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Suppression of Tomato Leaf Curl Virus in Tomato with Enhanced Growth, Yield and Fruit Quality by *Phyllanthus niruri* and *Boerhavia diffusa* under Field Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Aims: To evaluate promising antiviral botanicals such as *Phyllanthus niruri* and *Boerhavia diffusa* at low concentrations for managing tomato leaf curl disease (ToLCD) in tomato plants under pot culture and field conditions.
Study Design: Sequential pot culture and field experiments were conducted using a completely randomised design (CRD) in pot culture and a randomised block design (RBD) in field studies to evaluate pre- and post-treatment of promising antiviral botanicals for the management of ToLCD.
Place and Duration of Study: Pot experiments were conducted at the College of Agriculture, Vellayani, and field experiments were conducted at the College of Agriculture, Vellayani, and Coconut Research Station, Balaramapuram, Kerala, during summer and rabi seasons in 2023 and 2024.
Methodology: *P. niruri* and *B. diffusa* shoot extracts at 0.25, 0.5, 1, 5 and 10 per cent (w/v) were evaluated through pre- and post-application to tomato plants inoculated with tomato leaf curl virus (ToLCV) by wedge grafting using infected scions of disease grade 2. Disease incidence (DI), vulnerability index (VI), virus accumulation, plant growth, chlorophyll content, yield and fruit quality were assessed.
Results: Pre-application of 1 per cent *P. niruri* reduced DI from 100 to 57.69 per cent and VI from 68.34 to 33.12, representing reductions of 42.31 and 51.54 per cent, respectively, compared with the ToLCV control plants. One per cent *B. diffusa* reduced DI and VI by 34.62 and 44.02 per cent, respectively. Post-application of 1 per cent *P. niruri* reduced DI and VI by 34.62 and 41.70 per cent, respectively. One per cent botanical preparations resulted in reductions in DI and VI comparable to those obtained with the 5 and 10 per cent preparations; therefore, 1 per cent was considered practically and economically feasible. Serological and molecular analyses indicated reduced virus accumulation in the treated plants. Under field conditions, pre-application of 1 per cent *P. niruri* reduced DI by 22.72 and 21.95 per cent and VI by 33.07 and 35.87 per cent during summer and rabi, respectively. Botanical treatments also improved chlorophyll content, growth, yield and fruit quality, with the botanicals increasing the number of fruits per plant by 40.5 per cent compared with the control.
Conclusion: *P. niruri* and *B. diffusa* shoot extracts at 1 per cent could significantly reduce the DI and VI of ToLCD and could be used as a potential botanical component in the integrated management of the disease, with pre-application providing greater disease suppression than post-application.

Keywords: Tomato leaf curl virus; botanical extract; Boerhavia diffusa; Phyllanthus niruri; disease incidence; vulnerability index; Bemisia tabaci.

1. Introduction

Tomato *(Solanum lycopersicum L.)* is one of the most widely cultivated vegetable crops because of its high nutritional content, providing lycopene, β-carotene, and ascorbic acid, which are associated with the prevention of chronic diseases (Tilahun *et al.,* 2017). Productivity of tomato is affected by a diverse group of viral pathogens. Among these, whitefly-transmitted (*Bemisia tabaci)* begomoviruses are particularly destructive because infection can occur rapidly within the crop season and disease development is strongly influenced by the abundance and movement of the insect vector (Ghosh & Ghanim, 2021). Seasonal field observations in tomato have further shown that *B. tabaci* incidence varies over the crop period in relation to weather conditions, indicating that vector pressure can fluctuate during cultivation (Tomar *et al.,* 2024).

Tomato leaf curl virus (ToLCV) belongs to the genus Begomovirus of the family Geminiviridae. The circular single-stranded DNA viruses are characterised by twinned icosahedral virions. ToLCV occurs with either monopartite or bipartite genomes, with bipartite viruses possessing two genomic components, DNA-A and DNA-B, each of approximately 2.5–2.8 kb, whereas monopartite viruses contain a single DNA-A-like component (Seal *et al.,* 2006; Glick *et al.,* 2009). Tomato leaf curl disease (ToLCD) is characterised by leaf curling, reduction in leaf expansion, chlorosis, stunting and impaired reproductive development, with the severity of losses depending on the virus strain, host genotype, infection stage and environmental conditions (Mahmoud *et al.,* 2025).

Management of ToLCD is consequently challenging. The diversity in genome organisation, frequent recombination and pseudo-recombination, broad host range and efficient whitefly-mediated transmission contribute to the epidemiological complexity and management challenges associated with ToLCD (Saunders & Stanley, 1999). Conventional strategies have largely focused on reducing vector populations through insecticides, the use of resistant cultivars, cultural practices and combinations of these approaches. Integrated management studies have demonstrated that combining complementary approaches can provide greater disease suppression than reliance on a single measure. Further, insecticide-intensive management can exert selection pressure on vector populations and may not provide adequate protection once virus inoculation has occurred (Chatchawankanphanich & Maxwell, 2002; Prasanna & Rai, 2007; Pandey *et al.,* 2010). Recent fieldwork has also evaluated integrated treatment options aimed at reducing whitefly populations and ToLCV incidence in tomato (Gangele *et al.,* 2026).

An alternative approach is the use of plant-derived preparations capable of directly interfering with virus infection or multiplication, or indirectly activating host defence responses (Harb *et al.,* 2025). Plant-derived products are increasingly being investigated as sources of antiviral molecules and resistance-inducing compounds. Exogenous salicylic acid and jasmonic acid have been shown to enhance resistance against tomato yellow leaf curl virus (TYLCV), accompanied by changes in defence-associated signalling and gene expression (Wang *et al.,* 2022). More recently, plant-derived phytochemicals have also been investigated as candidate inhibitors of begomovirus proteins, highlighting the possibility of developing botanical compounds that act directly on virus-associated processes (Xu *et al.,* 2025). Hiremath *et al.* (2025) identified potential plant secondary metabolites targeting the βC1 virulence factor. Similarly, Sarkar *et al.* (2026) demonstrated the antiviral activity of the plant-derived phytochemical agathisflavone against tomato leaf curl New Delhi virus (ToLCNDV). Induced resistance has also been explored as a complementary approach, with *Beauveria bassiana*-derived proteins reported to reduce TYLCV disease severity in tomato (Basit *et al.,* 2024**).**

Among the antiviral botanicals, *Phyllanthus niruri* and *Boerhavia diffusa* have attracted considerable attention because of their diverse phytochemical composition and documented antiviral properties. Earlier studies demonstrated the antiviral activity of *P. niruri* extracts against animal viruses, including inhibition of hepatitis B virus-associated processes, providing evidence for the presence of biologically active antiviral constituents in the plant. Although these studies concern animal viruses, they support the broader antiviral potential of the species and provide a rationale for investigating its activity against plant viruses (Awasthi & Singh, 2015; Alex, 2017; Chandran, 2019). Aqueous root extracts of *B. diffusa* were reported to possess broad-spectrum antiviral activity against several plant viruses, and the antiviral effect was associated with a biologically active inhibitor present in the root extract. In addition, compounds derived from *B. diffusa*, including boeravinones, have demonstrated antiviral activity, further emphasising the phytochemical richness of this species (Najam *et al.,* 2017; Chandran, 2019).

The possibility that botanical treatments may influence both the virus and its insect vector population is particularly relevant to ToLCD. Tomato flavonoids reduce *B. tabaci* landing, settling and phloem feeding, thereby reducing virus acquisition, incubation and transmission and, consequently, the primary and secondary spread of TYLCV. This suggests that plant-derived compounds can potentially contribute to disease suppression through multiple mechanisms, including direct antiviral activity, induction of host resistance and interference with vector behaviour (Yang *et al.,* 2023; Hussein *et al.,* 2025). Plant-derived volatiles can also influence the virus-vector interaction; D-limonene altered *B. tabaci* feeding behaviour and reduced TYLCV acquisition and transmission in experimental assays (Wei *et al.,* 2024).

Despite these advances, there remains limited information on the systematic evaluation of *P. niruri* and *B. diffusa* against ToLCV using a progression from concentration optimisation to field validation and virus inhibition. The present investigation, therefore, aimed to determine effective concentrations of *P. niruri* and *B. diffusa* for the management of ToLCV and compare their efficacy when applied before and after virus inoculation; to determine the effects on disease development, plant growth, chlorophyll content, yield and fruit quality; and to validate the most effective concentration against the natural incidence of the disease during rabi and summer seasons under field conditions.

2. Materials and Methods

The experiments were conducted on tomato plants at the College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India. Tomato plants of the cultivar Vellayani Vijai, which is highly susceptible to ToLCV, were used throughout the experiments. Plants were maintained under recommended agronomic practices (Kerala Agricultural University [KAU], 2016).

2.1 Evaluation of the Promising Antiviral Botanicals, *Phyllanthus niruri* and *Boerhavia diffusa* against Tomato Leaf Curl Virus under Controlled Conditions

A pot culture experiment was conducted to evaluate the efficacy of *P. niruri* and *B. diffusa* at different concentrations against ToLCV following artificial inoculation through wedge-grafting. The treatments were imposed before and after virus inoculation to determine the effectiveness of the botanicals at different concentrations. Five aqueous concentrations of the antiviral botanicals, namely 0.25, 0.5, 1.0, 5.0 and 10.0 per cent (w/v), were evaluated.

