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Non Chemical Methods for Management of Chilli Leaf Curl Disease

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ABSTRACT

An experiment was conducted to evaluate the efficacy of aqueous extracts of certain plants with reported antiviral activity and biocontrol agents for the management of *Chilli leaf curl virus* (ChiLCV) disease naturally infecting chilli. It was observed that seed treatment followedby foliar sprays at 15 days interval using 10% *Azadirachta indica* and *Mirabilis jalapa* extracts significantly reduced the disease incidence as well as severity. Also, three treatments, namely *A. indica*, *Pseudomonas flourescens* and *Bacillus subtilis* were foundto efficiently check the number of whiteflies. Plant height was also found to be significantly higher in plants treated with *A.indica* and *M.jalapa*. *Bougainvillea spectabilis* and PGPR mix II treated plants. The presence of ChiLCV in the diseased plants was confirmed by performing PCR with virus specific primers.

Key Words: Agents Biocontrol, Botanicals, Chilli, Virus.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is considered as one of the most important commercial spice crops and is often called wonder spice. Viral diseases affecting the crop has emerged as a vicious threat that drastically reduces its production and quality. Among all, whitefly (*Bemisia tabaci*) transmitted Chilli leaf curl disease is thought of as the most destructive disease in terms of incidence and yield loss. In case of severe incidence, losses up to 100 per cent with regard to marketable fruit have been reported (Senanayake *et al,* 2007). The disease is characterized by typical leaf curl symptoms along with puckering, twisted petioles, stunted growth, reduced size of leaves, low or no fruit set *etc.*

Farmers currently resort to use of resistant varieties and large quantities of broad-spectrum insecticides in an attempt to check the disease. The later approach is costly, hazardous and certain insecticides are becoming less effective since the increase in populations of the highly virulent and insecticide-resistant `B' biotype of *B. tabaci*. Therefore, exploitation of the use of plant extracts with antiviral properties and induction of resistance using biocontrol agents assumes importance in this backdrop. Many scientists in India and abroad have reported virus inhibitorsin several plant species like *B. spectabilis, M. jalapa and B. diffusa* (Karthikeyan *et al,* 2009; Awasthi *et al,* 2016; Sharma *et al,* 2017)). Reports also show that use of bio control agents such as *Pseudomonas* spp. having diverse modes of action helps in induction of defense related enzymes such as peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) in virus infected plants (Venkatesan *et al,* 2010).

MATERIALS AND METHODS

The pot experiment aimed at evolving a suitable management strategy to contain the disease was conducted in open conditions relying on natural

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incidence of leaf curl disease in chilli in the year 2021-22 at KVK Palakkad. The experiment was laid out as a CRD including 9 treatments and 3 replications using the variety Byadagi Dabbi with 9 plants in each replication. The different treatments included are presented below:

T_1 : 10 % Leaf extract of *Bougainvillea spectabilis* - Seed treatment and foliar spray

T_2 : 10 % Root extract of *Boerhavia diffusa*- Seed treatment and foliar spray

T_3 : 10 % Leaf extract of *Azadirachta indica* - Seed treatment and foliar spray

T_4 : 10 % Leaf extract of *Mirabilis jalapa* - Seed treatment and foliar spray

T_5 : *Pseudomonas fluorescens* - Seed treatment (10g kg^{-1} seed) and foliar spray (20g l^{-1}) T_6 : *Bacillus subtilis* - Seed treatment (10g kg^{-1} seed) and foliar spray (20g l^{-1})

T_7 : PGPR – Seed treatment, seedling root dip and soil drenching @ 500g/ha

T_8 : Dimethoate 0.05%

T_9 : Untreated control

The extracts of T_1 to T_4 was prepared and sprayed at 10 per cent concentration according to the protocol suggested by Sharma *et al* (2017). Roots/leaves of the plants were collected, dried in shade at room temperature, ground to powder and stored at low temperature. The crude extracts were prepared by making the suspension of root or leaf powder separately in water (1g/10 ml). The pulp was stained through muslin cloth and the homogenate was clarified by centrifugation at 8,000 g for 15 minutes. The extract prepared was sprayed at 10 per cent concentration. Talc formulations of the biocontrol agents *viz., P. fluorescens, B. subtilis* and PGPR mix II were used in the study. All the treatments under study were appliedas seed treatment and then as foliar sprays/ soil drench at an interval of 15 d from 15 d after sowing. Six foliar sprays were given up to 90 DAS. The observations regarding plant height, whitefly count, DI and DS were recorded from 5 random plants at 15 days interval from 15 DAT. The plants were transplanted at 45 DAS.

