Role of antioxidants in the management of *Chilli leaf curl virus* in chilliusingbeneficial fungal root endophyte*Piriformosporaindica*

ABSTRACT

Chilli leaf curl virus disease caused by *Chilli leaf curl virus* (ChiLCV) is considered asthe most damage-causing for chilli crop, resulting in a cent per cent yield loss when affected at the early crop stage. *Piriformosporaindica* (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, Padannakkadfrom 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. Chlamyodspores 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.

Keywords: Chilli leaf curl virus: Piriformosporaindica; Endophyte; Reactive Oxygen Species; Begomovirus;

1. INTRODUCTION

Chilli (*Capsicumannum* 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].

Though India is the largest producer of chilli in the world, the production is limited by various pests and diseases. As the major threat, the chilli leaf curl disease was first observed in India Pushkar valley [3]. The presence of *Chilli leaf curl virus* (ChiLCV)was first identified in India in Rajasthan [4]. *Chilli*

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

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

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

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

2. MATERIAL AND METHODS

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

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

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

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

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

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

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

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

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

T_1: Absolute control

T_2: Chilli seedling colonized with *P. indica*

T_3: Graft transmission of the virus by the infected scion

T_4: *P. indica* priming followed by grafting of the virus infected scion after two days

T_5: *P. indica* priming followed by grafting of the virus infected scion after five days

T_6: *P. indica* priming followed by grafting of the virus infected scion after 10 days

T_7: *P. indica* priming followed by grafting of the virus infected scion after 15 days

Plants of T_2 were raised in *P. indica* mass multiplied media. Forty five days old seedlings of T_3 to T_7 were uprooted from the control medium, washed in sterile water and transplanted into *P. indica* mass multiplied medium, all at a time, which serves as rootstock. Wedge grafting was performed [9]with scions of disease severity grade 2 (disease score chart 0-6 of ChiLCV) [25] in time intervals according to treatments (T_4 to T_5). Plants of T_3 were also grafted at the same time, without *P. indica* inoculation. Grafted plants were covered with moist polythene cover to provide humid conditions.The disease severity (Vulnerability Index –V.I.) was calculated from the virus infected plants [26].

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

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

T_1: Absolute control

T_2: Chilli seedling colonized with *P. indica*

T_3: Graft transmission of the virus by the infectedscion

T_4: Graft transmission by the virus infected scion followed by *P.indica*priming after twodays

T_5: Graft transmission by the virus infected scionfollowed by *P.indica*priming after five days

T_6: Graft transmission by the virus infected scion followed by *P.indica*priming after 10 days

T_7: Graft transmission by the virus infected scionfollowed by *P.indica*priming after 15 days

Seedlings required for T_2 were raised in *P. indica* mass multiplied media and all other seedlings were grown in potting mixture without *P. indica*. Wedge grafting was performed [9] in the treatments $T_3$to T_7 at a time. Grafted plants of T_4 to T_7were transplanted to *P.indica* containing potting mixture according to the treatment intended.Observations were taken for disease severity calculation [26].

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

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

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

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

2.3.1 Nitroblue tetrazolium (NBT) and Diaminobenzidine (DAB) staining

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

2.3.2 Analysis of Total Soluble Protein content

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

2.3.3 Analysis of Catalase (CAT) activity

Fresh leaf tissue (1 g) was homogenized in 20 ml of 0.0067 M phosphate buffer and the mixture was centrifuged at 5000 rpm for 15 minutes at 4°C. The sample cuvette was filled with 40 µl of the extract and 3 ml H_2O_2-PO_4 buffer while H_2O_2-free PO_4 buffer was filled in the control cuvette. The time required for the change of absorbance (Δt) by 0.05 at 240 nm was recorded [30].