Thirty-day-old tomato seedlings were used for the experiment. Plants were maintained under protected and insect-proof conditions and were subjected to ToLCV inoculation through wedge-grafting using the infected scions obtained from ToLCV-infected tomato plants exhibiting disease grade 2. Scions possessing two to three actively growing axillary buds were selected from the symptomatic plants. The apical portion of the healthy tomato seedling used as rootstock was removed horizontally, retaining at least two nodes. A vertical incision was then made at the cut end of the rootstock. The basal end of the infected scion was shaped into a wedge and carefully inserted into the incision of the rootstock. The graft union was secured firmly using grafting tape/polythene strips and covered with a transparent polythene bag for approximately 3–4 days to facilitate graft union formation and minimise desiccation.

a. Prophylactic or pre-treatment with antiviral botanicals followed by artificial inoculation of tomato leaf curl virus by wedge-grafting

For the prophylactic or protective or pre-treatment, the respective concentrations of *P. niruri* and *B. diffusa* aqueous preparations were applied to healthy tomato seedlings prior to ToLCV inoculation. The botanical treatments were imposed as foliar sprays on the 12th and 30th days after sowing (DAS), followed by wedge-grafting with ToLCV-infected scions 2 days after the final spraying as described above. The treatments were designated as follows:

T_1 : 0.25 per cent *P. niruri* followed by wedge-grafting inoculation of ToLCV
T_2 : 0.25 per cent *B. diffusa* followed by wedge-grafting inoculation of ToLCV
T_3 : 0.5 per cent *P. niruri* followed by wedge-grafting inoculation of ToLCV
T_4 : 0.5 per cent *B. diffusa* followed by wedge-grafting inoculation of ToLCV
T_5 : 1 per cent *P. niruri* followed by wedge-grafting inoculation of ToLCV
T_6 : 1 per cent *B. diffusa* followed by wedge-grafting inoculation of ToLCV
T_7 : 5 per cent *P. niruri* followed by wedge-grafting inoculation of ToLCV
T_8 : 5 per cent *B. diffusa* followed by wedge-grafting inoculation of ToLCV
T_9 : 10 per cent *P. niruri* followed by wedge-grafting inoculation of ToLCV
T_{10} : 10 per cent *B. diffusa* followed by wedge-grafting inoculation of ToLCV
T_{11} : Graft transmission of ToLCV without botanical treatment
T_{12} : Absolute control (without botanical treatment and ToLCV inoculation)

b. Pre-inoculation of tomato leaf curl virus by wedge-grafting followed by curative or post-treatment with antiviral botanicals

To evaluate the curative or therapeutic or post-treatment effect of the antiviral botanicals, healthy 30-day-old tomato seedlings were first inoculated with ToLCV through wedge-grafting using infected scions exhibiting disease grade 2. The respective concentrations of *P. niruri* and *B. diffusa* were subsequently applied as two foliar sprays at 2 and 20 days after wedge-grafting. The post-treatments of the antiviral botanicals were designated as follows:

T_1 : Wedge grafting of ToLCV followed by spraying of 0.25 per cent *P. niruri*
T_2 : Wedge grafting of ToLCV followed by spraying of 0.25 per cent *B. diffusa*
T_3 : Wedge grafting of ToLCV followed by spraying of 0.5 per cent *P. niruri*
T_4 : Wedge grafting of ToLCV followed by spraying of 0.5 per cent *B. diffusa*
T_5 : Wedge grafting of ToLCV followed by spraying of 1 per cent *P. niruri*
T_6 : Wedge grafting of ToLCV followed by spraying of 1 per cent *B. diffusa*
T_7 : Wedge grafting of ToLCV followed by spraying of 5 per cent *P. niruri*
T_8 : Wedge grafting of ToLCV followed by spraying of 5 per cent *B. diffusa*
T_9 : Wedge grafting of ToLCV followed by spraying of 10 per cent *P. niruri*
T_{10} : Wedge grafting of ToLCV followed by spraying of 10 per cent *B. diffusa*
T_{11} : Wedge grafting of ToLCV without botanical treatment
T_{12} : Absolute control (without botanical treatment and ToLCV inoculation)

The botanical preparations were applied using the standardised method of Chandran (2019), in which aqueous solutions containing the respective percentages of shoot extracts were used. Each treatment consisted of 13 plants, and the experiment was maintained under insect-proof conditions to prevent accidental transmission of ToLCV by insect vectors.

2.2 Assessment of Tomato Leaf Curl Disease Development

The treated plants were observed periodically for the appearance of characteristic ToLCV symptoms. The number of days required for the first appearance of symptoms following the virus inoculation was recorded for each treatment. Disease incidence (DI) was calculated as the number of plants exhibiting visible symptoms relative to the total number of plants assessed.

$$\text{Disease incidence (DI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

The disease severity was assessed using the vulnerability index (VI) based on the 0–5 disease grading scale developed for ToLCV assessment (Bos, 1982). The grades were assigned according to the severity of visible symptoms as follows:

0 - No symptom
1- Yellow speckle on leaf
2- Initial leaf curling and chlorosis
3- Severe leaf curling and blister development
4- Purplish discolouration
5- Stunted growth with negligible or no flowering, small sized leaves and fruit

$$\text{Vulnerability Index (VI)} = \frac{(0n_0+1n_1+2n_2+3n_3+4n_4+5n_5)}{n_t(n_c-1)} \times 100$$

$n_0, n_1 \ldots\ldots n_5$ - number of plants in the category of 0, 1, 2, 3, 4, 5
n_t - total number of plants
n_c - number of categories

2.3 Field Studies with Antiviral Botanical-treated Plants under Natural Incidence of Tomato Leaf Curl Virus Disease

Field experiments were conducted to evaluate the efficacy of the selected concentration of *P. niruri* and *B. diffusa* against the natural incidence of ToLCV at the Instructional Farm, College of Agriculture, Vellayani, and Coconut Research Station, Balaramapuram during the rabi and summer seasons (2023 and 2024). The concentrations selected for field evaluation were based on the results of the pot culture experiment. Separate experimental blocks were maintained for each treatment to avoid treatment interference.

Thirty-day-old seedlings were transplanted to the field at a spacing of 60 cm × 60 cm. The treatments selected for field evaluation were as follows:

T_1 : best treatment of *P. niruri* from prophylactic or protective or pre-treatment of the botanical studies
T_2 : best treatment of *B. diffusa* from prophylactic or protective or pre-treatment of the botanical studies
T_3 : best treatment of *P. niruri* from curative or therapeutic or post-treatment of the botanical studies
T_4 : best treatment of *B. diffusa* from curative or therapeutic or post-treatment of the botanical studies
T_5 : control

The respective botanical preparations of *P. niruri* and *B. diffusa* at 1 per cent concentration (w/v) were applied as foliar sprays at 5 and 25 days after transplanting (DAT) for pre-application and at 5 and 25 days after ToLCV symptoms were expressed in 5 per cent of tomato plants in the field for post-application. All other agronomic and intercultural operations were carried out according to the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016). Two independent experiments were conducted during each season at each location. Fifteen plants were maintained as replicates for each treatment in each season. Biometric observations, including the days required for initiation of flowering and the number of fruits per plant, were recorded. In addition to ToLCD, the incidence of *B. tabaci* and other pests was also recorded to assess the influence of antiviral botanicals under natural field conditions. Data obtained from the independent experiments were subjected to analysis of variance using a pooled randomised block design (RBD). Treatment means were compared using the appropriate statistical test at the 5 per cent level of significance.

2.4 Serological Detection and Relative Quantification of ToLCV

The accumulation of ToLCV in antiviral botanical-treated and untreated plants was assessed using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). A polyclonal antiserum specific to ToLCV obtained from DSMZ, Leibniz-Institut, Germany, was used for virus detection. The primary antibody was diluted at the recommended dilution of 1:1000 in carbonate coating buffer containing Na_2CO_3, $NaHCO_3$ and NaN_3, adjusted to pH 9.6. Approximately 200 µL of the prepared antibody solution was dispensed into each well of a microtitre plate and incubated at 37°C for 2–4 h. Following the incubation, the wells were washed thoroughly with phosphate-buffered saline containing Tween-20 (PBS-T). Leaf samples were homogenised in extraction buffer consisting of PBS-T supplemented with 2 per cent polyvinyl pyrrolidone (PVP). The prepared plant extracts were added to the antibody-coated wells and incubated overnight at 4°C. After washing, the enzyme-conjugated antibody was added, and the plates were incubated at 37°C for 2–4 h. Following another washing step, 200 µL of freshly prepared substrate solution containing *p*-nitrophenyl phosphate in substrate buffer comprising diethanolamine and NaN_3 was added to each well. The reaction was allowed to develop at 37°C for 30–60 min. Absorbance was subsequently recorded at 405 nm using an ELISA microplate reader. The absorbance values obtained from botanical-treated plants were compared with those of ToLCV-infected untreated plants to determine the relative reduction in virus accumulation.

2.5 Molecular Detection of ToLCV by PCR

Molecular detection of ToLCV was carried out using total genomic DNA extracted from leaf samples of the virus-inoculated tomato plants. DNA extraction was performed using a modified cetyltrimethylammonium bromide (CTAB) protocol. PCR amplification was performed using universal begomovirus primers, including Deng and AV/AC, along with ToLCV-specific primers. The primer sequences, expected amplicon sizes and corresponding target regions were presented in Table 1. The PCR reaction was performed in a final volume of 10 µL containing 5 µL of 10× PCR master mix, 0.5 µL each of forward and reverse primers at a concentration of 10 pM, 1 µL of template DNA containing approximately 50 ng DNA and nuclease-free water to make up the final volume. The amplification was carried out in a thermal cycler (BIO-RAD, USA). The reaction cycle consisted of an initial denaturation at 94°C for 4 min, followed by 32 cycles of denaturation at 94°C for 30 s, primer annealing for 45 s and extension at 72°C for 30 s. A final extension was performed at 72°C for 7 min. The amplified products were separated by electrophoresis on 1.2 per cent agarose gel and visualised using a gel documentation system (Bio-Rad Gel Doc™ XR+ with Image Lab™ software, USA). The presence or absence of ToLCV-specific amplification was compared among botanical-treated, virus-inoculated control and healthy control plants.

Table 1. Primer details used for the detection of tomato leaf curl virus

Gene	Primers		Tm (°C)	Amplicon size (bp)
Deng	LP	TAATATTACCKGWKGVCCSC	55.4°C	520
	RP	TGGACYTTRCAWGGBCCTTCACA		
AV/AC	LP	GCCHATRTAYAGRAAGCCMAGRAT	54.6°C	575
	RP	GGRTTDGARGCATGHGTACANGCC		
ToLCV	LP	GCCCTCAACCAATGAAATTCAC	57°C	1180
	RP	GAGAGTCTTCAAAACCCAGGTCC		

2.6 Estimation of Total Chlorophyll Content

Chlorophyll content was determined according to the procedure of Arnon (1949). Fresh leaf tissue (1 g) collected from each treatment in the field study was homogenised thoroughly with 20 mL of 80 per cent acetone. The homogenate was centrifuged at 5000 rpm for 5 min, and the clear supernatant was collected in a 100 mL volumetric flask. The remaining residue was repeatedly extracted with 80 per cent acetone, followed by centrifugation, until the residue became completely colourless. The combined extracts were made up to 100 mL with 80 per cent acetone. Absorbance was recorded at 645 and 663 nm using 80 per cent acetone as the blank. Total chlorophyll, chlorophyll a and chlorophyll b contents were calculated according to the following equations:

$$\text{Chlorophyll a (mg/g)} = \frac{12.7\,(A663) - 2.69\,(A645) \times V}{1000 \times W}$$

$$\text{Chlorophyll b (mg/g)} = \frac{22.9\,(A645) - 4.68\,(A663) \times V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/g)} = \frac{20.2\,(A645) + 8.02\,(A663) \times V}{1000 \times W}$$

V - final volume of chlorophyll extract, W - fresh weight of the leaves

2.7 Determination of Fruit Quality Parameters

2.7.1 Estimation of Fruit pH

The pH of fully ripe tomato fruits was determined using a calibrated digital pH meter. A 10 g sample of pericarp tissue was homogenised with 20 mL of distilled water using a chilled mortar and pestle. The homogenate was passed first through two layers of muslin cloth and subsequently through Whatman No. 1 filter paper. The pH of the resulting filtrate was measured using the calibrated pH meter.

