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2 **Role of antioxidants in the management of**  
3 ***Chilli leaf curl virus* in chilli using beneficial**  
4 **fungal root endophyte *Piriformospora indica***

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11 **ABSTRACT**

12 Chilli leaf curl virus disease caused by *Chilli leaf curl virus* (ChiLCV) is considered as the most damage-causing for chilli crop, resulting in a cent per cent yield loss when affected at the early crop stage. *Piriformospora indica* (synonym *Serendipita indica*), a root endophytic fungus, enhances plant resilience against biotic and abiotic stresses. This study analyses the effect of *P. indica* against ChiLCV as a pot culture study under green net house conditions using Completely Randomized Design (CRD) layout in the Department of Plant Pathology, College of Agriculture, Padannakkad from August 2022 to January 2023. Chilli seeds colonized in *P. indica*-enriched potting mixtures exhibited earlier germination than non-colonized seeds. ChiLCV was introduced to chilli plants pre and post-colonized with *P. indica* and observed for disease incidence. Chlamyospores of *P. indica* were observed in the root cortical region five days after co-cultivation (DAC). In the two pot culture experiments conducted, plants pre-colonized with *P. indica* followed by graft transmission of the virus after 15 days expressed a low vulnerability index (V.I. - 25) against non-colonized, grafted plants (V.I. - 64). Colonization of *P. indica* (2 days) after graft transmission of the virus recorded a V.I. (36), while non-colonized grafted plants recorded a V.I. of 65. The endophyte colonized plants exhibited elevated ROS scavenging enzyme activity (Catalase, Peroxidase, Superoxide dismutase and Phosphatase) compared to non-colonized ones which was confirmed by enzyme activity analyses and ROS staining techniques. Endophyte colonized chilli plants had exhibited more resilience towards ChiLCV thereby proving that it could be exploited in future towards cultivating disease resistant plants. This study underscores the role of *P. indica* in managing chilli leaf curl disease by boosting ROS-scavenging enzyme production, offering a promising avenue for disease mitigation.

13  
14 **Keywords:** *Chilli leaf curl virus; Piriformospora indica; Endophyte; Reactive Oxygen Species; Begomovirus;*

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18 **1. INTRODUCTION**

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20 Chilli (*Capsicum annum* L.), popularly known as wonder spice, is a major vegetable from the family *Solanaceae*. Chilli is very much peculiar for its pungency and this flavour is imparted by the alkaloid capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) contained in the fruit's pericarp and placenta. The fruit is an excellent source of Vitamin C, Vitamin E, carotenoids and phenols [1]. In India, chilli accounts for an area of 4.18 lakh ha, producing 4.5 million metric tonnes [2].

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28 *leaf curl virus* is a bipartite begomovirus grouped under the family *Geminiviridae*. Begomoviruses are  
29 quasi-icosahedral particles with a genome size of 2.6-2.8 kb and approximately 18\*30 nm. The genomic  
30 component of begomoviruses may be either monopartite (DNA-A) or bipartite (DNA-A and DNA-B) [5].  
31 The primary field symptoms of chilli leaf curl disease include leaf curling, puckering, mottling, and stunted  
32 plant growth. Additional visible symptoms include thickened veins, enations on the leaves, and premature  
33 wilting of flowers. Affected plants exhibit a bushy appearance due to the proliferation of shorter branches  
34 with numerous small, curled leaves concentrated in the upper portion of the plant. Fruit production is  
35 significantly reduced or absent in these plants. Infection at an early stage can lead to total yield loss [6].  
36 The ChiLCV was exclusively transmitted in a persistent circulative manner by whiteflies (*Bemisia tabaci*)  
37 under natural conditions [7]. Being polyphagous pests, whiteflies are evolving continually which makes  
38 their management difficult. As the viruses are co-evolving with their vectors and hosts, several new  
39 strains of viruses are being formed which can cause severe destruction [8]. ChiLCV is also found to be  
40 graft-transmitted experimentally [6, 9, 10].

41 The appearance of novel recombinant strains of viruses and the evolution of insecticide-resistant  
42 vectors make virus disease management tedious. Management using beneficial micro-organisms is  
43 considered one of the best strategies for virus disease management. *Piriformospora indica*, a beneficial  
44 fungal root endophyte was isolated from the Thar Desert of India for the first time [11]. It was classified  
45 under the order *Sebacinales* of Basidiomycota due to its ultrastructure [12]. *P. indica* was proven culturable  
46 in potato dextrose agar (PDA) and potato dextrose broth [13, 14]. Mass multiplication of *P. indica* in the  
47 potting mixture is done by culturing a sterilised mixture of coir pith, dried farmyard manure (1:1) w/w and 2  
48 per cent gram flour [15].

49 This fungus undergoes symbiosis within the root cortical region of higher plants and promotes  
50 plant growth, increases the resistance of colonized plants against biotic and abiotic stress and acts as a  
51 bio-regulator for plant growth development [16]. *P. indica* inoculated tomato plants showed improved  
52 growth characteristics compared to non-inoculated plants [17]. The growth promotion in barley inoculated  
53 with *P. indica* was found as a result of the reprogramming of the micro-RNA (mi-RNA) profile; and the  
54 target genes of these mi-RNAs involve transcription, cell division, auxin signal perception and  
55 transduction, photosynthesis and hormone stimulus [18].