2.3.4 Analysis of Peroxidase (PO) activity

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

2.3.5 Analysis of superoxide dismutase (SOD) activity

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

2.3.6 Analysis of phosphatase activity

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

3. RESULTS AND DISCUSSION

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

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

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

Table 1. Effect of *P. indica* on germination of chilli seeds var. VellayaniAthulya

Treatment	Days taken to initiate	Days for 50 per cent

	germination	germination
- *P. indica*	10.32 ± 1.15	16.18 ± 0.72
+ *P. indica*	7.42 ± 0.49	10.48 ± 0.61
SE (m) ±	0.33	0.25
CV (0.05)	10.00	5.02

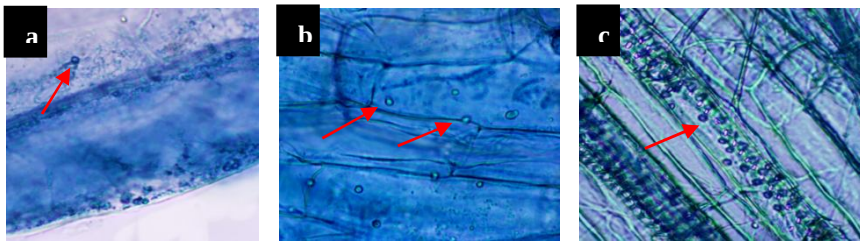


Plate 1.Rootcolonization of *P. indica* in chilli seedlings a) 8 DAC b) 15 DAC c) 30 DAC

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

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

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

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

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

P. indica alone	0	**	
ChiLCV alone	64	12.82 ± 1.07	
P. indica+ ChiLCV (2 days interval)	57	14.37 ±0.91	
P. indica+ ChiLCV (5 days interval))	55	15.4 ± 0.85	
P. indica+ ChiLCV (10 days interval)	42	23.6 ± 0.86	
P. indica+ ChiLCV (15 days interval)	25	30.17 ± 1.71	
SE (m) ±		0.56	
CD (0.05)		1.71	

3.2.2 Potculture experiment with *P. indica* post-colonized seedlings

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

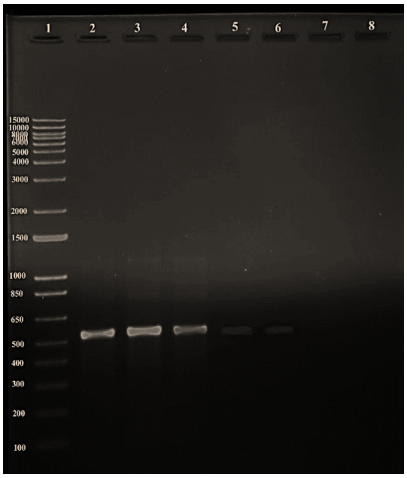
The results suggest that *P. indica* colonization significantly reduced disease occurrence, severity, and time for ChiLCV to appear under green net house conditions. In the study conducted on *Chenopodium* plants, the plants colonized with *P. indica* showed a significant reduction in the number of days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

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

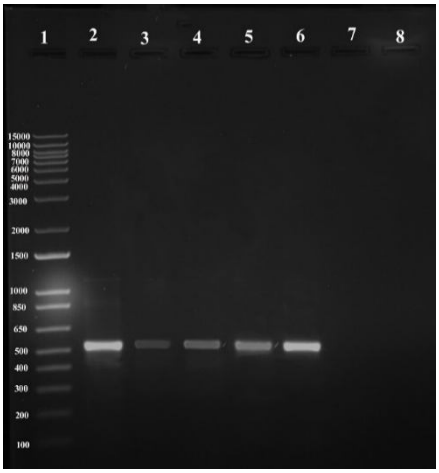
Treatment	V.I.(45 DAT)	Days for symptom appearance (days after treatment)
Healthy (control)	0	**
P. indica alone	0	**
ChiLCV alone	65	11.44 ± 1.23
ChiLCV + *P. indica* (2 days interval)	36	17.65 ± 1.05
ChiLCV + *P. indica* (5 days interval))	42	14.9 ± 1.1
ChiLCV + *P. indica* (10 days interval)	53	13.32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

