endophytic colonisation of the fungus in bitter gourd plants promoted shoot and root growth, with enhanced shoot and root biomass of 67.66 and 95.46 per cent respectively over control plants. The fungus also modified root architecture with more secondary and tertiary roots. The colonised plants produced more leaves with higher leaf area (218.50 leaves with average leaf area of 94.19 cm<sup>2</sup> at 60 DAG) compared to non-colonised plants (156.50 leaves with average area of 57.01 cm<sup>2</sup> at 60 DAG). *P. indica* colonisation enhanced the yield in bitter gourd by 30.30 per cent. *P. indica*-colonised plants produced 23.10 fruits weighing 445.20 g fruit<sup>-1</sup> having 44.10 seeds fruit<sup>-1</sup>, whereas the control plants produced only 16.10 fruits weighing 286.50 g fruit<sup>-1</sup> with 30.5 seeds fruit<sup>-1</sup>. These findings suggest that, *P. indica* is a promising growth promoting endophyte that can positively influence seed germination, growth, development and yield of bitter gourd plants. Hence, *P. indica* can be used in bitter gourd production system as a sustainable agricultural practice.

**Keywords:** *Piriformospora indica*; root endophyte; bitter gourd; biometric parameters; growth promotion; fruit parameters.

## 1. INTRODUCTION

*Piriformospora indica* is a beneficial root endophytic fungus belonging to the order Sebaciales of Basidiomycota phylum. The fungus was discovered in the Indian Thar Desert, and has significant attention due to its unique ability to establish symbiotic relationships with a wide variety of agriculturally important crop plants. Unlike mycorrhizal fungi, *P. indica* exhibits a broad host range, making it a versatile and valuable organism in the study of plant-microbe interactions [1]. One of the remarkable features of *P. indica* is its capability to promote plant growth, enhance nutrient uptake, and improve stress tolerance [2,3,4,5,6,7,8].

Bitter gourd (*Momordica charantia*) is a vegetable crop with significant medicinal properties and nutritional value. Cultivation of bitter gourd faces challenges due to abiotic and biotic stresses, leading to significant yield loss and reduced crop quality. To address the challenges, there is a growing interest in exploring sustainable and eco-friendly approaches to enhance crop productivity and stress tolerance. One promising approach involves harnessing the symbiotic relationship between plants and beneficial endophytic

microorganisms, such as root endophytic fungi. Among these fungi, *P. indica* has emerged as promising due to its ability to colonize plant roots and promote growth under various environmental conditions [9, 10]. *P. indica* is known to establish mutualistic associations with a wide range of host plants, leading to improvements in nutrient uptake and overall plant performance [11,12, 13]. Bitter gourd is a valuable vegetable cum cash crop in Kerala, contributing to the livelihood of farmers. Improving its growth and yield can lead to higher incomes for farmers and boost the agricultural economy.

In recent years, several studies have investigated the potential of *P. indica* to enhance the growth and physiological responses of various crop species. However, there is limited information regarding its effect on bitter gourd, particularly on biometric parameters such as plant height, leaf area, leaf number and root length. Understanding the impact of *P. indica* colonization on these biometric parameters in bitter gourd could provide valuable insights into its potential as a bioinoculant for improving bitter gourd cultivation practices. Therefore, the present study aims to evaluate the influence of *P. indica* on germination, seedling vigour, and growth and fruit parameters in bitter gourd

especially in a popular variety of the state, Preethi.

## 2. MATERIALS AND METHODS

**Maintenance of the fungal root endophyte, *P. indica*:** *P. indica* culture was maintained in potato dextrose agar (PDA) medium, multiplied in potato dextrose broth (PDB) and mass multiplied in coir pith-dried cow dung mixture amended with 2 per cent gram flour. Five mm discs from actively growing culture of *P. indica* was sub-cultured in PDA plates and incubated for three weeks at room temperature. The culture discs from these plates were transferred into 100 ml PDB prepared in 250 ml conical flask (4 discs per flask) and kept in orbital shaker at 40 rpm for two weeks at room temperature. This broth served as the source of *P. indica* for mass multiplication.