Disease incidence was calculated as,

Disease severity was calculated as,

Where, G_i: Number of plants with disease score i (i varies from 0 to 5)

A plant was considered infected as soon as a visible leaf curl symptom was observed. The genomic DNA of randomly collected fresh leaf samples from the experimental plot were isolated by modified Gem CTAB method (Rouhibhaksh *et al,* 2008) with slight modifications. The isolated DNA was amplified by PCR using virus specific primers to confirm the incidence of ChiLCV. The incidence of Begomovirus was first confirmed using DENG primer with a forward sequence of 5'- TAATATTACCKGWKGVCCSC -3' and reverse sequence of 5'- TGGACYTTRCAWGGBCCTTCACA -3'. The specific primer capable of amplifying the CP gene of ChiLCV was of the forward sequence 5'- AGAATTATGTCCAAGCGACCA-3' and reverse sequence of 5'- AAGCGTTGGGGATACACAAA-3'.

The statistical analysis for the data recorded from the pot experiment was analyzed by ANOVA (Analysis of Variance) for Completely Randomized Design (CRD) making use of WASP statistical software.

RESULTS AND DISCUSSION

Effect of treatments on disease incidence and severity

The Disease Incidence (DI) and Disease Severity (DS) recorded from the experimental plot is presented in Table 1. At 15 DAT, the incidence and severity were maximum in the untreated control plot. All the other treatments except that of T_5 (*P. flourescens*) exhibited a significant reduction in disease incidence when compared to T9, the untreated control. With respect to DS, the effect of *P.*

Table 1. Effect of treatments on disease incidence and severity

Sr. No.	Treatment	DI and DS			
		15 DAT	30 DAT	45 DAT	60 DAT
1	T_1 - Leaf extract of *B. spectabilis*	0.00 [a]	40.00 [bcd]	66.67 [bc]	73.33[c]
		0.00 [a]	12.00 [bc]	8.67[ab]	30.67[ab]
2	T_2 - Root extract of *B. diffusa*	0.00 [a]	33.33 [bc]	73.33[c]	73.33 [c]
		0.00 [a]	10.67 [bc]	6.67[bc]	30.67 [ab]
3	T_3 - Leaf extract of *A. indica*	6.67 [a]	26.67 [ab]	46.67 [ab]	66.67[ab]
		1.33 [ab]	5.33 [ab]	12.00[a]	22.67 [ab]
4	T_4 - Leaf extract of *M. jalapa*	6.67 [a]	26.67 [ab]	66.67 [bc]	80.00[bc]
		1.33 [ab]	5.33 [ab]	16.00[ab]	29.33 [ab]
5	T_5 - *P. fluorescens*	20.00[b]	46.67[cd]	73.33[bc]	93.33[cd]
		4.00[b]	12.00 [bc]	26.67[bc]	46.67 [c]
6	T_6 - *B. subtilis*	6.67 [a]	33.33[bc]	46.67 [ab]	73.33 [b]
		1.33[ab]	9.33 [ab]	18.67[ab]	32.00 [b]
7	T_7 - PGPR	0.00 [a]	53.33[d]	86.67[cd]	100.00[d]
		0.00 [a]	17.33 [c]	40.00[cd]	49.33[c]
8	T_8 - Dimethoate	0.00 [a]	13.33[a]	40.00[a]	53.33[a]
		0.00[a]	2.67 [a]	9.33[a]	18.67[a]
9	T_9 - Untreated Control	26.67[b]	93.33[e]	100.00[d]	100.00[d]
		8.00[c]	29.33 [d]	49.33[d]	54.67[c]
	CD (0.05)	13.20	18.67	22.8	17.46
		3.962	6.98	13.53	12.54