2.7.2 Estimation of Total Soluble Solids

The total soluble solids (TSS) of ripe tomato fruits were determined using a digital hand-held refractometer and expressed in °Brix. Fresh fruit pulp was gently crushed to obtain juice, which was filtered through muslin cloth to remove suspended tissue particles. A drop of the clarified juice was placed on the prism of the refractometer, and the TSS reading was recorded.

2.7.3 Estimation of Ascorbic Acid

Ascorbic acid content was estimated following the titrimetric procedure of Harris and Olliver (1942). Fresh tomato pulp weighing 10 g was homogenised in 3 per cent meta-phosphoric acid and the volume was adjusted to 100 mL. The homogenate was filtered through Whatman No. 1 filter paper, and a 10 mL aliquot of the filtrate was titrated against standardised 2,6-dichlorophenolindophenol (DCPIP) solution. The end point was taken as the appearance of a light pink colour that persisted for approximately 15 s. Ascorbic acid content was calculated from the titre value and expressed as mg 100 g^{-1} fresh weight (FW).

2.7.4 Estimation of Lycopene

Lycopene content was estimated following the method of Nagata and Yamashita (1992). Fresh tomato pericarp (1 g) was homogenised with 8 mL of a hexane:acetone:ethanol solvent mixture in a 2:1:1 (v/v/v) ratio. The homogenate was mixed thoroughly for 15 min under reduced-light conditions. Subsequently, 2 mL of distilled water was added to promote phase separation. The mixture was allowed to stand until distinct organic and aqueous phases were obtained. The upper hexane layer containing the extracted carotenoids was carefully collected, and its absorbance was measured at 503 nm using a UV-visible spectrophotometer with hexane as the blank. Lycopene content was expressed as mg 100 g^{-1} fresh weight.

2.7.5 Estimation of β-Carotene

The β-carotene content was determined from the hexane extract prepared for carotenoid estimation. Absorbance was recorded at 453, 505 and 663 nm using a UV–visible spectrophotometer. The β-carotene concentration was calculated and expressed as mg 100 g^{-1} fresh weight.

2.8 Statistical Analysis

The experimental data obtained for DI, VI, symptom development, plant growth, chlorophyll content, fruit quality and virus detection assays were subjected to statistical analysis using RASINS. Analysis of variance (ANOVA) was performed according to the experimental design adopted. Treatment means were compared using p-values at the 5 per cent probability level.

3. Results

3.1 Prophylactic or Pre-treatment Effect of Different Concentrations of Promising Antiviral Botanicals, *P. niruri* and *B. diffusa* on Tomato Leaf Curl Virus

The prophylactic or pre-treatment effect of different concentrations of *P. niruri* and *B. diffusa* on ToLCV-inoculated plants was assessed based on the time required for symptom development, DI and VI. The response varied with the concentration of the

botanicals (Table 2). In *P. niruri*-treated plants, symptom appearance was delayed from 15.36 days at 0.25 per cent to 17.24 days at 0.5 per cent and 21.18 days at 1 per cent. Further increase in concentration to 5 and 10 per cent resulted in symptom appearance at 21.46 and 21.62 days, respectively. In *B. diffusa*, symptoms appeared at 14.78 days at 0.25 per cent, 16.42 days at 0.5 per cent and 19.64 days at 1 per cent. At 5 and 10 per cent, symptom development occurred at 19.82 and 20.06 days, respectively. The ToLCV-alone treatment recorded symptom appearance at 12.42 days.

Table 2. Effect of different concentrations of *Phyllanthus niruri* and *Boerhavia diffusa* on its prophylactic or pre-application on symptom development, disease incidence and vulnerability index of tomato leaf curl disease caused by ToLCV inoculation through wedge-grafting

Treatments		Days taken for symptom development	Disease incidence (%) at 75 DAS	Vulnerability index at 75 DAS
T_1	0.25 % *P. niruri* + ToLCV	$15.36^{e} \pm 1.35$	$88.46^{f} \pm 2.18$	$55.28^{e} \pm 5.72$
T_2	0.25 % *B. diffusa* + ToLCV	$14.78^{e} \pm 1.72$	$92.31^{g} \pm 2.78$	$59.46^{f} \pm 6.91$
T_3	0.5 % *P. niruri* + ToLCV	$17.24^{c} \pm 1.13$	$73.08^{d} \pm 3.45$	$45.72^{c} \pm 7.38$
T_4	0.5 % *B. diffusa* + ToLCV	$16.42^{d} \pm 1.98$	$80.77^{e} \pm 3.98$	$50.36^{d} \pm 4.57$
T_5	1 % *P. niruri* + ToLCV	$21.18^{a} \pm 1.44$	$57.69^{b} \pm 5.22$	$33.12^{a} \pm 5.74$
T_6	1 % *B. diffusa* + ToLCV	$19.64^{b} \pm 1.89$	$65.38^{c} \pm 8.14$	$38.26^{b} \pm 5.91$
T_7	5 % *P. niruri* + ToLCV	$21.46^{a} \pm 1.21$	$53.85^{a} \pm 4.42$	$32.78^{a} \pm 4.69$
T_8	5 % *B. diffusa* + ToLCV	$19.82^{b} \pm 1.63$	$61.54^{b} \pm 6.94$	$38.02^{b} \pm 6.88$
T_9	10 % *P. niruri* + ToLCV	$21.62^{a} \pm 1.36$	$53.85^{a} \pm 5.12$	$32.46^{a} \pm 5.86$
T_{10}	10 % *B. diffusa* + ToLCV	$20.06^{b} \pm 1.00$	$60.00^{b} \pm 7.14$	$37.84^{b} \pm 6.02$
T_{11}	ToLCV alone	$12.42^{f} \pm 1.66$	$100.00^{h} \pm 0.00$	$68.34^{g} \pm 5.41$
T_{12}	Absolute control	–	0.00 ± 0.00	0.00 ± 0.00
	SE (m)	0.29	0.98	1.17
	CD (0.05)	0.82	2.74	3.26

Botanicals sprayings and ToLCV inoculation was done to the tomato plants as per the methods. The values are mean of 26 plants ± standard deviation. Two independent experiments were conducted. Superscripts with same letters indicate on-par values, and those with different letters indicate a significant difference at the 5 per cent level of significance. Pooled CRD. SE: standard error. CD: critical difference at the 5% level of significance. DAS: days after sowing. ToLCV: tomato leaf curl virus

ToLCD incidence showed a reduction with increasing concentration up to 1 per cent. In *P. niruri*, the DI decreased from 88.46 per cent at 0.25 per cent to 73.08 per cent at 0.5 per cent and 57.69 per cent at 1 per cent at 75 DAS. The incidence remained at 53.85 per cent at both 5 and 10 per cent. In *B. diffusa*, the corresponding incidence values were 92.31, 80.77 and 65.38 per cent at 0.25, 0.5 and 1 per cent, respectively. The incidence at 5 and 10 per cent was 61.54 and 60.00 per cent, respectively. In comparison, all plants in the ToLCV-alone treatment developed disease (Table 2).

ToLCD severity assessed as VI followed the same pattern. The VI in *P. niruri*-treated plants decreased from 55.28 at 0.25 per cent to 45.72 at 0.5 per cent and 33.12 at 1 per cent at 75 DAS. The VI values recorded at 5 and 10 per cent were 32.78 and 32.46 per cent, respectively. In *B. diffusa*, VI decreased from 59.46 at 0.25 per cent to 50.36 at 0.5 per cent and 38.26 at 1 per cent, followed by 38.02 and 37.84 at 5 and 10 per cent. The ToLCV-alone treatment recorded the highest VI of 68.34. The disease parameters recorded at 1, 5 and 10 per cent were comparable, particularly for DI and VI (Table 2; Plate 1). Hence, 1 per cent was taken forward for the subsequent studies rather than increasing the botanical concentration further.

Plate 1. Effect of pre- and post-treatment of 1 per cent *P. niruri* and *B. diffusa* on symptom severity of tomato leaf curl disease in tomato plants against artificial inoculation of ToLCV through wedge-grafting

3.2 Curative or Post-treatment Effect of Different Concentrations of Promising Antiviral Botanicals, *P. niruri* and *B. diffusa* on Tomato Leaf Curl Virus

The botanicals were also applied after ToLCV inoculation at different concentrations. In *P. niruri*, symptom appearance was recorded at 14.24, 16.12 and 20.08 days at 0.25, 0.5 and 1 per cent, respectively. The corresponding values at 5 and 10 per cent were 20.24 and 20.46 days. With *B. diffusa*, symptoms appeared after 13.68 days at 0.25 per cent, 15.36 days at 0.5 per cent and 19.02 days at 1 per cent. At 5 and 10 per cent, symptom appearance was recorded at 19.18 and 19.42 days, respectively.

At 0.25 per cent *P. niruri*, DI was 92.31 per cent, which decreased to 88.46 per cent at 0.5 per cent and 65.38 per cent at 1 per cent. The incidence remained at 65.38 per cent at 5 per cent; whereas DI was 61.54 per cent at 10 per cent. For *B. diffusa*, DI decreased from 96.15 per cent at 0.25 per cent to 84.62 per cent at 0.5 per cent and 73.08 per cent at 1 per cent. The values at 5 and 10 per cent were 69.23 per cent (Table 3).