56 Root colonization of *P. indica* also improves the tolerance of plants towards various biotic and  
57 abiotic stresses by upregulating multiple biochemical pathways. Studies of the effects of *P. indica* in  
58 *Tomato yellow leaf curl virus* affected tomato plants and found that the PR genes such as PAL, PR1a,  
59 PR3a, PR3b, and PR5 had their expressions increased in the leaves of the susceptible cultivar T07-1  
60 [19]. Increased activity of defence enzymes such as chitinase, PAL, catalase, peroxidase, superoxide  
61 dismutase, glutathione reductase and glutathione S transferases was observed in *P. indica* colonised taro  
62 plants upon *Phytophthora colocasiae* infection [20]. Various studies on plants inoculated with viral  
63 diseases revealed that the viral disease symptoms and disease severity were reduced in *P. indica* treated  
64 plants than in control plants [21, 22, 23, 24]. This study was therefore conducted to explore the tolerance  
65 of chill plants imparted by *P. indica* against chilli leaf curl virus disease.

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## 67 **2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY**

68

### 69 **2.1 Maintenance and mass multiplication of *P. indica* and co-cultivation with chilli seeds**

70 *Piriformospora indica* was maintained in PDA with subculturing at fortnight intervals. Fungal discs  
71 (6 mm) from actively growing regions of PDA plates are cut and inoculated into 100 ml potato dextrose  
72 broth in 250 ml Erlenmeyer flasks. The flasks are then incubated at room temperature with continuous  
73 shaking at 40 rpm for 15 days to obtain a mycelial mat.

74 As per the standardized protocol for *P. indica* mass multiplication [15], an equal quantity of dried  
75 coir pith compost and dried, powdered cow dung mixture is amended with two per cent gram flour (w/w)  
76 and moistened with distilled water to its field capacity. The mixture was then autoclaved for three  
77 consecutive days. Fungal mycelium from 18 days old broth culture was filtered through two layers of  
78 cheesecloth and washed thrice with sterile distilled water. The autoclaved potting mixture was transferred  
79 to surface sterilized plastic trays and one per cent (w/w) fungal mats were added and mixed into it. Sterile

80 water was then sprayed onto the mixture to field capacity. The trays were then covered with surface  
81 sterilized cling film and kept undisturbed in hygienic conditions to obtain complete mycelial covering over  
82 the medium.

83 After seven days of inoculation, the *P. indica* mass multiplied mixture was filled in cleaned and  
84 dried portrays. Surface sterilized chilli seeds of the variety Vellayani Athulya were sown onto the media  
85 and kept undisturbed for germination.

86 For analysing the root colonization of *P. indica*, the seedlings were uprooted, cleaned and the  
87 roots were cut into small pieces and subjected to softening with 10 per cent KOH and one per cent HCl.  
88 The softened root pieces were rinsed with water and dipped in lactophenol cotton blue dye for two  
89 minutes. The stained root bites were observed under Carl Zeiss Axiocamcolor microscope with 1000X  
90 magnification.

## 91 **2.2 Analysis of the effect of *P. indica* against chilli leaf curl virus disease**

### 92 **2.2.1 Pot culture experiment to study the effect of *P. indica* pre-colonization against ChiLCV**

93 The layout for the experiment was a completely randomized design (CRD) with seven treatments  
94 and eight replication with three plants per replication (Variety: Vellayani Athulya).

95 T<sub>1</sub>: Absolute control

96 T<sub>2</sub>: Chilli seedling colonized with *P. indica*

97 T<sub>3</sub>: Graft transmission of the virus by the infected scion

98 T<sub>4</sub>: *P. indica* priming followed by grafting of the virus infected scion after two days

99 T<sub>5</sub>: *P. indica* priming followed by grafting of the virus infected scion after five days

100 T<sub>6</sub>: *P. indica* priming followed by grafting of the virus infected scion after 10 days

101 T<sub>7</sub>: *P. indica* priming followed by grafting of the virus infected scion after 15 days

102 Plants of T<sub>2</sub> were raised in *P. indica* mass multiplied media. Forty five days old seedlings of T<sub>3</sub> to T<sub>7</sub> were  
103 uprooted from the control medium, washed in sterile water and transplanted into *P. indica* mass multiplied  
104 medium, all at a time, which serves as rootstock. Wedge grafting was performed [9]with scions of disease  
105 severity grade 2 (disease score chart 0-6 of ChiLCV) [25] in time intervals according to treatments (T<sub>4</sub> to  
106 T<sub>5</sub>). Plants of T<sub>3</sub> were also grafted at the same time, without *P. indica* inoculation. Grafted plants were  
107 covered with moist polythene cover to provide humid conditions. The disease severity (Vulnerability Index  
108 –V.I.) was calculated from the virus infected plants [26].

### 109 **2.2.2 Pot culture experiment to study the effect of *P. indica* post-colonization against ChiLCV**

110 The layout for the experiment was a completely randomized design (CRD) with seven treatments and  
111 eight replication with three plants per replication (Variety: Vellayani Athulya).

112 T<sub>1</sub>: Absolute control

113 T<sub>2</sub>: Chilli seedling colonized with *P. indica*

114 T<sub>3</sub>: Graft transmission of the virus by the infectedscion

115 T<sub>4</sub>: Graft transmission by the virus infected scion followed by *P.indic*priming after twodays

116 T<sub>5</sub>: Graft transmission by the virus infected scion followed by *P.indica* priming after five days

117 T<sub>6</sub>: Graft transmission by the virus infected scion followed by *P.indica* priming after 10 days

118 T<sub>7</sub>: Graft transmission by the virus infected scion followed by *P.indica* priming after 15 days

119 Seedlings required for T<sub>2</sub> were raised in *P. indica* mass multiplied media and all other seedlings were  
120 grown in potting mixture without *P. indica*. Wedge grafting was performed [9] in the treatments T<sub>3</sub> to T<sub>7</sub> at  
121 a time. Grafted plants of T<sub>4</sub> to T<sub>7</sub> were transplanted to *P.indica* containing potting mixture according to the  
122 treatment intended. Observations were taken for disease severity calculation [26].