flourescens was observed to be significant from that of the untreated control. At 15 DAT plants treated with *B. spectabilis*, *B. diffusa*, PGPR and Dimethoate were free of disease incidence suggesting their effect in delaying the disease initiation. Six percent disease incidence was recorded in plants treated with *A. indica*, *M. jalapa* and *B. subtilis* but the DI as well as DS was less than that of the untreated controlplot. As the disease progressed, at 30 DAT, the highest values of DI and DS were recorded in treatment T9, the untreated control. Treatment T8, the insecticide check apparently exhibited the best performance and treatments *A. indica* and *M. jalapa* were on par with the insecticide check. At 45 DAT, the disease incidence recorded in *A. indica* and *B. subtilis* treated plants wereon par with the insecticide check but plants treated with *B. spectabilis*, *M. jalapa, B. diffusa* and *P. flourescens* also showed reduction in DS and DI when compared to the untreated control. At 60 DAT, with respect to DI, the only treatment that was on par with the effect of insecticide check was *A. indica*.

The effect of treatments *M. jalapa, B. subtilis, B. spectabilis* and *B. diffusa* were significant when compared to the untreated control. Though the appearance of symptoms were very much delayed in the plants treated with *B. spectabilis* and *B. diffusa*, at 60 DAT, the disease incidence was the least in *A. indica* treatedplants (Plate 1). With regard to DS, the effect of all the plant extracts was significant from the untreated control. Researchers from a long time have been reporting the antiviral effect of various plant extracts. Apart from the antiviral properties, these are also known to positively influence many

other plant growth parameters. Leaf extracts of *A. indica* is a suitable control strategy against chilli leaf curl in the light of this study.

In a study conducted on the management of viral diseases of watermelon, Sharma *et al* (2017) observed that a combination of seed treatment and 6 foliar sprays of leaf extract of *A. indica* delayed the onset of symptom expression till 58 DAS against 18.5 DAS in the untreated control resulting in an appreciable reduction in disease incidence to the tune of 52.08 per cent. The disease severity also was tremendously reduced by timely sprays of *A. indica*. A per cent reduction in DS to the tune of 75.75 % at 45 DAT compared to the untreated control was observed in this study. Though, reports of antiviral effects of *A. indica* have been widely studied about, there are no reports of any antiviral proteins isolated from the plants. In fact, adding confidence to the findings of this study, studies reflecting the effect of *A. indica* in containing human viruses have been reported. Faccin-Galhardi *et al* (2012) reported the antiviral effect of two polysaccharides, P1and P2 isolated from the leaves of neem tree in containing the poliovirus type 1. They suggested that the prominent antiviral effect of this plant that assumes very much importance on an ethno medical background was by inhibition of viral replication at initial stages. In the present study, the leaf extracts of *M. jalapa* had a significant effect on reducing the disease incidence and the disease severity and the effect was on par with that of *A. indica* most of the time. Though the number of days to disease initiation wasn't delayed, the further spread was thoroughly checked. Karthikeyan *et al* (2009) on his studies with ULCV infecting blackgram observed a satisfactory reduction (90 %) in infection of by *M. jalapa* and *B. spectabilis* over the control when applied 24 h before inoculation. It was reported that the number of incubation days in the former was 28 days and latter was 21 days compared to 14 days in the control. But in this study, symptom expression was visible by 15 DAT in *M. jalapa* and 30 DAT in *B. spectabilis*. It has been suggested that higher levels of the defence related enzymes, PAL, PPO, PO and total phenols could be responsible for the activation of several defence mechanisms resulting in the reduced disease infection.

M. jalapa is a member of family Nyctaginaceae whose antiviral effect is attributed to the presence of a ribosome inactivating protein (RIP) termed as Mirabilis antiviral protein (MAP).Vivanco *et al* (1999) believed that the MAP behaved as a signal molecule that could signal a cascade response which in turn activated a series of defence mechanisms well in advance of the viral infection. The supreme effect of leaf extract of *B. spectabilis* with respect to delay in onset of disease and further spread along with significant reduction in disease severity in the present study is worth mentioning. Symptoms were not expressed in 15DAT in *Bougainvillea* treated plants when a DI of 26.67 per cent was observed in the control plot.Similar observations were made by Ashfaq *et al* (2006) wherein spraying leaf extract of *Bougainvillea* delayed the incidence of Urdbean Leaf Crinkle Virus in blackgram by10- 14 days and reduced the disease incidence to 20-30 per cent against the 80 per cent in control. Guller *et al* (2017) isolated, cloned and expressed one of the ribosome inactivating proteins, Bouganin antiviral protein (BAP) from *B. Spectabilis* Willd. The influence of defence related peroxidase enzymes in polymerization reactions and cross linking of structural cell wall proteins on the cell walls of the sprayed plant which could possibly be negate the movement of viruses was suggested by Fry (1986). *B. diffusa* showcased an activity on par with *Bougainvillea* in the study. The disease initiation was delayed. A reduction to the tune of 34 % was observed. The observations made from this study was in accordance with that of Sharma *et al* (2017) who observed a commendable decrease (54.24 %) in incidence of viral diseases in watermelon when compared to the control when treated with root extract of *B. diffusa* followedby *A. indica* (52.02 %) both of which were comparable to that of the disease reduction in the insecticide treated plot (57.07 %). Production of a Virus Inhibitory Agent (VIA) in healthy but susceptible plant was observed