3.2.3 Confirmation of the presence of begomovirus by PCR reaction

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



520 bp



2 **3.**

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

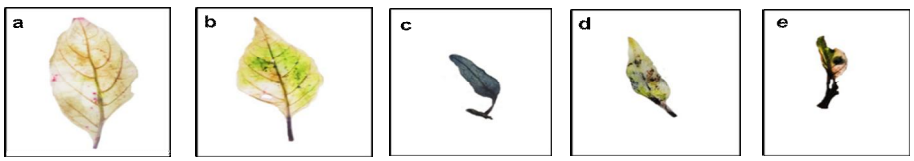
Plate 3. Electrophoresis gel image of amplified coat protein region of ChiLCV using Deng primer. Lane 1) 1 kb marker 2) ChiLCV alone; graft transmission of the virus infected scion followed by *P. indica*-priming after 3) 2 days 4) 5 days 5) 10 days 6) 15 days 7) *P. indica* alone 8) Absolute control

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

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

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

NBT



DAB

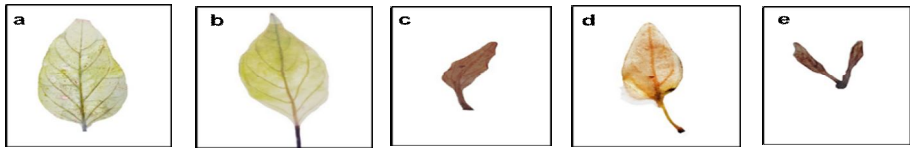


Plate 4. Effect of *P. indica* colonization on ROS accumulation at 15 DAT a) Control b) *P. indica*primed c) ChiLCV alone by graft transmission, d) *P. indica* colonization followed by graft transmission e) graft transmission followed by *P. indica* colonization

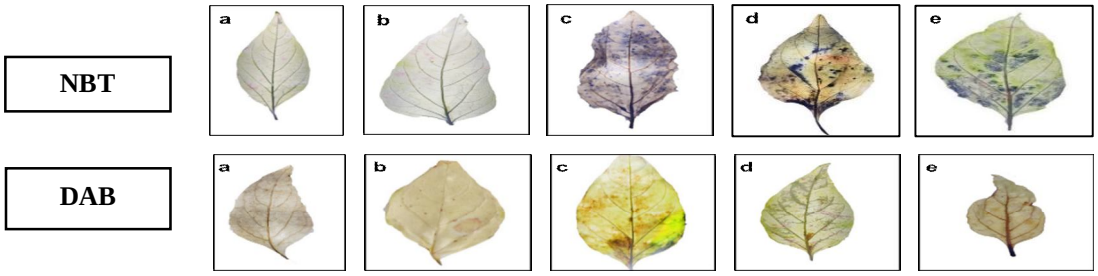


Plate 5. Effect of *P. indica*-colonization on ROS accumulation at 60 DAT a) Control b) *P. indica*-primed c) ChiLCV alone by graft transmission, d) *P. indica* colonization followed by graft transmission e) graft transmission followed by *P. indica* colonization