Table 3. Effect of different concentrations of *Phyllanthus niruri* and *Boerhavia diffusa* on its curative or post-application on symptom development, disease incidence and vulnerability index of tomato leaf curl disease caused by ToLCV inoculation through wedge-grafting

Treatments		Days taken for symptom development	Disease incidence (%) at 75 DAS	Vulnerability index at 75 DAS
T_1	ToLCV + 0.25 % *P. niruri*	$14.24^{f} \pm 1.48$	$92.31^{g} \pm 2.58$	$59.84^{e} \pm 4.76$
T_2	ToLCV 0.25 % + *B. diffusa*	$13.68^{f} \pm 1.55$	$96.15^{h} \pm 2.58$	$62.16^{e} \pm 4.83$
T_3	ToLCV + 0.5 % *P. niruri*	$16.12^{e} \pm 1.61$	$88.46^{f} \pm 3.65$	$51.28^{c} \pm 4.42$
T_4	ToLCV + 0.5 % *B. diffusa*	$15.36^{e} \pm 1.57$	$84.62^{e} \pm 3.65$	$54.72^{d} \pm 4.51$
T_5	ToLCV + 1 % *P. niruri*	$20.08^{a} \pm 1.72$	$65.38^{b} \pm 5.212$	$39.84^{a} \pm 3.82$
T_6	ToLCV + 1 % *B. diffusa*	$19.02^{b} \pm 1.66$	$73.08^{d} \pm 4.14$	$43.72^{b} \pm 3.96$
T_7	ToLCV + 5 % *P. niruri*	$20.24^{a} \pm 1.78$	$65.38^{b} \pm 3.01$	$39.56^{a} \pm 3.88$
T_8	ToLCV + 5 % *B. diffusa*	$19.18^{b} \pm 1.69$	$69.23^{c} \pm 4.14$	$43.48^{b} \pm 4.02$
T_9	ToLCV + 10 % *P. niruri*	$20.46^{a} \pm 1.75$	$61.54^{a} \pm 3.67$	$39.18^{a} \pm 3.94$
T_{10}	ToLCV +10 % *B. diffusa*	$19.42^{b} \pm 1.71$	$69.23^{c} \pm 4.14$	$43.26^{b} \pm 4.08$
T_{11}	ToLCV alone	$12.42^{g} \pm 1.56$	$100.00^{i} \pm 0.00$	$68.34^{f} \pm 5.41$
T_{12}	Absolute control		0.00 ± 0.00	0.00 ± 0.00
	SE (m)	0.32	0.71	0.85
	CD (0.05)	0.91	2.02	2.42

Botanicals sprayings and ToLCV inoculation was done to the tomato plants as per the methods. The values are mean of 26 plants ± standard deviation. Two independent experiments were conducted. Superscripts with same letters indicate on-par values, and those with different letters indicate a significant difference at the 5 per cent level of significance. Pooled CRD. SE: standard error. CD: critical difference at the 5% level of significance. DAS: days after sowing. ToLCV: tomato leaf curl virus

A reduction in tomato leaf curl disease severity, assessed as VI, was recorded with increasing concentrations of the botanicals. *P. niruri* recorded VI values of 59.84, 51.28 and 39.84 at 0.25, 0.5 and 1 per cent, respectively, at 75 DAS. The values at 5 and 10 per cent were 39.56 and 39.18. In *B. diffusa*, VI decreased from 62.16 at 0.25 per cent to 54.72 at 0.5 per cent and 43.72 at 1 per cent, followed by 43.48 and 43.26 at 5 and 10 per cent. The ToLCV-alone treatment recorded a VI of 68.34. The DI and VI values at 5 and 10 per cent were close to those obtained with 1 per cent for both botanicals. Therefore, 1 per cent was selected as the concentration for the field studies (Table 3; Plate 1).

Virus accumulation was assessed by DAS-ELISA at 75 DAS for the best treatments from pre- and post-application of botanicals. The OD values at 405 nm were lower in botanical-treated plants than in the ToLCV control. In the pre-application treatment, *P. niruri* recorded an OD value of 0.046, while the corresponding value for *B. diffusa* was 0.061. Following post-application, the OD values for *P. niruri* and *B. diffusa* were 0.079 and 0.098, respectively. The ToLCV control recorded an OD value of 0.149 (Table 4).

Table 4. Effect of pre- and post-application of 1 per cent *P. niruri* and *B. diffusa* on ToLCV titre assessed by DAS-ELISA on tomato plants artificially inoculated with ToLCV through wedge-grafting at 75 DAS

Treatments		OD value at 405 nm
T_1	1 % *P. niruri* + ToLCV	$0.046^{a} \pm 0.002$
T_2	1 % *B. diffusa* + ToLCV	$0.061^{b} \pm 0.004$
T_3	ToLCV + 1 % *P. niruri*	$0.079^{c} \pm 0.004$
T_4	ToLCV + 1 % *B. diffusa*	$0.098^{d} \pm 0.005$
T_5	ToLCV alone	$0.149^{e} \pm 0.005$
T_6	Absolute control	0.021 ± 0.001
T_7	Blank	0.014 ± 0.001
	SE(m) ±	0.001
	CD (0.05)	0.004

The values are the mean of nine OD values ± standard deviation. Two independent experiments were conducted. Superscripts with same letters indicate on-par values, and those with different letters indicate a significant difference at the 5 per cent level of significance. Pooled CRD. SE: standard error. CD: critical difference at the 5% level of significance. DAS: days after sowing. ToLCV: tomato leaf curl virus

PCR analysis using Deng, AV/AC and ToLCV-specific primers confirmed the presence of ToLCV in the inoculated treatments. Amplification was observed in the botanical-treated plants as well as the ToLCV control. The amplification was lower in botanical-treated plants compared with the control (Plate 2).

3.3 Field Evaluation of Promising Antiviral Botanicals, *P. niruri* and *B. diffusa* against Natural Incidence of Tomato Leaf Curl Virus

The selected 1 per cent treatments were evaluated during summer and rabi under field conditions. Symptom appearance was delayed in 1 per cent botanical-treated plants compared with the control. During summer, ToLCD symptoms appeared at 13.99 days in

plants sprayed with 1 per cent *P. niruri* and at 14.10 days with *B. diffusa* before natural virus infection. During rabi, symptoms appeared after 16.41 and 18.13 days with pre-application of the botanical treatments. The control recorded symptom appearance after 8.20 days during summer and 9.24 days during rabi (Fig 1).

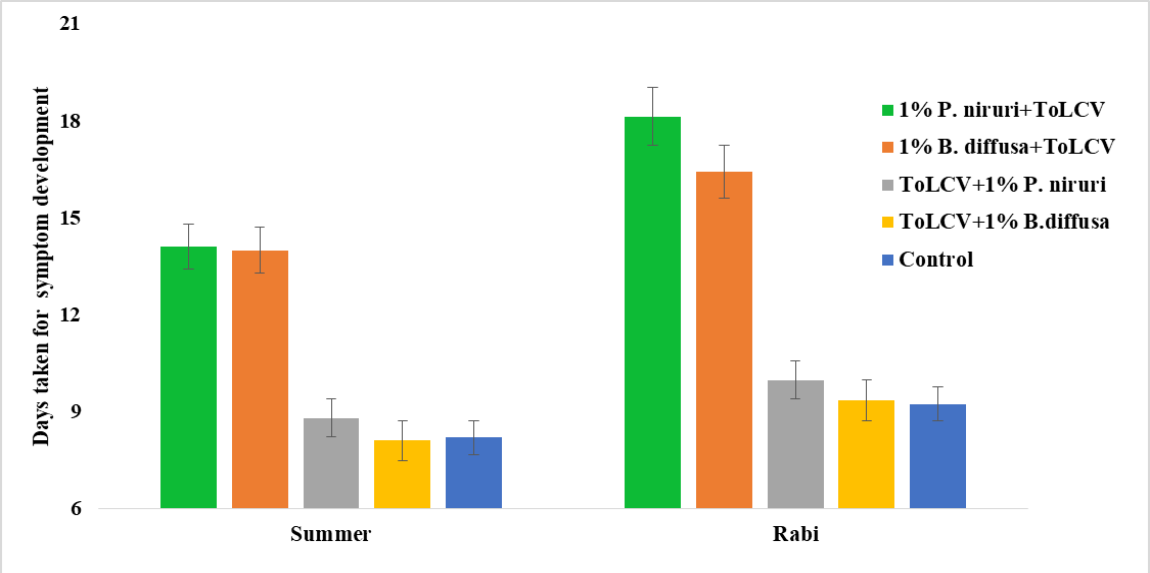

Fig. 1. Effect of pre- and post-treatment of 1 per cent *P. niruri* and *B. diffusa* to tomato plants on days taken for symptom development of ToLCD under field conditions

ToLCD incidence increased with crop age in all the treatments. At 40 DAS, no disease was recorded in the prophylactic or protective or pre-treatments of the botanicals in either season. The post-infection treatments recorded 6.67 and 10.00 per cent DI during summer and 3.33 and 8.34 per cent during rabi for *P. niruri* and *B. diffusa*, respectively. The control recorded a DI of 20.00 per cent during summer and 16.67 per cent during rabi. At 75 DAS, the prophylactic or protective spraying of *P. niruri* recorded DI of 26.67 per cent during summer and 23.33 per cent during rabi, while *B. diffusa* recorded 28.33 per cent during summer and 25.04 per cent during rabi seasons. The post-infection treatments recorded 36.67 and 35.81 per cent with *P. niruri* and 43.31 and 38.33 per cent with *B. diffusa* during summer and rabi, respectively. The corresponding values in the control were 58.33 and 46.67 per cent. At 90 DAS, DI reached 56.67 and 53.33 per cent in pre-*P. niruri*, 58.33 and 56.67 per cent in pre-*B. diffusa*, 65.00 and 61.67 per cent in post-*P. niruri*, and 66.67 and 63.33 per cent in post-*B. diffusa* treatments during summer and rabi, respectively. The control recorded 73.33 and 68.33 per cent (Table 5).

Visual assessment of ToLCV symptoms revealed the occurrence and greater severity of yellow and dark-green speckling, upward leaf curling, chlorosis, blister formation and stunting in the virus-infected plants. The untreated ToLCV control exhibited higher symptom grades, while plants sprayed with *P. niruri* and *B. diffusa* recorded comparatively lower symptom grades (Table 6).

The VI also increased with crop age. At 40 DAS, no disease was recorded in the prophylactic or protective or pre-treatments of the botanicals in either season. The post-infection treatments recorded 3.86 and 4.28 per cent VI during summer and 3.24 and 3.72 per cent during rabi for *P. niruri* and *B. diffusa*, respectively. The control plants recorded a VI of 8.62 during summer and 6.98 during rabi. At 75 DAS, VI values in the prophylactic or protective or pre-treatments ranged from 13.84 to 15.72 during summer and from 11.56 to 13.43 during rabi. The curative or post-treatments recorded values between 21.68 and 23.42 during summer and 18.74 and 20.36 during rabi. The control plants recorded VI of 42.26 during summer and 32.54 during rabi. At 90 DAS, prophylactic spraying of *P. niruri* at 1 per cent recorded the lowest VI among the botanical treatments, with values of 41.82 during summer and 37.68 during rabi. The corresponding values for prophylactic spraying of *B. diffusa* were 44.96 and 40.52. *P. niruri* recorded 49.64 and 45.86, while post-*B. diffusa* recorded 51.28 and 47.42 in the curative treatments. The control plants recorded 62.48 and 58.76 (Table 7).