### 123 **2.2.3. Detection of begomovirus by PCR reaction using coat protein specific primer**

124 Total plant genomic DNA was isolated using OMEGA E.Z.N.A Plant DNA Kit by following the  
125 manufacturer's protocol. The primer pair used in the study amplifies explicitly the coat protein region of  
126 begomovirus [DENG541-F (TAATATTACCKGWKGVCCSC)/ DENG540-R  
127 (TGGACYTTRCAWGGBCCTTCACA)] [27]. The PCR reaction conditions followed as initial denaturation  
128 (94°C) for 1 min, followed by denaturation (94°C) for 50 s, annealing (52°C) for 45 s, extension (72°C) for  
129 2 min and final extension (72°C) for 10 min (SimpliAmp Thermal Cycler™, Thermo Scientific). The PCR  
130 product obtained was undergone 1.2 per cent agarose gel electrophoresis (Cleaver Scientific, UK). The  
131 electrophoresed gels were visualized and images were documented under UV light using a Gel  
132 documentation system (Bio-Rad, USA).

## 133 **2.3 Elucidation of reactive oxygen species (ROS) and its scavenging enzymes in *P. indica*** 134 **mediated tolerance to chilli leaf curl virus complex**

135 Leaf samples from absolute control, plants with ChiLCV alone, plants with *P. indica* alone and the  
136 best treatments from pot experiments 2.3.1 and 2.3.2 were taken 15, 45, and 60 days after treatment and  
137 at final harvest (90 DAT) to analyse ROS and its scavenging enzymes present.

### 138 **2.3.1 Nitroblue tetrazolium (NBT) and Diaminobenzidine (DAB) staining**

139 NBT and DAB staining protocol was used to detect hydrogen peroxide and superoxide anion  
140 accumulation [28]. 50 mg of DAB was dissolved in 50 ml of distilled water (pH 3.8) to prepare the staining  
141 solution. For NBT staining, 0.1 g of NBT was dissolved in 50 ml 50 mM sodium phosphate buffer (pH 7.5).  
142 Chilli leaves were cleaned and immersed in staining solutions overnight, treated with absolute alcohol to  
143 remove chlorophyll, and placed on a 60 per cent glycerol-saturated paper towel for 15 minutes. The  
144 leaves were arranged on white paper to visualize the colour change.

### 145 **2.3.2 Analysis of Total Soluble Protein content**

146 Total soluble proteins were estimated using the Bradford protocol [29]. A dye concentrate was  
147 prepared beforehand with Coomassie brilliant blue G 250, ethanol and concentrated orthophosphoric  
148 acid. Fresh chilli leaf (1 g) was homogenized with 10 ml of phosphate-buffered saline (PBS) and  
149 centrifuged at 5000 rpm for 15 minutes at 4°C, the supernatant was collected for further analysis. One  
150 part of the dye concentrate was mixed with four parts of distilled water for the assay. In tubes, 0.5 ml  
151 supernatant, 0.5 ml double distilled water, and 5 ml dye solution were mixed and used for analysing the  
152 absorbance at 595 nm against reagent blank.

### 153 **2.3.3 Analysis of Catalase (CAT) activity**

154 Fresh leaf tissue (1 g) was homogenized in 20 ml of 0.0067 M phosphate buffer and the mixture  
155 was centrifuged at 5000 rpm for 15 minutes at 4°C. The sample cuvette was filled with 40 µl of the extract  
156 and 3 ml H<sub>2</sub>O<sub>2</sub>-PO<sub>4</sub> buffer while H<sub>2</sub>O<sub>2</sub>-free PO<sub>4</sub> buffer was filled in the control cuvette. The time required  
157 for the change of absorbance (Δt) by 0.05 at 240 nm was recorded [30].

158 **2.3.4 Analysis of Peroxidase (PO) activity**

159 Fresh chilli leaf (1 g) was homogenized with 5 ml of sodium phosphate buffer (pH 6.5) along with  
160 a pinch of polyvinyl pyrrolidone, centrifuged at 6000 rpm for 15 minutes at 4°C. The reaction mixture  
161 consists of 3 ml of 0.05 M pyrogallol and 50 µl of enzyme extract in the sample cuvette and pyrogallol in  
162 the reference cuvette. The reaction was initiated when one per cent hydrogen peroxide (1 ml) was added  
163 to sample cuvettes and changes in absorbance readings were measured in a spectrophotometer at 420  
164 nm at an interval of 30 seconds and continued for 180 seconds [31].

165 **2.3.5 Analysis of superoxide dismutase (SOD) activity**

166 Fresh chilli leaf (1 g) was homogenized with 10 ml of ice-cold 50 mM potassium phosphate buffer  
167 (pH 7.8) and centrifuged at 10000 rpm for 10 minutes at 4°C. The reaction cocktail contained potassium  
168 phosphate buffer, methionine, riboflavin, EDTA and crude enzyme extract. A blank solution was made  
169 without adding the enzyme extract and NBT and an additional reference was made without adding NBT,  
170 but by adding enzyme extract. All the tubes were exposed to 400 W bulbs for 15 minutes. The  
171 absorbance readings were taken immediately at 560 nm [32].

172 **2.3.6 Analysis of phosphatase activity**

173 Fresh chilli leaf (1 g) was homogenized in 10 ml of chilled 50 mM citrate buffer (pH 5.3) and  
174 centrifuged at 12000 rpm for 10 min at 4°C. 3 ml of substrate solution was incubated for 5 min at 37°C.  
175 0.5 ml of enzyme extract was added to this and mixed thoroughly. From this mixture, 0.5 ml was pipetted  
176 out immediately to mix with 9.5 ml of 0.085 N sodium hydroxide, corresponding to a blank solution. The  
177 remaining mixture was incubated at 37°C for 15 min. 0.5 ml of this mixture was taken again and mixed  
178 well with 9.5 ml of sodium hydroxide. The absorbance of this solution was checked at 405 nm against the  
179 blank solution [33].