**Mass multiplication of *P. indica* in potting mixture:** *P. indica* was mass-multiplied in coir pith-cow dung mixture. The mixture contained coir pith, finely powdered and dried farmyard manure (1:1), and gram flour 2 per cent. The mixture was prepared following the protocol of Joji et al. [14]. Coir-pith blocks were soaked in water overnight and drained on the subsequent day. The soaking and draining was followed 2 to 3 times and shade-dried. To 1 kg of partially dried coir-pith, 1 kg of dried and powdered farm yard manure and 20 g of gram flour were added; and thoroughly mixed and moistened to field capacity. This mixture was packed in poly propylene covers and autoclaved for three consecutive days at 121°C, 15 psi for two hours each.

For mass multiplication of *P. indica*, plastic trays (42 cm x 30 cm x 9 cm) were taken and surface sterilised with 70 per cent ethanol. The autoclaved coir pith mixture (1 kg) was set in the tray as a layer. Two-week-old *P. indica* broth culture was poured into the 1 kg mix in tray and thoroughly mixed (1 % w/w) with the mixture. The moisture was maintained to field capacity, if necessary, using sterile water. The inoculated mix was spread evenly in the tray as a layer (3-5 cm thick). The trays were then covered using sterile cling films and incubated at room temperature. The same mixture without *P. indica* was used as potting mixture for growing the control plants.

**Co-cultivation of *P. indica* with bitter gourd:** Once the mycelial run was complete (1 week), the mix was used for filling pro-trays. The mix

without *P. indica* was used for filling control pro-trays. Kerala Agricultural University released bitter gourd variety, Preethi, was used in this study. The seeds were surface-sterilized in 1 per cent sodium hypochlorite solution for 1 min followed by washing thrice in sterile water for 5 min each and soaked in sterile water over night. The seeds were sown in trays filled with *P. indica*-multiplied and control potting mixtures. The pro-trays were kept in insect proof chamber under temperature and humidity-controlled conditions for uniform germination and growth.

**Germination of bitter gourd seeds in *P. indica* mass multiplied medium:** The experiment was conducted with four treatments and five replications in completely randomized design. Bitter gourd seeds were soaked in sterile water and in filter sterilised 1:10 solution of 150 ppm potassium nitrate for 3 h. These seeds were sown separately in *P. indica*-multiplied potting mixtures and potting mixtures without *P. indica*, and kept under controlled and insect proof conditions. In this study, days taken for seed germination in each treatment were recorded. At 15 days after sowing, seedling vigour was calculated in terms of Seedling Vigour Index using the following formula [15].

$$\text{Seedling Vigor Index (VI)} = (\text{Root length} + \text{shoot length}) \times \text{Germination percent}$$

***P. indica* on growth and yield parameters of bitter gourd plants:** The seedlings were transferred from pro-trays to grow bags filled with sterilised soil on 30 DAG for recording different growth parameters viz., shoot length, shoot weight, root length, root weight, number of secondary roots, number of tertiary roots, number of leaves and leaf area at 5, 15, 30, and 60 days after germination (DAG) in *P. indica*-colonized and control plants. The yield parameters viz., number of fruits per plant, average weight per fruit, average length of fruit, average girth of fruit, number of seeds per fruits and 100 seed weight were also recorded in both the treatments. Fifteen plants were maintained for each treatment. The statistical design followed for the experiment was paired t-test.

**Statistical analysis:** The data were analysed using paired t-test for all experiments and ANOVA for seed germination test. Statistical significance between the treatments was compared by least significant difference (LSD) test at  $p < 0.05$  probability level on different plant

growth promoting parameters. All the statistical analyses were performed using GRAPES 1.0.0, developed in Kerala Agricultural University [16].