Further, pre-application of *P. niruri* recorded the lowest incidence of leaf-eating caterpillars (0.81 and 0.73 pests plant^{-1}), whiteflies (2.48 and 2.10 pests plant^{-1}), aphids (2.00 and 1.81 pests plant^{-1}) and mealybugs (1.21 and 1.15 pests plant^{-1}) during summer and rabi, respectively. Pre-application of *B. diffusa* recorded 0.93 and 0.85 leaf-eating caterpillars, 2.65 and 2.38 whiteflies, 2.10 and 1.95 aphids, and 1.30 and 1.23 mealybugs plant^{-1} during summer and rabi, respectively. The post-application treatments generally recorded intermediate pest incidence, while the control recorded the highest incidence of most pests. For short-horned grasshoppers, the lowest incidence was recorded with pre-application of *B. diffusa* during summer (0.21 pests plant^{-1}), whereas pre-application of *P. niruri* recorded the lowest value during rabi (0.21 pests plant^{-1}). Leaf roller incidence was lowest in pre-application of *P. niruri* during both summer and rabi, with values of 0.33 and 0.28 pests plant^{-1}, respectively. The control recorded the highest incidence of leaf-eating caterpillars, leaf rollers, whiteflies, aphids and mealybugs in both seasons. Overall, pre-application of the botanicals was associated with lower pest incidence compared with post-application treatments and the control (Table 8).

Table 5. Effect of pre- and post-application of 1 per cent *P. niruri* and *B. diffusa* on natural incidence of tomato leaf curl disease caused by ToLCV in tomato during summer and rabi seasons under field conditions

Treatments		40 DAS		75 DAS		90 DAS	
		Summer	Rabi	Summer	Rabi	Summer	Rabi
T_1	1% *P. niruri* + ToLCV	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$26.67^{a} \pm 4.43$	$23.33^{a} \pm 2.42$	$56.67^{a} \pm 6.61$	$53.33^{a} \pm 2.43$
T_2	1% *B. diffusa* + ToLCV	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$28.33^{a} \pm 2.57$	$25.04^{a} \pm 2.49$	$58.33^{a} \pm 2.24$	$56.67^{a} \pm 6.12$
T_3	ToLCV + 1 % *P. niruri*	$6.67^{b} \pm 2.00$	$3.33^{b} \pm 2.43$	$36.67^{b} \pm 4.83$	$35.81^{b} \pm 2.04$	$65.00^{b} \pm 4.43$	$61.67^{b} \pm 4.49$
T_4	ToLCV + 1 % *B. diffusa*	$10.00^{b} \pm 2.07$	$8.34^{b} \pm 2.67$	$43.31^{b} \pm 4.64$	$38.33^{b} \pm 2.94$	$66.67^{b} \pm 4.24$	$63.33^{b} \pm 4.48$
T_5	Control	$20.00^{c} \pm 2.10$	$16.67^{c} \pm 2.17$	$58.33^{c} \pm 4.91$	$46.67^{c} \pm 2.48$	$73.33^{c} \pm 2.61$	$68.33^{c} \pm 4.15$
	SE (m) ±	0.46	0.54	1.26	0.72	1.25	1.30
	CD (0.05)	1.31	1.55	3.59	2.05	3.55	3.69

The values are the mean of sixty plants ± standard deviation. Four independent experiments at multi-locations were conducted. Superscripts with same letters indicate on-par values, and those with different letters indicate a significant difference at the 5 per cent level of significance. Pooled RBD. SE: standard error. CD: critical difference at the 5% level of significance. DAS: days after sowing. ToLCV: tomato leaf curl virus

Table 6. Effect of pre- and post-application of 1 per cent *P. niruri* and *B. diffusa* on severity of symptoms of tomato leaf curl disease caused by natural infection of ToLCV during summer and rabi seasons under field conditions at 75 DAS

Symptoms	1% *P. niruri* + ToLCV		1% *B. diffusa* + ToLCV		ToLCV + 1% *P. niruri*		ToLCV + 1% *B. diffusa*		Control	
	Summer	Rabi	Summer	Rabi	Summer	Rabi	Summer	Rabi	Summer	Rabi
Yellow and dark green speckles	++++	++	++++	++	+++++	+++	+++++	+++	+++++	+++++
Upward curling without chlorosis	++	++	++	++	+++	+++	+++	+++	+++++	+++++
Upward curling with chlorosis	++	++	++	++	+++	+++	+++	+++	+++++	+++++
Blister development	++	+	++	+	+++	++	+++	++	+++++	++++
Stunted growth	+	-	+	-	++	+	++	+	+++++	++++

Four independent experiments at multi-locations were carried out and fifteen plants were used for taking observation per treatment per experiment. +++++ very severe, ++++ severe, +++ moderately severe, ++ moderate, + mild, - symptoms absent. DAS: days after sowing. ToLCV: tomato leaf curl virus

Table 7. Effect of pre- and post-application of 1 per cent *P. niruri* and *B. diffusa* on tomato leaf curl disease severity on natural incidence of tomato leaf curl disease during summer and rabi seasons under field conditions

Treatments		40 DAS		75 DAS		90 DAS	
		Summer	Rabi	Summer	Rabi	Summer	Rabi
T_1	1 % *P. niruri* + ToLCV	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$13.84^{a} \pm 3.66$	$11.56^{a} \pm 3.16$	$41.82^{a} \pm 4.74$	$37.68^{a} \pm 4.28$
T_2	1 % *B. diffusa* + ToLCV	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$15.72^{a} \pm 3.98$	$13.43^{a} \pm 4.02$	$44.96^{a} \pm 4.18$	$40.52^{a} \pm 5.64$
T_3	ToLCV + 1 % *P. niruri*	$3.86^{b} \pm 0.84$	$3.24^{b} \pm 1.72$	$21.68^{b} \pm 4.26$	$18.74^{b} \pm 3.84$	$49.64^{b} \pm 5.12$	$45.86^{b} \pm 5.72$
T_4	ToLCV + 1 % *B. diffusa*	$4.28^{b} \pm 0.96$	$3.72^{b} \pm 1.84$	$23.42^{b} \pm 4.48$	$20.36^{b} \pm 4.12$	$51.28^{b} \pm 5.36$	$47.42^{b} \pm 5.94$
T_5	Control	$8.62^{c} \pm 2.26$	$6.98^{c} \pm 2.92$	$42.26^{c} \pm 6.52$	$32.54^{c} \pm 5.74$	$62.48^{c} \pm 5.64$	$58.76^{c} \pm 4.18$
	SE(m) ±	0.82	0.86	1.36	1.28	2.18	1.94
	CD (0.05)	2.44	2.56	4.06	3.82	6.49	5.78

The values are the mean of sixty plants ± standard deviation. Four independent experiments at multi-locations were conducted. Superscripts with same letters indicate on-par values, and those with different letters indicate a significant difference at the 5 per cent level of significance. Pooled RBD. SE: standard error. CD: critical difference at the 5% level of significance. DAS: days after sowing. ToLCV: tomato leaf curl virus

Table 8. Effect of pre- and post-application of 1 per cent *P. niruri* and *B. diffusa* on natural pest infestation during summer and rabi seasons under field conditions

Treatments		Pest incidence at 75 DAS (Number of pests plant^{-1})						
		Season	Leaf eating caterpillar	Short horned GH	Leaf roller	White fly	Aphids	Mealy bugs
T1	1 % *P. niruri* + ToLCV	Summer	$0.81^{a} \pm 0.07$	$0.28^{b} \pm 0.02$	$0.33^{a} \pm 0.03$	$2.48^{a} \pm 0.19$	$2.00^{a} \pm 0.16$	$1.21^{a} \pm 0.10$
		Rabi	$0.73^{a} \pm 0.06$	$0.21^{a} \pm 0.01$	$0.28^{a} \pm 0.02$	$2.10^{a} \pm 0.17$	$1.81^{a} \pm 0.14$	$1.15^{a} \pm 0.09$
T2	1 % *B. diffusa* + ToLCV	Summer	$0.93^{b} \pm 0.07$	$0.21^{a} \pm 0.02$	$0.38^{b} \pm 0.03$	$2.65^{a} \pm 0.21$	$2.10^{a} \pm 0.17$	$1.30^{a} \pm 0.10$
		Rabi	$0.85^{b} \pm 0.07$	$0.23^{a} \pm 0.02$	$0.23^{a} \pm 0.02$	$2.38^{b} \pm 0.18$	$1.95^{a} \pm 0.15$	$1.23^{a} \pm 0.10$
T3	ToLCV + 1 % *P. niruri*	Summer	$1.10^{c} \pm 0.09$	$0.25^{a} \pm 0.02$	$0.41^{b} \pm 0.03$	$2.83^{b} \pm 0.22$	$2.30^{b} \pm 0.18$	$1.58^{b} \pm 0.12$
		Rabi	$0.95^{c} \pm 0.08$	$0.23^{a} \pm 0.02$	$0.32^{b} \pm 0.02$	$2.50^{b} \pm 0.20$	$2.13^{b} \pm 0.17$	$1.40^{b} \pm 0.11$
T4	ToLCV + 1 % *B. diffusa*	Summer	$1.33^{d} \pm 0.11$	$0.28^{b} \pm 0.02$	$0.45^{b} \pm 0.03$	$3.01^{b} \pm 0.24$	$2.50^{c} \pm 0.20$	$1.71^{c} \pm 0.14$
		Rabi	$1.13^{d} \pm 0.09$	$0.25^{a} \pm 0.02$	$0.43^{c} \pm 0.03$	$2.70^{c} \pm 0.22$	$2.38^{c} \pm 0.18$	$1.60^{c} \pm 0.13$
T5	Control	Summer	$2.05^{e} \pm 0.16$	$0.31^{c} \pm 0.03$	$0.61^{c} \pm 0.05$	$3.88^{c} \pm 0.30$	$3.10^{d} \pm 0.25$	$2.55^{d} \pm 0.20$
		Rabi	$2.27^{e} \pm 0.18$	$0.30^{b} \pm 0.02$	$0.70^{d} \pm 0.06$	$3.40^{d} \pm 0.27$	$2.88^{d} \pm 0.22$	$2.83^{d} \pm 0.22$
	SE(m) ±	Summer	0.034	0.016	0.020	0.068	0.056	0.041
		Rabi	0.035	0.016	0.010	0.051	0.060	0.044
	CD	Summer	0.084	0.018	0.029	0.193	0.160	0.113
		Rabi	0.087	0.016	0.028	0.174	0.143	0.114

Sixty plants were observed. Four independent experiments at multi-locations were conducted. DAS: days after sowing, GH: grasshopper