180 **3. RESULTS AND DISCUSSION**

181

182 Chilli leaf curl disease is a major viral disease faced by chilli farmers which causes up to cent per  
183 cent yield loss in the early crop stage. Due to the increased white fly proliferation brought on by climate  
184 change, the disease spreads more quickly across the nation, resulting in a significant economic loss. The  
185 use of fungal root endophyte *P. indica* is a novel strategy for managing ChiLCV because it forms a  
186 symbiotic relationship with plants and positively modifies their transcriptomes, proteomes, and  
187 metabolomes, including phytohormone synthesis and signalling that affects growth, nutrient uptake,  
188 flowering, seed production, and defence against biotic and abiotic stresses [34].

189

190 **3.1 Co-cultivation of *P. indica* with chilli seeds**

191 Seeds of chilli var. Vellayani Athulya sown on *P. indica* mass multiplied medium germinated early  
192 (seven days) and completed 50 per cent germination within ten days compared with untreated seeds (ten  
193 days for germination and 17 days for 50 per cent germination) (Table 1). After staining the root bits with  
194 lactophenol cotton blue, chlamydospores were visible in the cortical region five days after co-cultivation  
195 (DAC) and a chain of spores could be found 30 DAC (Plate 1). 35.75 per cent root colonization efficiency  
196 in chilli roots after one week of co-cultivation of *P. indica*[35]. In tomato roots, fungal spores were found  
197 after three days of co-cultivation [24].

198

199 Table 1. Effect of *P. indica* on germination of chilli seeds var. Vellayani Athulya

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Treatment	Days taken to initiate	Days for 50 per cent
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	germination	germination
- <i>P. indica</i>	10.32 ± 1.15	16.18 ± 0.72
+ <i>P. indica</i>	7.42 ± 0.49	10.48 ± 0.61
SE (m) ±	0.33	0.25
CV (0.05)	10.00	5.02

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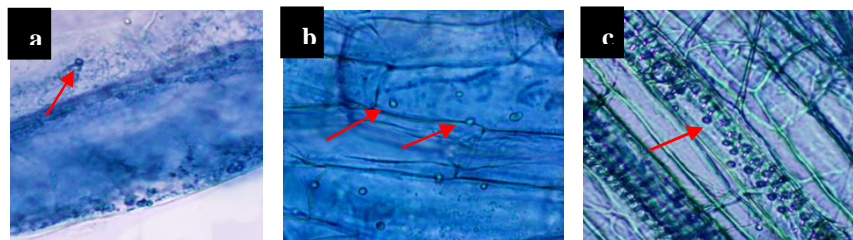


Plate 1. Root colonization of *P. indica* in chilli seedlings a) 8 DAC b) 15 DAC c) 30 DAC

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### 213 3.2 Effect of *P. indica* against chilli leaf curl virus disease

#### 214 3.2.1 Pot culture experiment with *P. indica* pre-colonized seedlings

215 In this experiment, *P. indicapre*-colonized chilli plants grafted after 15 days have recorded a  
 216 reduced vulnerability index (V.I. - 25) against grafted plants without *P. indica* colonization (V.I.- 64) when  
 217 observed at 45 days after treatment (DAT), and the former recorded a significant increase in the number  
 218 of days taken for symptom development (30.17) against the latter (12.82) (Table 2). *P. indica* colonization  
 219 followed by graft transmission of the virus at 15 days, recorded a maximum reduction in disease severity  
 220 (61 per cent) 45 days after treatment.

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224 Table 2. Effect of *P. indica* pre-colonization on vulnerability index initiation and symptom appearance of  
 225 ChiLCV in chilli var. Vellyani Athulya upon graft transmission

Treatment	V.I.(45 DAT)	Days taken for symptom appearance (DAT)
Healthy (control)	0	**

<i>P. indica</i> alone	0	**	226
			227
ChiLCV alone	64	12.82 ± 1.07	228
<i>P. indica</i> + ChiLCV (2 days interval)	57	14.37 ± 0.91	229
			230
<i>P. indica</i> + ChiLCV (5 days interval))	55	15.4 ± 0.85	231
			232
			233
			234
<i>P. indica</i> + ChiLCV (10 days interval)	42	23.6 ± 0.86	235
			236
			237
<i>P. indica</i> + ChiLCV (15 days interval)	25	30.17 ± 1.71	238
			239
			240
SE (m) ±		0.56	241
			242
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CD (0.05)		1.71	244
			245
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### 254 3.2.2 Potculture experiment with *P. indica* post-colonized seedlings

255 In this experiment, plants colonized with *P. indica* two days after grafting recorded reduced V. I.  
256 (36) against grafted plants without *P. indica* colonization (V.I. - 65) when observed at 45 DAT and the  
257 former recorded a significant increase in the number of days taken for symptom expression (17.65)  
258 against the latter (11.44) (Table 3).