### 3. RESULTS AND DISCUSSION

***P. indica* enhanced seed germination and seedling vigour of bitter gourd:** *P. indica* in potting medium induced early germination of bitter gourd seeds (Plate 1). Bitter gourd seeds treated with 150 ppm KNO<sub>3</sub> required 6.60 days to germinate compared to 10.80 days for the control (Table 1). Surprisingly, seeds soaked in water and sown in *P. indica*-multiplied medium took only 3.60 days to germinate. Similarly, a comparable result was obtained with seeds treated with KNO<sub>3</sub> grown in *P. indica*-multiplied medium (3.20 days). The above results indicated that the presence of *P. indica* significantly promoted the seed germination compared to the control.

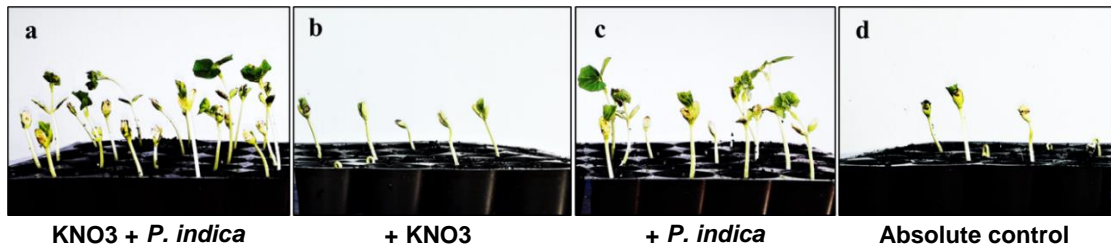
Seedling vigour index (SVI) was also enhanced in the colonised plants compared to other treatments (Fig. 1). Seeds soaked in KNO<sub>3</sub> as well as in plain water, and germinated in *P.*

*indica*-multiplied potting medium produced seedlings with comparable SVI of 3025.54 and 3061.81 respectively. KNO<sub>3</sub> treated seeds produced less vigorous seedlings (SVI 2191.36) compared to *P. indica*-colonised plants; but better seedlings compared to the control (SVI 1840.38) (Table 1). Hence, seeds sown in *P. indica*-multiplied medium, whether soaked in KNO<sub>3</sub> or in water, germinated faster and showed enhanced seedling vigour index.

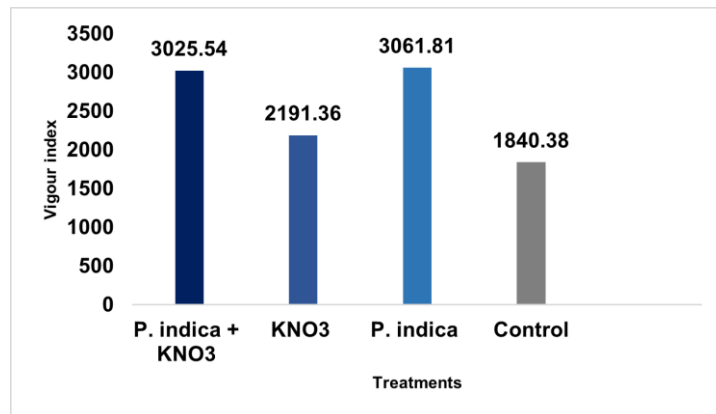
**Table 1. Effect of *P. indica* root colonization on germination of bitter gourd seeds**

Treatments	Days taken for germination*
<i>P. indica</i> + KNO <sub>3</sub>	3.20 <sup>a</sup> ± 0.402
KNO <sub>3</sub>	6.60 <sup>c</sup> ± 0.492
<i>P. indica</i>	3.60 <sup>b</sup> ± 0.492
Control	10.80 <sup>d</sup> ± 1.172
LSD (0.05)	0.198

\* Values are mean of observations taken from 100 plants/seeds sown ± SD; # Observation taken at 15 days after sowing. Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance.