Table 9. Effect of pre- and post-application of 1 per cent *P. niruri* and *B. diffusa* on flowering parameters of tomato plants on natural incidence of tomato leaf curl disease caused by ToLCV during summer and rabi seasons under field conditions

Treatments		Days taken for flowering*		Number of flowers per plant*		Number of fruits per plant	
		Summer	Rabi	Summer	Rabi	Summer	Rabi
T_1	1 % *P. niruri* + ToLCV	$32.75^{a} \pm 2.74$	$31.86^{a} \pm 2.62$	$33.43^{a} \pm 3.76$	$34.28^{a} \pm 3.94$	$24.00^{a} \pm 2.48$	$25.40^{a} \pm 2.74$
T_2	1 % *B. diffusa* + ToLCV	$32.01^{a} \pm 2.61$	$31.42^{a} \pm 2.57$	$33.41^{a} \pm 3.82$	$34.05^{a} \pm 3.71$	$24.20^{a} \pm 2.36$	$25.50^{a} \pm 2.68$
T_3	ToLCV + 1 % *P. niruri*	$32.46^{a} \pm 2.68$	$31.74^{a} \pm 2.60$	$31.86^{a} \pm 3.64$	$32.74^{a} \pm 3.78$	$22.80^{a} \pm 2.28$	$24.20^{a} \pm 2.56$
T_4	ToLCV + 1 % *B. diffusa*	$32.18^{a} \pm 2.65$	$31.58^{a} \pm 2.58$	$31.42^{a} \pm 3.58$	$32.38^{a} \pm 3.69$	$22.40^{a} \pm 2.24$	$23.90^{a} \pm 2.52$
T_5	Control	$32.01^{a} \pm 2.72$	$31.84^{a} \pm 2.68$	$27.41^{b} \pm 3.46$	$28.16^{b} \pm 3.52$	$17.00^{b} \pm 1.92$	$18.00^{b} \pm 2.18$
	SE (m) ±	0.70	0.61	1.03	1.08	0.82	0.90
	CD (0.05)	1.96	1.71	2.88	3.02	2.29	2.52

The values are the mean of sixty plants ± standard deviation. Four independent experiments at multi-locations were conducted. Superscripts with same letters indicate on-par values, and those with different letters indicate a significant difference at the 5 per cent level of significance. Pooled RBD. SE: standard error. CD: critical difference at the 5% level of significance. DAS: days after sowing. ToLCV: tomato leaf curl virus

3.4 Effect of Promising Antiviral Botanicals, *P. niruri* and *B. diffusa* on Chlorophyll Content of Tomato Plants on Natural Incidence of ToLCV under Field Conditions

The chlorophyll content of tomato leaves varied between the treatments under field conditions (Fig 2). Pre-application of 1 per cent *P. niruri* recorded the highest chlorophyll a content of 1.85 mg g^{-1} FW, followed by pre-application of 1 per cent *B. diffusa* with 1.72 mg g^{-1} FW. Post-application of 1 per cent *P. niruri* recorded a chlorophyll a content of 1.70 mg g^{-1} FW, while post-application of 1 per cent *B. diffusa* recorded 1.59 mg g^{-1} FW. The control recorded the lowest chlorophyll a content of 1.41 mg g^{-1} FW. A similar trend was observed for chlorophyll b content. Pre-application of 1 per cent *P. niruri* recorded the highest chlorophyll b content of 0.65 mg g^{-1} FW, followed by pre-application of *B. diffusa* with 0.51 mg g^{-1} FW. Post-application of *P. niruri* and *B. diffusa* recorded chlorophyll b contents of 0.58 and 0.38 mg g^{-1} FW, respectively, while the control recorded the lowest value of 0.31 mg g^{-1} FW. The total chlorophyll content followed a similar pattern. The highest total chlorophyll content of 2.48 mg g^{-1} FW was recorded in plants subjected to pre-application of 1 per cent *P. niruri*, followed by pre-application of *B. diffusa* with 2.35 mg g^{-1} FW. Post-application of *P. niruri* and *B. diffusa* recorded total chlorophyll contents of 2.32 and 1.94 mg g^{-1} FW, respectively. The control recorded the lowest total chlorophyll content of 1.64 mg g^{-1} FW.

3.5 Effect of Promising Antiviral Botanicals, *P. niruri* and *B. diffusa* on Biometric and Reproductive Parameters of Tomato Plants on Natural Incidence of ToLCV under Field Conditions

The botanical treatments showed significant differences in plant growth during the field experiment. Plant height at 40 DAS was 60.96 cm during summer and 61.74 cm during rabi following pre-application of *P. niruri*. The corresponding values for pre-application of *B. diffusa* were 59.90 and 60.20 cm. Post-application of *P. niruri* recorded 59.42 and 59.40 cm, while post-application of *B. diffusa* recorded 58.36 and 59.18 cm. The control plants recorded 57.62 and 58.94 cm during summer and rabi, respectively (Fig 3a; Plate 3). The number of leaflets was also higher in the botanical-treated plants. At 40 DAS, pre-application of *P. niruri* recorded 458.80 leaflets during summer and 472.40 during rabi, followed by pre-application of *B. diffusa* with 452.60 and 468.80 leaflets, respectively (Fig 3b). Post-application of *P. niruri* recorded 441.60 and 455.80 leaflets, while post-application of *B. diffusa* recorded 434.20 and 448.60 leaflets during summer and rabi, respectively. The control recorded 411.80 and 438.60 leaflets during summer and rabi, respectively. Similarly, pre-application of *P. niruri* recorded 10.16 cm^2 and 10.62 cm^2 leaflet area during summer and rabi, respectively, followed by pre-application of *B. diffusa* with 10.04 cm^2 and 10.48 cm^2 (Fig 3c). Post-application of *P. niruri* recorded 9.82 cm^2 and 10.18 cm^2, while post-application of *B. diffusa* recorded 9.68 cm^2 and 10.02 cm^2. The control recorded 8.28 cm^2 and 8.96 cm^2 during summer and rabi, respectively.

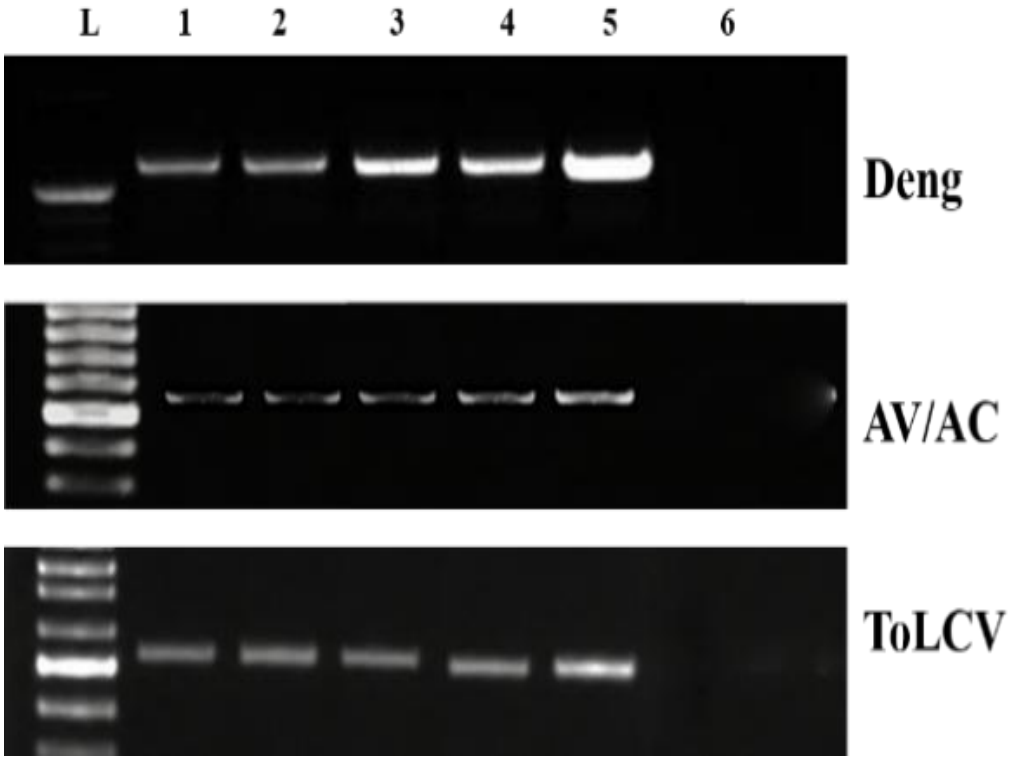

Plate 2. Electrophoresis gel image of pre- and post-treatment of 1 per cent *P. niruri* and *B. diffusa* on amplification of ToLCV using Deng, AV/AC and ToLCV primers against artificial inoculation of ToLCV through wedge-grafting. L – molecular marker, 1 - 1 % *P. niruri* + ToLCV, 2 - 1 % *B. diffusa* + ToLCV, 3 - ToLCV + 1 % *P. niruri* 4 - ToLCV + 1 % *B. diffusa*, 5 – ToLCV alone, 6 – Absolute control