259 The results suggest that *P. indica* colonization significantly reduced disease occurrence, severity,  
260 and time for ChiLCV to appear under green net house conditions. In the study conducted on  
261 *Chenopodium* plants, the plants colonized with *P. indica* showed a significant reduction in the number of  
262 days taken for symptom development and lesion size; and an increment of 68 per cent in per cent  
263 inhibition of lesion size when compared to plants in control. In yard-long beans, plants colonized with *P.*  
264 *indica* before virus inoculation have an increased reduction in disease (71 per cent) and reduced V.I.  
265 compared with post-inoculated plants [36]. The effect of *P. indica* on *Tomato leaf curl virus* (ToLCV) in  
266 tomato variety Vellayani Vijay was evaluated. *P. indica* colonized tomato plants had shown more  
267 improvement in germination, vegetative and reproductive character than non-colonized plants. *P. indica*  
268 colonized plants had exhibited a significantly less virus titre against non-colonized plants such that *P.*  
269 *indica* reduced the disease severity of ToLCV by 58 per cent in field conditions than plants in control [24].  
270

271 Table 3. Effect of *P. indica* post-colonization on vulnerability index initiation and symptom appearance of  
272 ChiLCV in chilli var. Vellyani Athulya upon graft transmission

Treatment	V.I.(45 DAT)	Days for symptom appearance (days after treatment)
Healthy (control)	0	**
<i>P. indica</i> alone	0	**
ChiLCV alone	65	11.44 ± 1.23
ChiLCV + <i>P. indica</i> (2 days interval)	36	17.65 ± 1.05
ChiLCV + <i>P. indica</i> (5 days interval))	42	14.9 ± 1.1
ChiLCV + <i>P. indica</i> (10 days interval)	53	13.32 ± 0.96
ChiLCV + <i>P. indica</i> (15 days interval)	62	12.37 ± 0.75
SE (m) ±		0.51
CD (0.05)		1.55

274

### 275 3.2.3 Confirmation of the presence of begomovirus by PCR reaction

276 Total genomic DNA was extracted from experiments 2.3.1 and 2.3.2 plants and subjected to PCR  
 277 amplification using Deng primer. Amplification at 520 bp was obtained for all the grafted plants from the  
 278 pot culture experiment which confirms the presence of begomovirus in such plants (Plate 2, Plate 3). The  
 279 samples from the infected plants showed positive amplification to Deng primer, yielding an amplicon of  
 280 520 bp as it is specific for the coat protein region of begomovirus. Deng primers were to amplify DNA from  
 281 chilli and tomato plants to screen for begomovirus infection. They observed that 49 out of 99 samples  
 282 tested positive for PCR at the expected amplicon size of approximately 530 bp [37].

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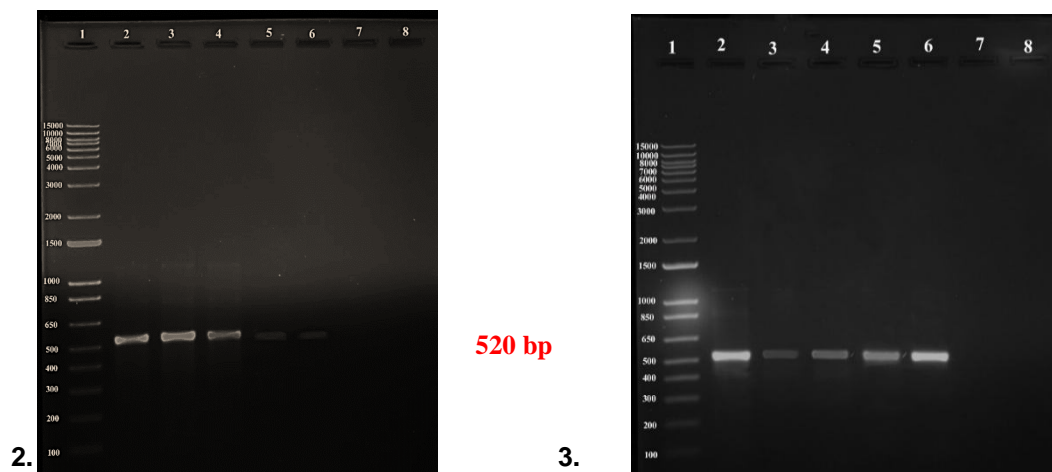
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298 Plate 2. Electrophoresis gel image of amplified coat protein region of ChiLCV using Deng primer. Lane 1)  
299 1 kb marker 2)ChiLCV alone; *P. indica*-priming followed by graft transmission of the virus infected scion  
300 after 3) 2 days 4) 5 days 5) 10 days 6) 15 days 7) *P. indica* alone 8) absolute control

301 Plate 3. Electrophoresis gel image of amplified coat protein region of ChiLCV using Deng primer. Lane 1)  
302 1 kb marker 2) ChiLCV alone; graft transmission of the virus infected scion followed by *P. indica*-priming  
303 after 3) 2 days 4) 5 days 5) 10 days 6) 15 days 7) *P. indica* alone 8) Absolute control

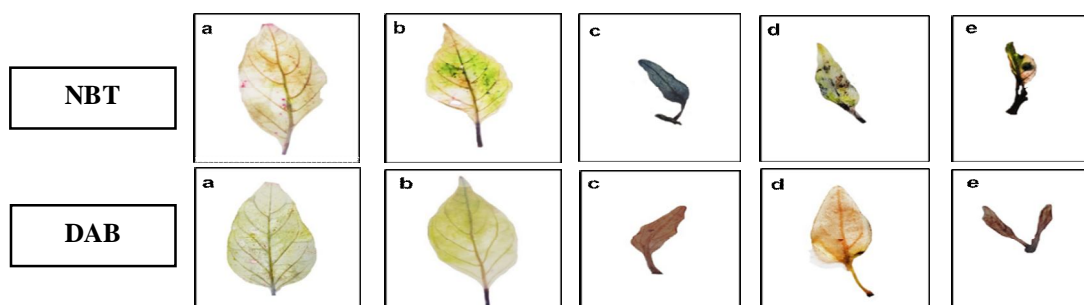
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### 305 3.3 Elucidation of reactive oxygen species (ROS) and its scavenging enzymes in *P. indica* 306 mediated tolerance to chilli leaf curl virus complex

#### 307 3.3.1 Nitro blue tetrazolium (NBT) and Diaminobenzidine (DAB) staining

308 The presence of ROS, such as superoxide anion and hydrogen peroxide was assessed using  
309 nitroblue tetrazolium (NBT) and diaminobenzidine (DAB) respectively. Virus-inoculated plants without *P.*  
310 *indica* colonization recorded the highest stain intensity as ROS production is highest in such plants. The  
311 presence of *P. indica* in virus-inoculated plants decreased ROS production, as evidenced by the reduced  
312 stain intensity. *P. indica* pre-colonization expressed a better suppression of ROS production than post-  
313 colonization (Plate 4, Plate 5).