**Plate 1. Effect of *P. indica* on germination of bitter gourd seeds at 10 days after sowing (DAS). (a) Seeds were soaked in KNO<sub>3</sub> and sown in *P. indica*-multiplied potting mix, (b) soaked in KNO<sub>3</sub> and sown in potting mix without *P. indica*, (c) soaked in water and sown in *P. indica*-multiplied potting mix, (d) soaked in water and sown in potting mix without *P. indica***



**Fig. 1. Effect of *P. indica* root colonization on seedling vigour of bitter gourd (at 15 DAS)**

Varma et al. [17,18] reported that *P. indica* and its culture filtrate could enhance seed germination in crops like rice, maize, *Brassica* sp., sunflower and common beans. Sam [19] studied the effect of *P. indica* on germination in tomato var. Vellayani Vijay and found that the seeds in *P. indica*-multiplied medium germinated on 3<sup>rd</sup> day whereas the seeds in control medium germinated on 5<sup>th</sup> day.

*P. indica* improves water use efficiency [9] as well as nutrient acquisition [20,21] in the colonized plants, which can reduce the time required for seeds to germinate. Moreover, *P. indica* is also known to induce systemic changes in the plant's hormonal balance, particularly increasing auxin production, which could be another probable reason for earlier germination [21, 22, 23]. *P. indica* promotes faster and more uniform seed germination by lowering ABA levels and boosting growth-promoting hormones [24, 25]. *P. indica* also produces various bioactive compounds (enzymes or signaling molecules) that can break seed dormancy or promote faster germination [2, 3].

***P. indica* promoted shoot and root growth in bitter gourd plants:** *P. indica* colonisation in bitter gourd plants displayed promising results in vegetative growth of plants. All biometric parameters were enhanced by *P. indica* colonization (Plate 2). The positive effect of *P. indica* on shoot length was noticeable even at 5 DAG. The shoot length of *P. indica*-colonised plant was 8.93 cm compared to 7.02 cm in control plants at 5 DAG. The shoot lengths in *P. indica*-colonised plants increased further to 20.88, 129.58 and 326.90 cm at 15, 30 and 60 DAG respectively which accounted for 23.40, 47.25 and 51.19 per cent increase over control plants (Table 2). A similar increase was observed in case of shoot weight. The shoot biomass was found to be higher for *P. indica*-colonised plants compared to the control plants. The shoot weights in *P. indica*-colonised plants were 1.92, 7.42, 42.30 and 177.50 cm compared to 1.40, 3.84, 29.30 and 90.60 cm in control plants at 5, 15, 30 and 60 DAG respectively. A substantial increase of 37.14, 93.23, 44.37 and 95.92 per cent were observed in the *P. indica*-colonised plants at 5, 15, 30, and 60 DAG respectively (Table 2). In nutshell, significant increase in shoot growth was observed in the *P. indica*-colonised plants throughout the crop period with an average increase of 37.26 per cent in shoot length and 67.66 per cent increase in shoot biomass compared to that of the control plants.

*P. indica*-colonisation in bitter gourd also resulted drastic changes in root length and biomass as well as its architecture (Plate 2). The root length was 6.99, 10.11, 15.86 and 22.65 cm in the fungus colonised plants compared to 4.10, 7.95, 12.05 and 15.50 cm in control plants with the per cent increase of 70.49, 27.17, 31.62 and 46.13 at 5, 15, 30 and 60 DAG respectively. Similarly, root weight in the *P. indica*-colonised plants were 0.28, 0.59, 3.89 and 6.29 g with an increase of 100, 47.50, 150.97 and 83.38 per cent compared to the control plants at 5, 15, 30 and 60 DAG respectively. The above results reinstate that *P. indica* improved root and shoot growth in bitter gourd seedlings resulting the increased root and shoot biomass (Table 2).

Rai et al. [26] demonstrated that *P. indica* colonization significantly enhances nutrient uptake and water use efficiency, leading to greater biomass accumulation in plants. *P. indica* also influences auxins, gibberellin, and cytokinins biosynthesis which are crucial for promoting cell division, elongation, and differentiation. These hormones are directly involved in root and shoot growth, leading to increased biomass [21, 27, 28]. Li et al. [29] have demonstrated that *P. indica* treatment leads to enhanced photosynthetic efficiency and, consequently, increased biomass production in plants.