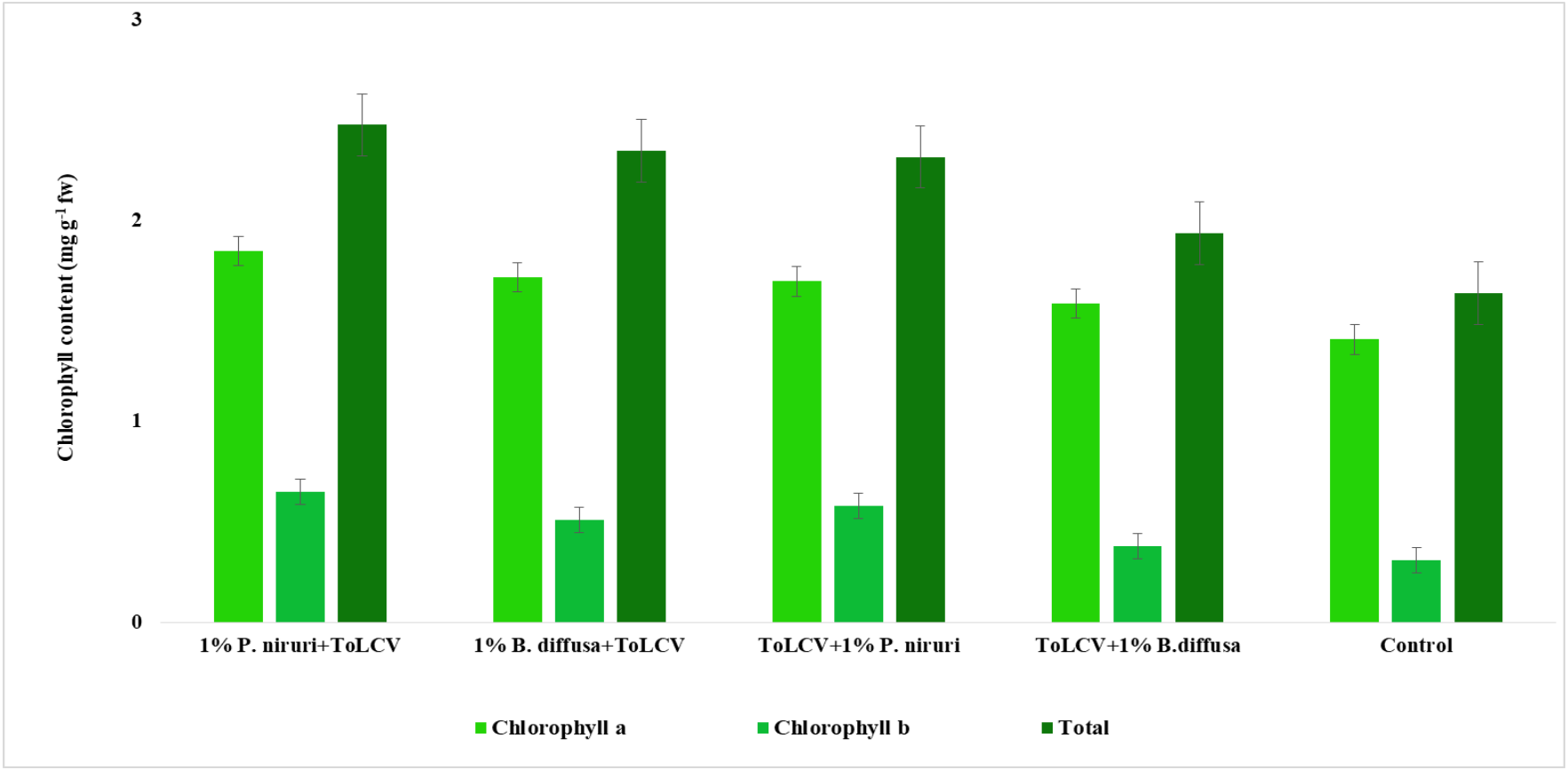

Fig. 2. Effect of pre- and post-treatment of 1 per cent *P. niruri* and *B. diffusa* on chlorophyll content of tomato plants under field conditions

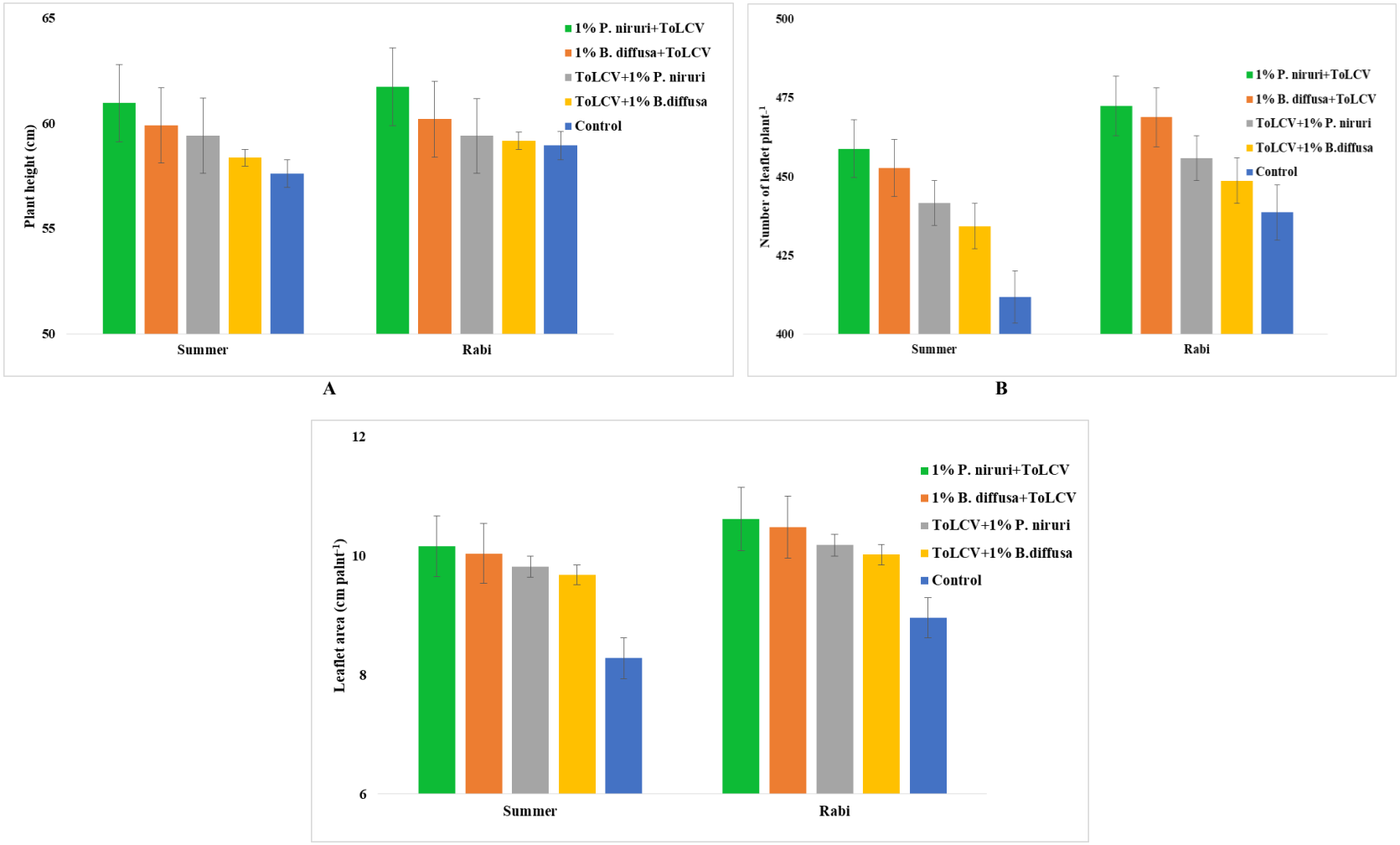

Fig. 3. Effect of pre- and post-treatment of 1 per cent *P. niruri* and *B. diffusa* on a) plant height, b) number of leaflets c) leaflet area of tomato plants under field conditions

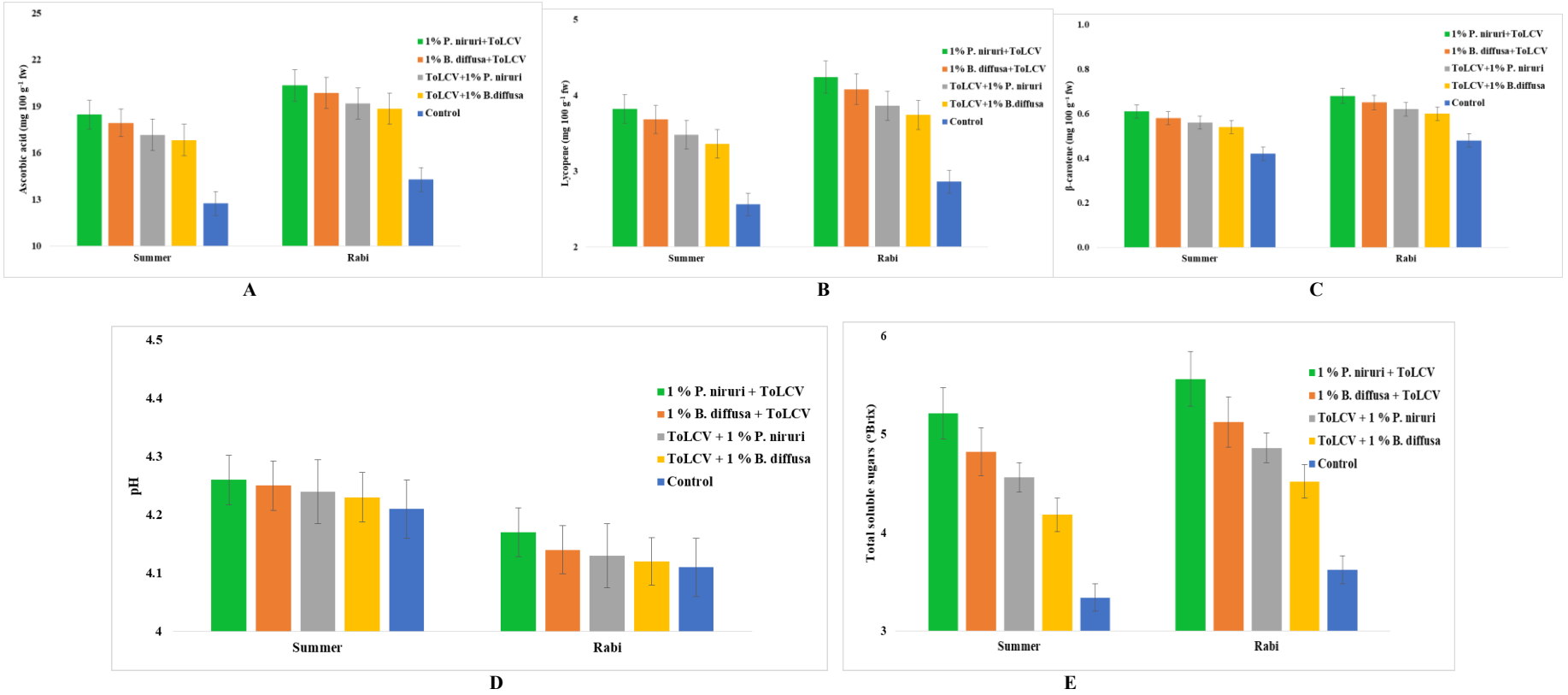

Fig. 4. Effect of pre- and post-treatment of 1 per cent *P. niruri* and *B. diffusa* on A) ascorbic acid, B) lycopene C) β -carotene D) pH E) total soluble solids of tomato plants under field conditions

The number of days taken for flowering showed minimal differences among treatments. *P. niruri*-pre-applied plants flowered at 32.75 days during summer and 31.86 days during rabi, while *B. diffusa*-pre-applied plants flowered at 32.01 and 31.42 days, respectively. Post-application of *P. niruri* recorded 32.46 and 31.74 days, while post-application of *B. diffusa* recorded 32.18 and 31.58 days during summer and rabi, respectively. The control plants flowered at 32.01 and 31.84 days during summer and rabi, respectively. The number of flowers per plant was higher in botanical-treated plants. Pre-application of *P. niruri* recorded 33.43 and 34.28 flowers per plant during summer and rabi, respectively, followed by pre-application of *B. diffusa* with 33.41 and 34.05 flowers per plant. Post-application of *P. niruri* recorded 31.86 and 32.74 flowers per plant, while post-application of *B. diffusa* recorded 31.42 and 32.38 flowers per plant during summer and rabi, respectively, compared with 27.41 and 28.16 in the control (Table 9). Fruit production followed the same pattern. The number of fruits per plant following pre-application of *P. niruri* was 24.00 during summer and 25.40 during rabi, while pre-application of *B. diffusa* recorded 24.20 and 25.50. Post-application of *P. niruri* and post-application of *B. diffusa* recorded 22.80 and 24.20 fruits per plant and 22.40 and 23.90 fruits per plant, respectively. The control recorded 17.00 and 18.00 fruits per plant during summer and rabi, respectively (Table 9).