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325 Plate 4. Effect of *P. indica* colonization on ROS accumulation at 15 DAT a) Control b) *P. indica* primed c)  
326 ChiLCV alone by graft transmission, d) *P. indica* colonization followed by graft transmission e) graft  
327 transmission followed by *P. indica* colonization

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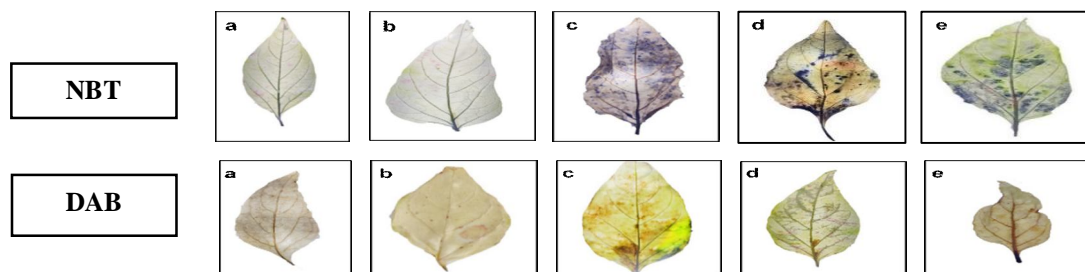
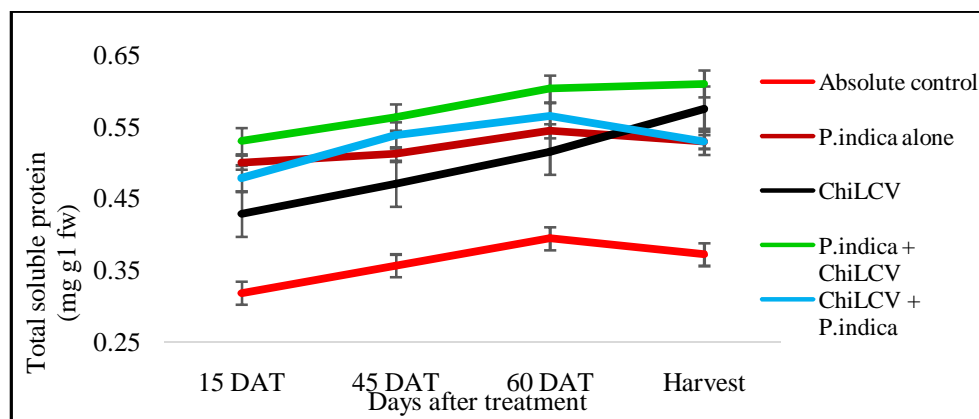


Plate 5. Effect of *P. indica*-colonization on ROS accumulation at 60 DAT a) Control b) *P. indica*-primed c) ChiLCV alone by graft transmission, d) *P. indica* colonization followed by graft transmission e) graft transmission followed by *P. indica* colonization

346 When ChiLCV is artificially transmitted to chilli plants, those plants colonized by *P. indica* showed  
347 a significant decrease in the accumulation of reactive oxygen species (ROS) as assessed through  
348 nitroblue tetrazolium (NBT) and diaminobenzidine (DAB) staining for H<sub>2</sub>O<sub>2</sub>, compared to non-colonized  
349 infected plants. The staining intensity of leaves colonized by *P. indica* was less pronounced than non-  
350 colonized plants. Non-colonized plants that were inoculated with the virus displayed intense colour  
351 development. At the same time, the staining intensity showed a decreasing trend in plants that were  
352 colonized before and after the virus challenge inoculation. This suggests a decrease in the accumulation  
353 of hydrogen peroxide and superoxide anion in colonized plants. Throughout the tested time intervals, the  
354 pre-colonized plants with *P. indica* exhibited a more significant colour intensity reduction than the post-  
355 colonized plants. A similar observation was recorded in Alternaria leaf spot on chilli regarding the role of  
356 nitric oxide in inducing defence response [38]. They observed that the susceptible cultivar was showing a  
357 higher intensity of reddish brown colour upon DAB staining at pathogen penetration sites, which indicated  
358 the production of more H<sub>2</sub>O<sub>2</sub> at such sites. In contrast, the resistant cultivar shows lesser brown  
359 colouration. This suggests that the susceptible cultivar is more prone to cell damage caused by the H<sub>2</sub>O<sub>2</sub>  
360 than the resistant cultivar. The accumulation of malondialdehyde (MDA), H<sub>2</sub>O<sub>2</sub> and O<sup>2-</sup> were significantly  
361 lower in soybean plants colonized with *P. indica* than plants in control (under salt stress), which indicates  
362 less oxidative damage in the former plants, which in turn yield lesser colour development when treated  
363 with NBT and DAB [39].