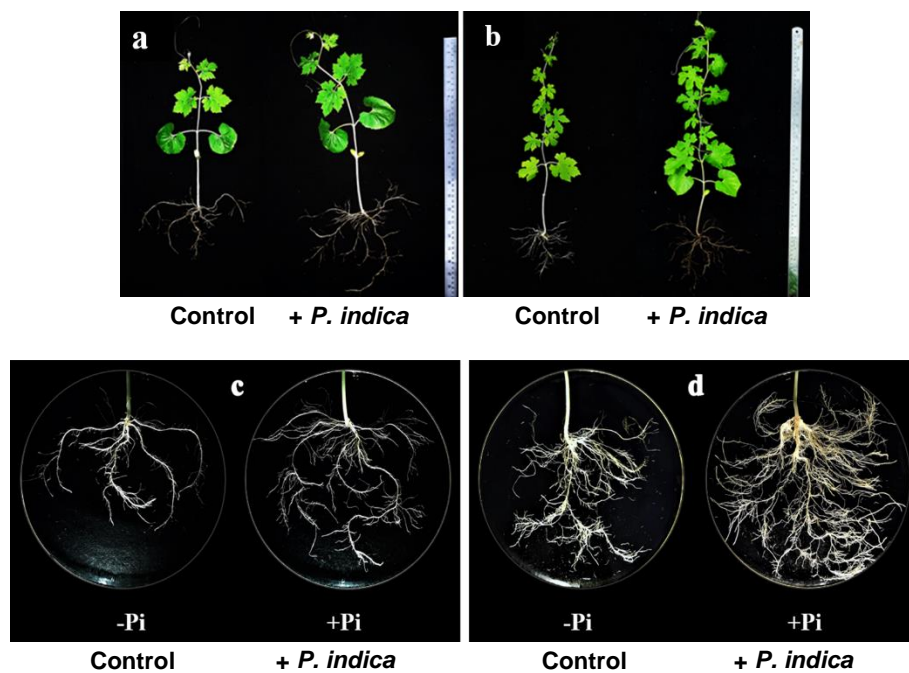
***P. indica* modified root architecture of bitter gourd plants:** *P. indica*-colonised plants showed increased number of secondary and tertiary roots compared to control plants (Plate 2). In *P. indica*-colonised plants, secondary roots were observed to be 7.10 at 5 DAG compared to 5.40 in control. At 15 DAG a prominent increase was noticed with 16.00 roots in the *P. indica*-colonised plants compared to 8.60 in control plants. Number of secondary roots at 30 and 60 DAG were 85.70 and 92.30 in the *P. indica*- colonised plants and 54.90 and 66.10 for control plants. Tertiary roots were observed only by 10 DAG in both treatments. Surprisingly, there was tremendous increase in number of tertiary roots in the *P. indica*-colonised plants. At 15 DAG, 55.40 tertiary roots were observed in the colonised plants compared to 16.2 in control plants. Moreover, 232.40 and 269.60 tertiary roots were found in the fungus colonized plants against 89.0 and 119.20 roots in control plants at 30 and 60 DAG respectively. There was up to 241.98 per cent increase in tertiary root formation in the *P. indica*-colonised plants. *P. indica*-colonised plants; thus, displayed an average increase of 53.31 per cent

in secondary and 176.42 per cent in tertiary roots over control in bitter gourd root system (Table 2).

Sam [16] reported an increase in plant height by 24 per cent, shoot biomass by 216 per cent, root biomass by 139 per cent, number of secondary and tertiary roots by 73 and 98 per cent in *P. indica*-colonised tomato plants, compared to control plants at 30 DAS. Comparable results like increased shoot length, leaf area, and root biomass were also reported in *P. indica*-colonized pepper, tomato and cucumber plants by Kim et al. [30], Verma et al. [31] and Yadav et al. [32] respectively.