When ChiLCV is artificially transmitted to chilli plants, those plants colonized by *P. indica* showed a significant decrease in the accumulation of reactive oxygen species (ROS) as assessed through nitroblue tetrazolium (NBT) and diaminobenzidine (DAB) staining for H_2O_2, compared to non-colonized infected plants. The staining intensity of leaves colonized by *P. indica* was less pronounced than non-colonized plants. Non-colonized plants that were inoculated with the virus displayed intense colour development. At the same time, the staining intensity showed a decreasing trend in plants that were colonized before and after the virus challenge inoculation. This suggests a decrease in the accumulation of hydrogen peroxide and superoxide anion in colonized plants. Throughout the tested time intervals, the pre-colonized plants with *P. indica* exhibited a more significant colour intensity reduction than the post-colonized plants. A similar observation was recorded in Alternaria leaf spot on chilli regarding the role of nitric oxide in inducing defence response [38]. They observed that the susceptible cultivar was showing a higher intensity of reddish brown colour upon DAB staining at pathogen penetration sites, which indicated the production of more H_2O_2 at such sites. In contrast, the resistant cultivar shows lesser brown colouration. This suggests that the susceptible cultivar is more prone to cell damage caused by the H_2O_2 than the resistant cultivar. The accumulation of malondialdehyde (MDA), H_2O_2 and O^{2-} were significantly lower in soybean plants colonized with *P. indica* than plants in control (under salt stress), which indicates less oxidative damage in the former plants, which in turn yield lesser colour development when treated with NBT and DAB [39].

3.3.2 Elucidation of ROS scavenging enzymes and total soluble protein content

The total soluble protein content in virus-inoculated plants was recorded higher in all the plants that were colonized with *P. indica* compared to non-colonized plants (Fig. 1). At the final harvest, the total soluble protein content increased significantly in virus-inoculated plants without endophytic colonization. This might be due to this plant's increased accumulation of virus-related proteins. There was a noticeable increase in protein content in *P. indica* pre-colonized plants containing the virus compared to plants with ChiLCV alone. This might be due to the upregulation of growth-related enzymes, defense-related enzymes and PR proteins by the action of *P. indica*[18, 40]. In all the plants colonized by *P. indica*, an increase in CAT activity was observed compared to the absolute control and plants infected with ChiLCV alone (Fig. 2). In all the plants colonized by *P. indica*, an increase in CAT activity was observed compared to the absolute control and plants infected with ChiLCV alone (Fig. 2). Both *P. indica* pre-inoculated and post-inoculated plants showed peak activity at 60 DAT against the plants in control. Pre-inoculated plants showed an increment of 26.64 per cent while post-colonized plants showed an increment of 13 per cent

against the plants with ChiLCV alone. Similarly, a 45 per cent increase was observed in CATactivity in tomato plants inoculated with *P. indica* than in uninoculated plants[17]. In wheat plants infected with *R. cerealis*and *F. graminaeareum, P. indica* pre-colonization increased the production of CAT [18].

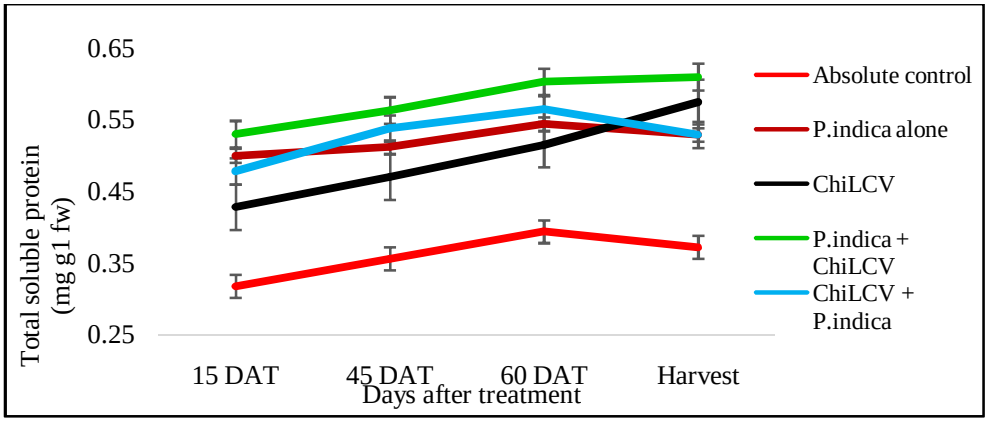


Fig 1. Effect of *P. indica* colonization on total soluble protein in chilli leaves against ChiLCV