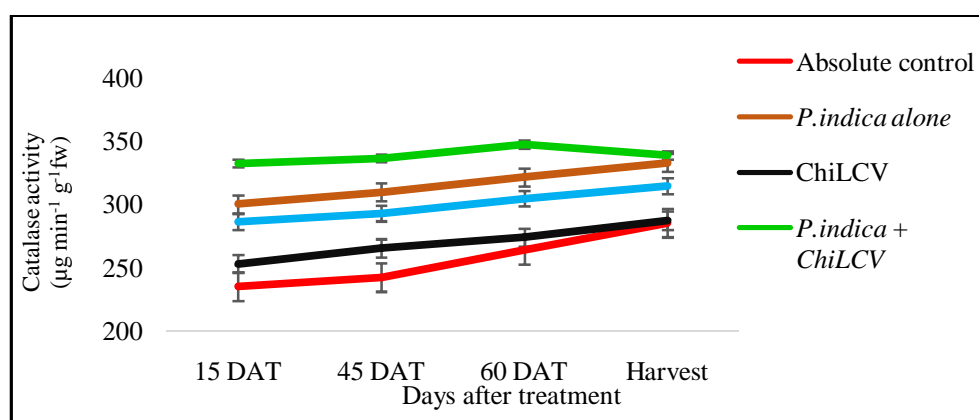
### 364 3.3.2 Elucidation of ROS scavenging enzymes and total soluble protein content

365 The total soluble protein content in virus-inoculated plants was recorded higher in all the plants  
366 that were colonized with *P. indica* compared to non-colonized plants (Fig. 1). At the final harvest, the total  
367 soluble protein content increased significantly in virus-inoculated plants without endophytic colonization.  
368 This might be due to this plant's increased accumulation of virus-related proteins. There was a noticeable  
369 increase in protein content in *P. indica* pre-colonized plants containing the virus compared to plants with  
370 ChiLCV alone. This might be due to the upregulation of growth-related enzymes, defense-related  
371 enzymes and PR proteins by the action of *P. indica*[18, 40]. In all the plants colonized by *P. indica*, an  
372 increase in CAT activity was observed compared to the absolute control and plants infected with ChiLCV  
373 alone (Fig. 2). In all the plants colonized by *P. indica*, an increase in CAT activity was observed compared  
374 to the absolute control and plants infected with ChiLCV alone (Fig. 2). Both *P. indica* pre-inoculated and  
375 post-inoculated plants showed peak activity at 60 DAT against the plants in control. Pre-inoculated plants  
376 showed an increment of 26.64 per cent while post-colonized plants showed an increment of 13 per cent  
377 against the plants with ChiLCV alone. Similarly, a 45 per cent increase was observed in CAT activity in  
378 tomato plants inoculated with *P. indica* than in uninoculated plants[17]. In wheat plants infected with *R.*  
379 *cerealis* and *F. gramineaeureum*, *P. indica* pre-colonization increased the production of CAT [18].



380

381 Fig 1. Effect of *P. indica* colonization on total soluble protein in chilli leaves against ChiLCV

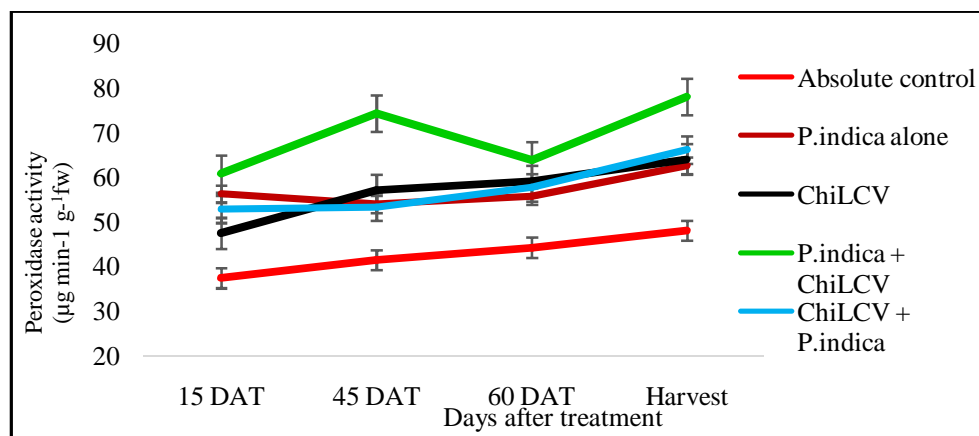


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383 Fig 2. Effect of *P. indica* colonization on catalase activity in chilli leaves against ChiLCV

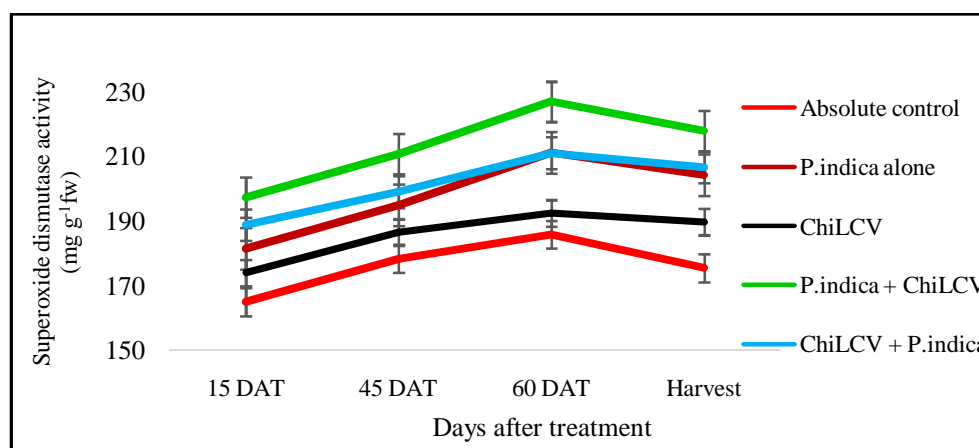
384 Plants pre-colonized by *P. indica* with ChiLCV recorded peak PO activity at 60 DAT. At this point,  
 385 the PO activity was 30 per cent higher than that of plants having ChiLCV alone. Plants with ChiLCV  
 386 displayed a significant increase in peroxidase activity from 15 DAT to 45 DAT, followed by a gradual  
 387 increase. A similar result was recorded when the chilli seeds were primed with PGPRs against chilli  
 388 anthracnose, the quantity of PO rapidly increased to 1.5 times that of the non-primed inoculated plants  
 389 [40]. Increased PO activity was observed in *P. indica* colonized cowpea plants inoculated with BICMV  
 390 [22] and in *P. indica* colonized tomato plants inoculated with ToLCV [24]. All the treatments recorded a  
 391 peak SOD activity at 60 DAT and decreased at the final harvest (Fig. 4). *P. indica* pre-colonized plants  
 392 upon graft transmission showed an 18 per cent increase in SOD activity plants with ChiLCV alone at 60  
 393 DAT. The activity in pre colonized plants was significantly higher than all other treatments throughout the  
 394 time intervals analysed. A gradual increase of SOD activity from PGPR-treated chilli seeds challenge  
 395 inoculated with *Colletotrichum truncatum* from zero hours post inoculation to 48 hours after inoculation  
 396 followed by a subsequent decrease up to 96 h.a.i [41]. A significant increase in SOD activity in soybean  
 397 plants colonized with *P. indica* under salt stress as the endophyte stimulated corresponding genes to  
 398 perform [39]. Both pre-colonized and post-colonized experiments had a significant increase of  
 399 phosphatase activity throughout the period against the plants with ChiLCV alone (Fig. 5). The highest  
 400 activity was recorded in pre-colonized plants (95.95 EU min<sup>-1</sup> g<sup>-1</sup>) against the plants have ChiLCV alone  
 401 (87.4 EU min<sup>-1</sup> g<sup>-1</sup>) at final harvest