Increased phosphorus availability can stimulate the growth of secondary and tertiary roots, and enhancing the root architecture. *P. indica* colonization enhances nutrient uptake, particularly phosphorus, which contributes to more extensive root system development [26, 33]. Likewise, *P. indica* also increases auxin levels in colonized plants, which directly promotes secondary and tertiary root formation in plants [22, 29]. Zhang et al. [34] revealed that *P. indica* enhances the expression of genes involved in strigolactone biosynthesis in rice, leading to increased lateral root formation and root hair density.

***P. indica*-colonised plants produced more leaves with higher leaf area:** *P. indica* significantly enhanced leaf number and leaf area in bitter gourd plants (Table 2). The colonised plants produced 4.20 leaves at 5 DAG; whereas control plants had only 3.60 leaves. Significant difference in leaf numbers were noticed from 15 DAG. In the colonised plants, 10.20, 40.20 and 218.50 leaves were recorded at 15, 30 and 60 DAG respectively, over 7.60, 28.20 and 156.50 leaves in control plants. Maximum per cent difference in leaf number over control was observed at 60 DAG (39.62). On an average, there was an increase of 33.26 per cent in leaf number in the colonised plants compared to that of the control. *P. indica* not only enhanced the number of the leaves but also the leaf area. Notable difference in leaf area was recorded in the colonised plants compared to control plants. The leaf area in the colonised plants was recorded as 36.00, 50.25, 58.11 and 94.19 cm<sup>2</sup> at 5, 15, 30 and 60 DAG respectively. On contrary, the leaf area of control plants was recorded as 24.00, 40.19, 40.33 and 57.01 cm<sup>2</sup> at 5, 15, 30 and 60 DAG respectively. Similar to leaf number, maximum per cent difference in leaf area over control was also observed at 60 DAG (65.22). Moreover, on an average, a per cent increase of 46.08 was noted in leaf area in the colonised plants compared to the control plants.



**Plate 2. Effect of *P. indica* on shoot and root growth in bitter gourd plants (a - *P. indica* colonized bitter gourd plant at 15 DAG, b - control plant at 15 DAG, c - *P. indica* colonized bitter gourd plant at 30 DAG, d - control plant at 30 DAG)**