(The first row against each treatment indicates DI and the second row indicates DS)

a.Effect of T_3- 10% *A. indica*

b. Effect of T_9- Untreated control

Plate 1. Effect of 10% *A. indica* over untreated control on disease incidence

post application of the systemic resistance inducing protein (BD-SRIP) identified from *B. diffusa*. This glycoprotein is known to function as a signal molecule that stifles the virus by stimulating the defense systems of plants. It is explained that the inhibitory effect is a due function of phytoproteins in these plants which is capable of preventing the formation of necrotic lesions in hypersensitive hostsas well as delaying the development of disease specific symptom in systemic hosts, by activating pathways responsible for Induced Systemic Resistance (ISR). Therefore, it could be inferred that the best performing plantextracts against the control of ChiLCV in chilli is *A. indica* and *M. jalapa* . The effects of the other two tested antiviral agents, *B. diffusa* and *B. spectabilis* is also appreciable in terms of delaying disease onset. The effect of *B. subtilis* onreducing the disease incidence was at par with the best performing treatments, *A. indica* and *M. jalapa* throughout the experiment but its effect on disease severity was less pronounced by 60 DAT. A 53.3 per cent reduction in disease incidence and 62.17 per centreduction in disease severity was observed in *B. subtilis* treated plants by 45 DAT. A similar disease reducing activity was observed by Lian *et al* (2010) wherein 52 and 71 percent reduction in

The gel pictures of amplification is presented as Fig. 1 and Fig. 2.