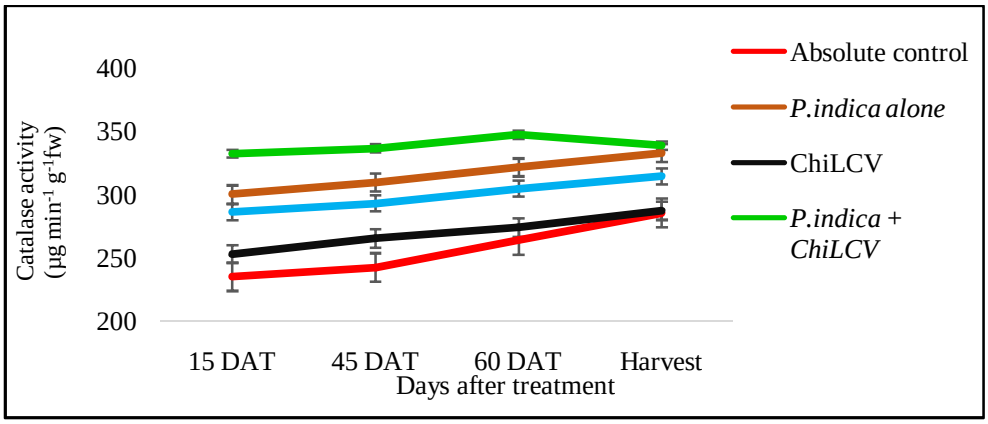


Fig 2. Effect of *P. indica* colonization on catalase activity in chilli leaves against ChiLCV

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

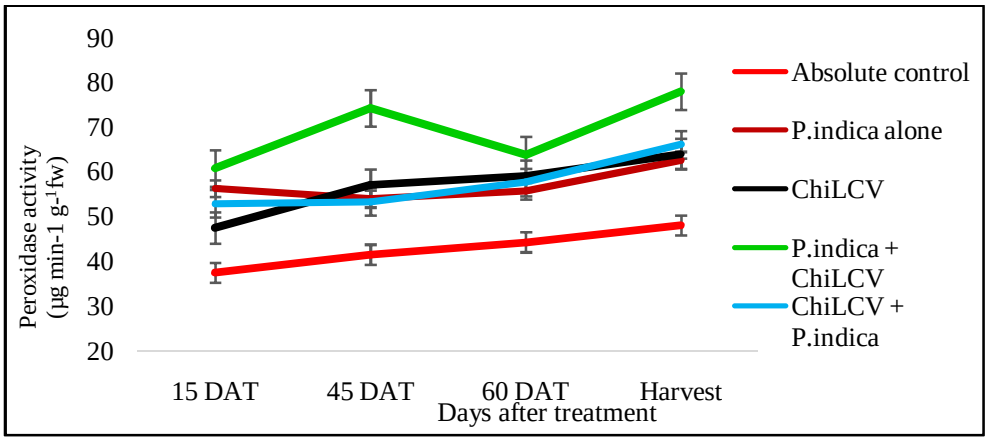


Fig 3. Effect of *P. indica* colonization on peroxidase activity in chilli leaves against ChiLCV