**Table 2. Effect of *P. indica* root colonization on root and shoot parameters of bitter melon plants var. Preethi**

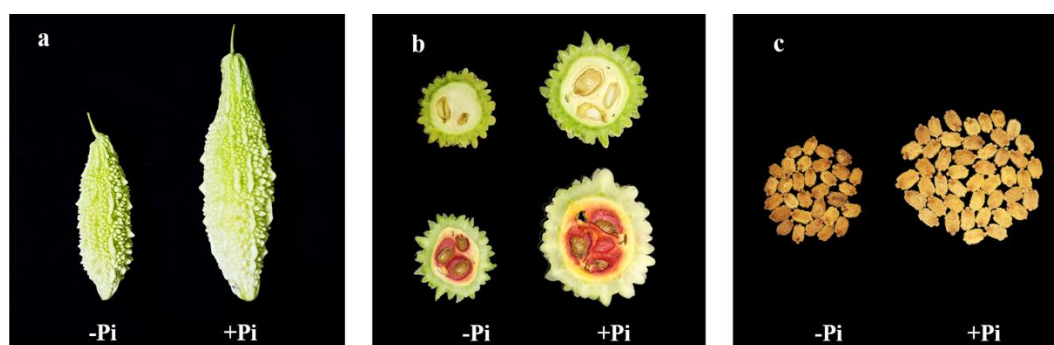
Observations*	5 DAG*		15 DAG*		30 DAG*		60 DAG*	
	- Pi	+ Pi	- Pi	+ Pi	- Pi	+ Pi	- Pi	+ Pi
Shoot length (cm)	7.02 <sup>b</sup> ± 0.10	8.93 <sup>a</sup> ± 0.16	16.92 <sup>b</sup> ± 0.16	20.88 <sup>a</sup> ± 0.54	88.0 <sup>b</sup> ± 9.93	129.58 <sup>a</sup> ± 0.80	216.22 <sup>b</sup> ± 8.10	326.9 <sup>a</sup> ± 9.02
Root length (cm)	4.10 <sup>b</sup> ± 0.19	6.99 <sup>a</sup> ± 0.11	7.95 <sup>b</sup> ± 0.12	10.11 <sup>a</sup> ± 0.16	12.05 <sup>b</sup> ± 0.15	15.86 <sup>a</sup> ± 0.33	15.5 <sup>b</sup> ± 1.59	22.65 <sup>a</sup> ± 2.55
Shoot weight (g)	1.40 <sup>b</sup> ± 0.06	1.92 <sup>a</sup> ± 0.07	3.84 <sup>b</sup> ± 0.08	7.42 <sup>a</sup> ± 0.10	29.30 <sup>b</sup> ± 2.83	42.30 <sup>a</sup> ± 2.30	90.60 <sup>b</sup> ± 6.09	177.50 <sup>a</sup> ± 9.31
Root weight (g)	0.14 <sup>b</sup> ± 0.03	0.28 <sup>a</sup> ± 0.02	0.40 <sup>b</sup> ± 0.03	0.59 <sup>a</sup> ± 0.01	1.55 <sup>b</sup> ± 0.62	3.89 <sup>a</sup> ± 1.30	3.43 <sup>b</sup> ± 1.15	6.29 <sup>a</sup> ± 1.60
Number of leaves	3.60 <sup>b</sup> ± 0.51	4.20 <sup>a</sup> ± 0.42	7.60 <sup>b</sup> ± 0.51	10.20 <sup>a</sup> ± 0.78	28.2 <sup>b</sup> ± 4.56	40.2 <sup>a</sup> ± 5.32	156.5 <sup>b</sup> ± 7.58	218.5 <sup>a</sup> ± 8.92
Leaf area (cm <sup>2</sup> )	24.00 <sup>b</sup> ± 0.33	36.00 <sup>a</sup> ± 0.57	40.19 <sup>b</sup> ± 0.20	50.25 <sup>a</sup> ± 0.16	40.33 <sup>b</sup> ± 2.06	58.11 <sup>a</sup> ± 4.96	57.01 <sup>b</sup> ± 5.05	94.19 <sup>a</sup> ± 2.87
Number of secondary roots	5.40 <sup>b</sup> ± 0.51	7.10 <sup>a</sup> ± 0.31	8.60 <sup>b</sup> ± 0.51	16.00 <sup>a</sup> ± 0.66	54.90 <sup>b</sup> ± 3.17	85.70 <sup>a</sup> ± 2.58	66.10 <sup>b</sup> ± 2.68	92.30 <sup>a</sup> ± 2.98
Number of tertiary roots	-	-	16.2 <sup>b</sup> ± 0.78	55.40 <sup>a</sup> ± 3.43	89.00 <sup>b</sup> ± 2.10	232.40 <sup>a</sup> ± 2.91	119.20 <sup>b</sup> ± 4.44	269.60 <sup>a</sup> ± 7.04
<b>T - Table (0.05)</b>	<b>2.977</b>							

\* DAG - Days after germination; Values are mean of 15 replications ± SD; Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance

Krishnan [35] reported that *P. indica*-colonised black pepper plants had 34 leaves compared to 22 leaves in control plants. Moreover, the colonised black pepper plants had 23 per cent more leaf area compared to non-colonised plants. Yadav et al. [32] in tomato plants and Verma et al. [31] in cucumber (*Cucumis sativus*) plants also reported comparable results. Gene expression studies associated with leaf development in bitter melon plants colonized by *P. indica* also showed upregulation of genes involved in leaf expansion, chlorophyll biosynthesis, and hormone signalling pathways, indicating the molecular basis for the observed improvements in leaf area and leaf number [36].

***P. indica* colonisation enhanced the yield and fruit parameters in bitter melon:** The yield and fruit parameters like number of fruits per plant, average weight of fruit, average length of fruit, average girth/diameter of fruit, number of seeds per fruit, test weight of seeds, and shelf life of fruits were assessed in the *P. indica*-colonised and control plants. There was a significant increase in yield and fruit parameters in *P. indica*-colonized bitter melon plants compared to non-colonized controls (Plate 3).