3.6 Effect of Promising Antiviral Botanicals, *P. niruri* and *B. diffusa* on Fruit Quality Parameters on Natural Incidence of ToLCV under Field Conditions

Fruit quality parameters varied among the treatments. Pre-application of 1 per cent *P. niruri* recorded the highest ascorbic acid, lycopene and β-carotene contents, with values of 18.46, 3.82 and 0.61 mg 100 g^{-1} FW during summer, respectively, and 20.34, 4.24 and 0.68 mg 100 g^{-1} FW during rabi. Pre-application of *B. diffusa* and post-application treatments also improved these quality parameters compared with the control, which recorded the lowest values (Fig 4a, Fig 4b, Fig 4c). Fruit pH showed only minor variation among treatments, ranging from 4.21 to 4.26 during summer and 4.11 to 4.17 during rabi. In contrast, total soluble solids varied considerably, with pre-application of 1 per cent *P. niruri* recording the highest TSS of 5.21 and 5.56 °Brix during summer and rabi, respectively, while the control recorded the lowest values of 3.34 and 3.62 °Brix (Fig 4d, Fig 4e).

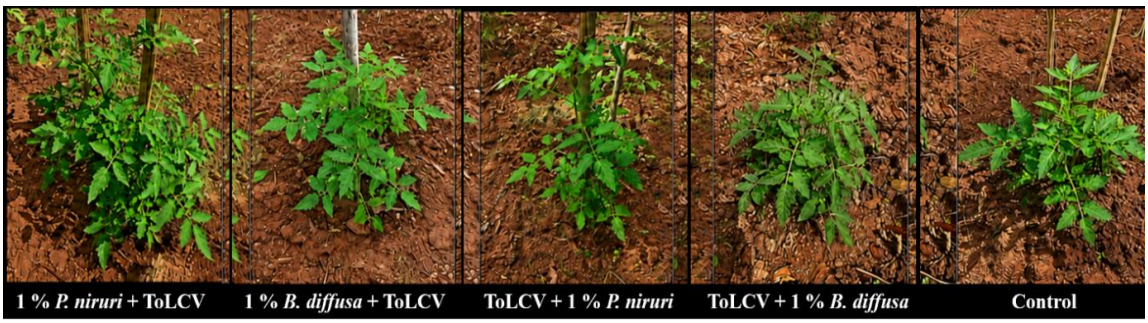

Plate 3. Effect of pre- and post-treatment of 1 per cent *P. niruri* and *B. diffusa* on tomato plants on natural incidence of tomato leaf curl disease caused by ToLCV under field conditions

4. Discussion

The present study demonstrated that the promising botanicals *P. niruri* and *B. diffusa* were effective in suppressing ToLCD. Under both pre- and post-treatment with the botanicals, increasing the concentration from 0.25 to 1 per cent progressively delayed symptom development and reduced DI and VI following ToLCV inoculation. However, further increases to 5 and 10 per cent resulted in only marginal improvement. The comparable disease responses at 1, 5 and 10 per cent justified the selection of 1 per cent for subsequent studies. This indicates that increasing the concentration beyond the effective level may not necessarily provide additional disease suppression and that 1 per cent was adequate for further evaluation.

Pre-application of the botanicals generally performed better than their post-application. The greater delay in symptom appearance and lower DI and VI observed with pre-application suggest that treatment before virus establishment provided better protection against subsequent disease development. The response was particularly evident with *P. niruri*, which recorded the lowest VI at 1 per cent. The better performance of prophylactic or protective application may be attributed to the opportunity for the botanical constituents to act before extensive virus establishment and disease development (Lohani *et al.,* 2007; Sharma & Awasthi*,* 2017; Wang *et al.,* 2022). In contrast, application of the botanicals after the inoculation of the virus was less effective because infection had already been initiated and was progressing. The results therefore emphasise the importance of application timing in achieving effective management of ToLCV using *P. niruri* and *B. diffusa*. Such suppression may be associated with the diverse secondary metabolites present in plant-derived preparations, as several classes of specialised plant metabolites have been reported to possess antiviral activity through multiple targets (Ponticelli *et al.,* 2023).

The DAS-ELISA results provided further evidence for the suppressive effect of the botanical treatments. Lower titre values were recorded in *P. niruri* and *B. diffusa*-treated plants than in the ToLCV control, with the lowest values generally observed in prophylactic application. The lower absorbance in the treated plants suggests that the extent of virus accumulation was reduced. PCR analysis further confirmed ToLCV in all ToLCV-inoculated plants, including botanical-treated plants, while the absolute control remained negative. The botanicals did not completely prevent virus infection but were associated with reduced virus accumulation and disease expression. Plant-derived compounds have also been shown to interfere directly with TYLCV infection (Bishnoi *et al.,* 2017). Arbutin, a naturally occurring hydroquinone glucoside, was recently reported to inhibit viral accumulation

and interact with the viral coat protein (Xu *et al.,* 2025). The combined ELISA and PCR observations indicate that the treatments functioned primarily through disease suppression rather than through complete elimination of the virus.

The field evaluation of the selected 1 per cent concentration of the promising botanicals further supported the observations obtained under controlled conditions. ToLCD symptom development was delayed in botanical-sprayed plants compared with the untreated control, and DI and VI increased progressively with crop age in all treatments. Nevertheless, the magnitude of disease development remained lower in the botanical-sprayed plants, particularly in those receiving pre-application. The consistently lower disease severity in botanical-pre-treated plants indicates that early application provided more sustained protection throughout crop development. Among the botanicals, *P. niruri* generally showed a better response than *B. diffusa*, particularly when applied before virus inoculation. Visual symptom assessment was consistent with the quantitative observations on the disease. Characteristic ToLCV symptoms such as yellow and dark-green speckling, upward curling, chlorosis, blister formation and stunting were observed in the infected plants. The more severe symptoms occurred in the untreated control plants naturally infected by the virus, whereas the botanical-treated plants exhibited comparatively lower symptom severity.

An additional response observed under field conditions was a reduction in the incidence of several insect pests. Leaf-eating caterpillars, leaf rollers, whiteflies, aphids and mealybugs generally occurred at lower levels in botanical-treated plants than in the untreated control. The effect on whitefly is particularly relevant to ToLCV management because whitefly is responsible for virus transmission. The comparatively lower whitefly incidence in the botanical-sprayed plants could therefore have contributed to the reduced secondary spread of ToLCV within the crop. The response also suggests that the botanical treatments may provide an additional pest-management benefit along with their disease-suppressive effect. This is consistent with reports that plant-derived essential oils can exert repellent, ovicidal and insecticidal effects against *B. tabaci* and reduce its colonisation on host plants (Santana *et al.,* 2022; Tang *et al.,* 2023). However, the present observations establish the association between botanical application and pest incidence, while the specific insecticidal or repellent mechanisms require further investigation.

The reduction in disease severity was accompanied by higher chlorophyll content and improved plant growth and yield performance. Botanical-treated plants generally maintained greater plant height and produced more leaflets than the untreated control, particularly at the later stages of the crop. The effect was most evident with pre-application of *P. niruri*. Improved vegetative growth under reduced disease pressure could have helped maintain a larger photosynthetically active canopy, thereby supporting reproductive development (El-Sappah *et al.,* 2022). Although days to flowering differed only slightly among treatments, the number of flowers and fruits per plant was higher in the treated plants. The higher fruit production, particularly following pre-application of *P. niruri* and *B. diffusa*, indicates that botanical treatment helped to maintain plant productivity under ToLCV pressure.

The beneficial effect of botanical treatment extended to fruit quality. Fruits from pre-application of *P. niruri* had the highest ascorbic acid, lycopene and β-carotene contents, whereas the untreated control recorded the lowest values. The same trend was observed for TSS. The higher accumulation of these quality attributes in the botanical-treated plants may be associated with better maintenance of plant physiological activity and assimilate production under reduced disease stress. In particular, the higher lycopene and β-carotene contents indicate better accumulation of important fruit pigments, while the increased TSS reflects greater accumulation of soluble constituents during fruit development. Fruit pH, in contrast, showed only narrow variation among treatments, indicating that it was comparatively less affected by botanical application.

5. Conclusion

The present study demonstrated the potential of *P. niruri* and *B. diffusa* as antiviral botanicals for the management of tomato leaf curl virus disease. Among the concentrations evaluated, 1 per cent was identified as the effective concentration, with higher concentrations offering no substantial additional benefit. Pre-application of the botanicals was more effective than post-application in delaying symptom development, reducing DI, disease severity and virus accumulation. Under field conditions, spraying with 1 per cent botanicals also supported better plant growth, fruit production and fruit quality, while being associated with a lower incidence of several insect pests, including whitefly, and significant reductions in the DI and VI of ToLCD. Overall, pre-application of 1 per cent *P. niruri* showed the most consistent performance and could be used as a promising component of an integrated and environmentally compatible strategy for tomato leaf curl disease management in tomato.

The study was conducted at Vellayani and Balaramapuram in Kerala, and further evaluation across multiple locations and farmers' fields is required to validate the consistency, field applicability and economic feasibility of the botanical treatments. Further studies are also needed to elucidate the biochemical and physiological mechanisms underlying the botanical-mediated disease suppression.

6. Limitation

The field validation was conducted at two locations in Kerala using the susceptible tomato cultivar Vellayani Vijai, so the consistency of the response across broader agroecological conditions and cultivars remains to be established. Field evaluation focused on the selected 1 per cent concentration after pot screening. Although lower virus accumulation and pest incidence were associated with botanical treatment, the specific biochemical, physiological, insecticidal or repellent mechanisms were not resolved. Further multi-location testing and economic evaluation are therefore required before broader field application.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference

formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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