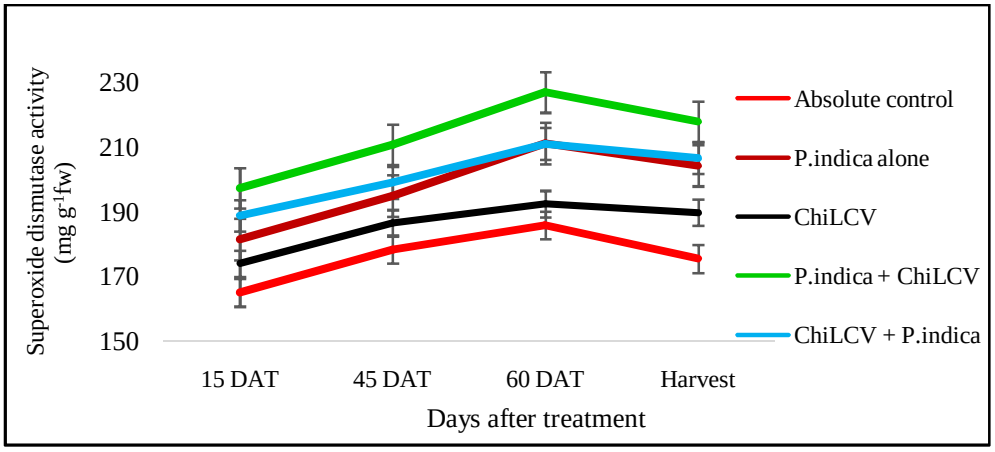


Fig 4. Effect of *P. indica* colonization on superoxide dismutase activity in chilli leaves against ChiLCV

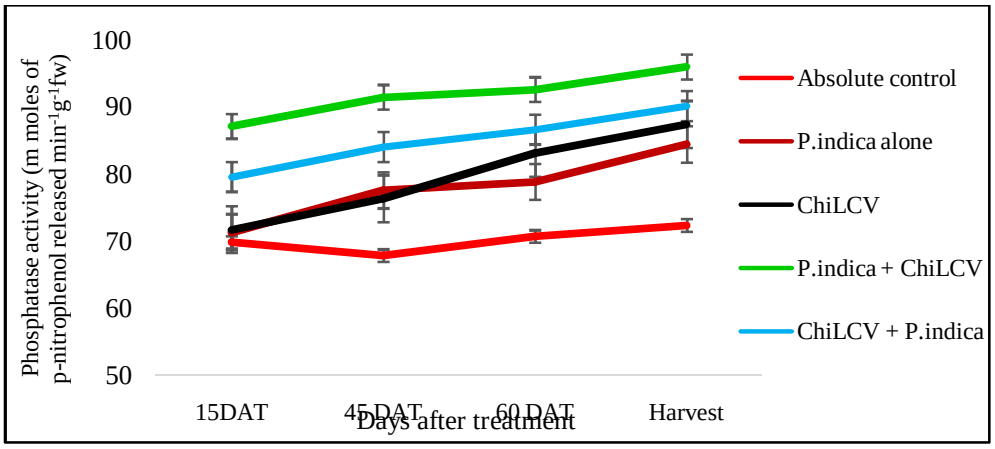


Fig 5. Effect of *P. indica* colonization on phosphatase activity in chilli leaves against ChiLCV

P. indica pre-colonized tomato plants showed a significant increase in phosphatase activity upon challenge inoculation with ToLCV[24].This might be due to the enhanced action of phytohormones which

P. indica could trigger. The reprogramming of different fundamental miRNAs and gene expressions in barley-*P. indica* interaction was studied [18]. They had identified 42 miRNAs from barley which were expressed as a result of *P. indica* colonization, and they predicted that the target genes of these miRNAs are mainly involved in transcription, cell division, auxin signal perception and transduction, photosynthesis, and hormone stimulus.The study conducted in *P. indica* colonized banana plants challenge inoculated with *Banana Bract Mosaic Virus* (BBrMV) indicated that the endophyte reduces the symptom expression through the inhibition of viral genes responsible for symptom development, downregulation of chlorophyll degrading genes, upregulation of chlorophyll synthesizing genes and by stimulating genes responsible for enhanced growth [42]. A talc based product of *P. indica* was developed at Amity University, Noida, India named Rootonic, which could be used for seed treatment purpose [43]. However, the development of commercial therapeutics based on *P. indica* is still under process. *P. indica* is an excellent solution for various biotic and abiotic stresses where the endophyte indirectly counteracts stress. It will provide more resilience to plants to thrive in adverse conditions by upregulating their beneficial genetic mechanisms, thereby making them more immune.

CONCLUSION

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

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Details of the AI usage are given below:

1.

2.

3.

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