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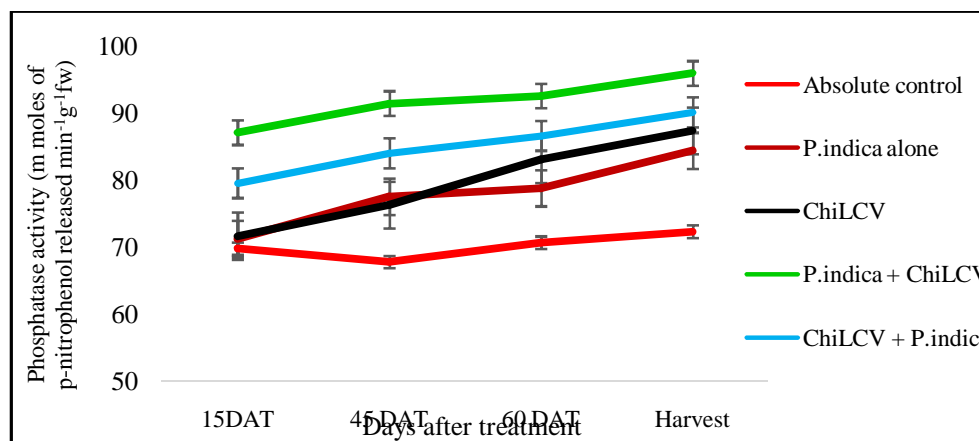
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404 Fig 3. Effect of *P. indica* colonization on peroxidase activity in chilli leaves against ChiLCV



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406 Fig 4. Effect of *P. indica* colonization on superoxide dismutase activity in chilli leaves against ChiLCV



407

408 Fig 5. Effect of *P. indica* colonization on phosphatase activity in chilli leaves against ChiLCV

409 *P. indica* pre-colonized tomato plants showed a significant increase in phosphatase activity upon  
 410 challenge inoculation with ToLCV [24]. This might be due to the enhanced action of phytohormones which  
 411 *P. indica* could trigger. The reprogramming of different fundamental miRNAs and gene expressions in  
 412 barley-*P. indica* interaction was studied [18]. They had identified 42 miRNAs from barley which were

413 expressed as a result of *P. indica* colonization, and they predicted that the target genes of these miRNAs  
414 are mainly involved in transcription, cell division, auxin signal perception and transduction,  
415 photosynthesis, and hormone stimulus. The study conducted in *P. indica* colonized banana plants  
416 challenge inoculated with *Banana Bract Mosaic Virus* (BBrMV) indicated that the endophyte reduces the  
417 symptom expression through the inhibition of viral genes responsible for symptom development,  
418 downregulation of chlorophyll degrading genes, upregulation of chlorophyll synthesizing genes and by  
419 stimulating genes responsible for enhanced growth [42]. A talc based product of *P. indica* was developed  
420 at Amity University, Noida, India named Rootonic, which could be used for seed treatment purpose [43].  
421 However, the development of commercial therapeutics based on *P. indica* is still under process. *P. indica*  
422 is an excellent solution for various biotic and abiotic stresses where the endophyte indirectly counteracts  
423 stress. It will provide more resilience to plants to thrive in adverse conditions by upregulating their  
424 beneficial genetic mechanisms, thereby making them more immune.

## 425 CONCLUSION

426 The colonization of *P. indica* can enhance plant biometric characteristics by activating multiple  
427 biosynthetic pathways. Furthermore, *P. indica* suppresses the production of reactive oxygen species  
428 (ROS) within plant cells, thereby reducing disease severity. These findings suggest that pre-colonization  
429 or priming of *P. indica* could be a valuable strategy for effectively managing chilli leaf curl disease.  
430 Exploitation of *P. indica* in plant stress management also aids in sustainable agriculture goals. Increased  
431 crop production with high quality produce will improve the food and nutritional security of the society also.  
432 Further studies are required in the field of understanding the out-of-sight relationships between the  
433 endophyte and various types of plant stresses. This could yield in better understanding of the mode of  
434 action of endophyte and thereby unlashes the possibilities of formulation of commercial therapeutics in  
435 which those could be efficiently and effortlessly applied to the standing crops by farmers.

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