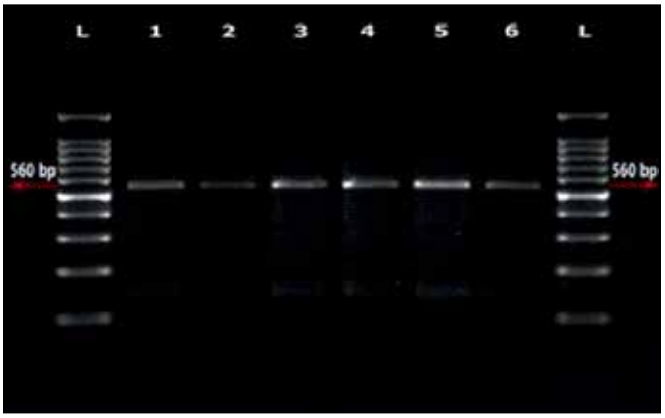

Fig. 1 Amplification using universal DENG primer

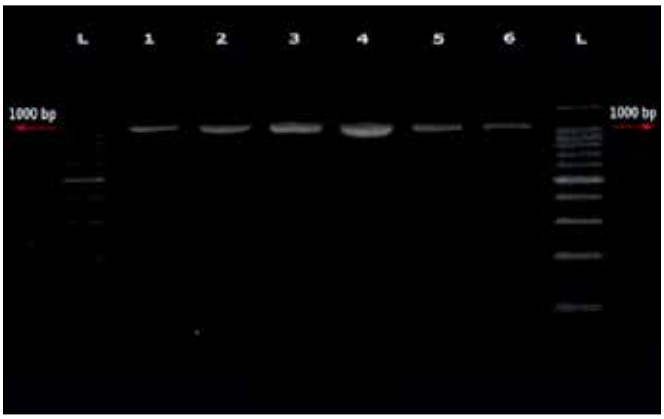

Fig. 2 Amplification using specific primer, ChiLCV specific primer

Table 2. Effect of treatments on height of plants and no. whiteflies

Sr. No.	Treatments	Height (Cm)				No. of whiteflies	
		15 DAT	30 DAT	45 DAT	60 DAT	15 DAT	30 DAT
1	T1 - Leaf extract of *B. spectabilis*	17.57^{bc}	30.83^{bc}	41.33^{abc}	49.27^{abc}	8.40^{d}	5.80^{b}
2	T2 - Root extract of *B. diffusa*	21.87^{ab}	31.14^{bc}	40.32^{bc}	46.00^{bcd}	6.00^{c}	5.60^{b}
3	T3 - Leaf extract of *A. indica*	17.73^{bc}	29.13^{cd}	41.73^{abc}	51.13^{ab}	1.67^{a}	1.93^{a}
4	T4 - Leaf extract of *M. jalapa*	25.60^{a}	37.57^{a}	46.93^{a}	53.53^{a}	7.73^{d}	6.00^{b}
5	T5 - *P. fluorescens*	18.07^{bc}	30.80^{bc}	39.13^{bcd}	43.93^{cde}	1.60^{a}	2.00^{a}
6	T6 - *B. subtilis*	17.13^{c}	27.46^{cd}	36.46^{cd}	43.73^{de}	3.80^{b}	1.82^{a}
7	T7 - PGPR	24.63^{a}	36.07^{ab}	42.85^{ab}	49.77^{ab}	8.13^{d}	6.93^{b}
8	T8 - Dimethoate	18.70^{bc}	28.34^{cd}	37.37^{bcd}	43.13^{de}	1.13^{a}	1.87^{a}
9	T9 - Untreated Control	15.93^{c}	24.33^{d}	33.20^{d}	39.06^{e}	9.20^{d}	6.73^{b}
	CD (0.05)	4.67	5.86	6.03	5.5	1.7	2.9

symptom expression was observed in plants treated with *B. subtilis* strain EN16 and SW1 respectively while working with the management of TMV in Tobacco. Later, it was evident from the enzyme analysis that there was a corresponding increase of defence related enzymes and pathogenesis-related (PR) proteins in *Bacillus* treated plants challenged with pathogen.

At 15 DAT, three treatments were noticed to have a significant inhibitory effect on the count of whiteflies, *A. indica, P. flourescens* and the insecticide check. The effect of treatment *B. subtilis* was also significant when compared to the untreated control. At 30 DAT, the effect of *A. indica, P. flourescens* and *B. subtilis* were on par with that of the insecticide check. All other treatments were on par with the untreated control indicating their insignificance in reducing the whitefly count one month after transplant. From this it could be inferred that the disease reducing activity of plant extracts like *B. spectabilis*, *M.jalapa* and *B. diffusa* is not by means of checking the insect vector but by virtue of some antiviral property it confers (Table 2).

Genomic DNA isolated from six random samples collected from infected plants in the experimental plot were subjected to PCR amplification using two sets of primers, a universal primer, DENG primer and another specific primer. All the samples tested positive confirming the incidence of Chilli leaf curl virus in the experimental plot.

CONCLUSION

The management of plant viruses is usually restricted to the utilization of resistantvarieties, the availability of such sources along with commercial acceptance is often a problem. In this experiment to identify the effective non- chemical strategy to contain Chilli leaf curl virus affecting chilli, leaf extract of *A. indica* (10%) and *M. jalapa* (10%) were found to have significant disease reducing effect. The effect of the other two plant extracts with reported antiviral properties, *B. spectabilis* (10%) and *B. diffusa* (10%) was also significant in reducing the chilli leaf curl disease incidence and severity. The disease severity recorded in these treatments ranged from 22.67 to 30.67 against the 54.67 in the untreated control. The only biocontrol agent with adisease reducing effect was *B. subtilis.* It may be proposed to include an environmentally sound and viable management strategy that includes seed treatment and timely foliar sprays at 15 days interval of the plant extracts of *A. indica* or *M. jalapa* in combination with the bioagent *B. subtilis* in effectively containing the ChiLCVD associated with the curling, crinkling, puckering and dwarfing

symptoms in chilli. This combination apart from disease reduction also is promising in reflecting increased plant height and health.

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