The colonized-plants consistently produced more and larger fruits compared to the control plants. *P. indica*-colonised plants produced 23.10 fruits per plant compared to 16.10 fruits in control plants. The trend was similar with fruit weight, fruit length and fruit girth. Fruit weight in the colonised plants was 445.20 g compared to 286.50 g in control plants (Table 3). Average length and diameter of fruits in the colonised plants were 32.80 cm and 29.10 cm respectively, over 23.70 cm and 21.50 cm in control plants. Hence, number of fruits per plant, and fruit weight, length and diameter were increased by 30.30, 35.64, 27.74 and 26.11 per cent respectively in the fungus-colonised plants over the control. Similarly, the seed parameters also showed positive results in the endophyte colonised plants. The colonised plants produced 44.10 seeds per fruit whereas only 30.5 seeds per fruit were found in control plants. Moreover, the seeds were bold and 100 seed weight or test weight was 25.0 g in the colonised plants compared to 19.6 g in control plants. All these results suggest the beneficial influence of *P. indica*-colonization on fruit parameters in bitter melon. It was also reported that fresh weights of tomato fruits per plant increased significantly up to 100 per cent in *P. indica* in plants over control plants [31, 37, 38].



**Plate 3. Comparison of fruit parameters of *P. indica*-colonized (+Pi) and control plants (-Pi). (a) Whole bitter melon fruit, (b) Cross section of fruit, and (c) seeds per fruit**

**Table 3. Effect of *P. indica* root colonization on fruit parameters of bitter melon plants**

Fruit parameters	Control*	+ <i>P. indica</i> *	% increase over control
Number of fruits per plant	16.10 <sup>b</sup> ± 3.52	23.10 <sup>a</sup> ± 3.20	30.30
Average weight per fruit	286.50 <sup>b</sup> ± 12.64	445.20 <sup>a</sup> ± 7.20	35.64
Average length of fruit (cm)	23.70 <sup>b</sup> ± 1.72	32.80 <sup>a</sup> ± 2.10	27.74
Average girth of fruit (cm)	21.50 <sup>b</sup> ± 1.89	29.10 <sup>a</sup> ± 2.01	26.11
Number of seeds per fruits	30.5 <sup>b</sup> ± 2.53	44.10 <sup>a</sup> ± 2.32	30.83
100 seed weight	19.6 <sup>b</sup> ± 1.65	25.00 <sup>a</sup> ± 0.96	21.60
T - Table (0.05)	2.977		

\* Values are mean of 15 replications ± SD; Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance



The influence of *P. indica* on nutrient uptake, water use efficiency as well as hormonal regulations can also result in improved yield parameters. Moreover, Waller, et al. [9] demonstrated that *P. indica* increases photosynthetic rates in the colonized plants, leading to enhanced biomass and yield. *P. indica* colonization can also enhance flower retention and improve pollination efficiency, leading to a higher fruit set and in turn higher yield [5, 39]. The observed increase in yield and fruit parameters in bitter gourd plants due to *P. indica* colonization highlights the potential of the fungal endophyte in enhancing plant growth and yield. The beneficial effects of *P. indica* could be attributed to various mechanisms, including improved nutrient availability, uptake and translocation, hormone modulation, and enhanced stress tolerance. Moreover, exploring the long-term effects of *P. indica* colonization in bitter gourd growth and yield could provide valuable insights for agricultural applications aimed at sustainable crop production.

#### 4. CONCLUSION

The study reveals the impact of *P. indica* colonization on enhanced growth and yield in bitter gourd. The use of beneficial endophytes like *P. indica* can reduce the dependency on chemical inputs for crop production, thereby minimizing environmental pollution and preserving soil health. Hence, the technology can be included in bitter gourd cultivation system to enhance the productivity and quality of bitter gourd; and also contribute to better health, economic prosperity, and environmental sustainability.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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