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ASSESSING THE RESISTANCE OF DIFFERENT CHILI GENOTYPES TO CHILI LEAF CURL VIRUS (CHILCV) AND EVALUATING INSECTICIDES FOR CONTROLLING ITS VECTOR, *BEMISIA TABACI*

[a]Azher Mustafa, [a]Saima Naseer, [b]Salman Ahmad, [c]Hafiz Muhammad Aatif, [d]Azhar Abbas Khan, [e]Zeshan Hassan, [d]Ch. Muhammad Shahid Hanif, [a]Saba Saeed, [a]Javed Anwar Shah, [c]Yasir Ali
[a] *Plant Pathology Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan.*
[b] *Department of Plant Pathology, University College of Agriculture, University of Sargodha, Sargodha, Pakistan.*
[c] *Department of Plant Pathology, College of Agriculture, University of Layyah, Layyah, Pakistan.*
[d] *Department of Entomology, College of Agriculture, University of Layyah, Layyah, Pakistan.*
[e] *Department of Plant Breeding and Genetics, College of Agriculture, University of Layyah, Layyah, Pakistan.*

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ABSTRACT

The production of chili in Pakistan is currently facing a significant threat from Chili leaf curl virus (ChiLCV). A study was conducted in the crop growing seasons 2019-2020 and 2020-2021 at Ayub Agriculture Research Institute Faisalabad to assess the susceptibility and resistance of nine chili genotypes to ChiLCV. Each genotype was replicated three times by using the Randomized Complete Block Design. Weekly documentation was carried out to record the incidence and severity of the disease. During the first and second crop growing seasons, it was observed that, Tarapuri (78%, 76.9%), CH 107 (67.8%, 63.7%), Talhari (65.7%, 56.8%), CH 109 (52.7%, 54.4%), CH 108 (50.8, 51.1), and GSL-111 (42.6%, 39.5%) genotypes showed susceptible response to ChiLCV. The two genotypes Hybrid-46 (17.7%, 18.5%) and Hot Queen (15.5%, 13.4%) exhibited a moderately resistant response and only one genotype CBS1292 (40.7%, 39.1%) showed a moderately susceptible response against ChiLCV. The results of the screening experiment indicated that a majority of chili varieties exhibit susceptibility to chili leaf curl disease and the most of the germplasm is deficient in terms of providing protection against the disease. In addition, another study was conducted to evaluate the effectiveness of five insecticides, namely Acephate 75 SP, Emamectin, Imidacloprid, Diafenthiuron, and a combination of these insecticides, in managing percent disease incidence, reducing whitefly population, and enhancing crop yield. Among the various insecticides evaluated, Diafenthiuron exhibited the highest efficacy in reducing disease incidence (7.70%, 6.66%), reducing white fly population (1.80, 1.56), and increasing the yield of green fruit (120.71, 132.52q/ha) followed by combination of all of these inecticides, Imidacloprid, Emamectin, and Acephate during both crop growing seasons. This approach has proved a high level of safety and ecological compatibility, and it appeared to be a practical disease management tool.

Corresponding Author: Yasir Ali
Email: yasirklasra.uca@gmail.com

INTRODUCTION

Pepper, or chili (*Capsicum annum* L.), is a vegetable crop belonging to the family Solanaceae (Mishra et al., 2022). It originated in Mexico 6000 years ago and was carried to Asia by Portuguese navigators in the 16th century (Kraft et al., 2014). According to Dorantes et al. (2000), the spiciness of chili peppers is attributed to capsaicin and other capsaicinoids. This crop is cultivated in regions with subtropical and tropical climates for both medicinal and dietary purposes. The cultivation of this crop is commercially practiced in all four provinces of Pakistan (Iqbal et al., 2012). Its production in Pakistan amounted to 463,860 metric tons, cultivated across an area of 171,800 hectares in the year 2020 (Anonymous, 2020).

Chili serves as a versatile ingredient in various culinary applications, including as a spice, vegetable, pickle, and sauce component (Verma et al., 2023). The utilization of oleoresin and other spiritual applications has been documented in previous investigations (Meghvansi et al., 2010). It is considered a healthy food source due to the presence of various antioxidants such as Vitamin A, Vitamin C, β-carotene, potassium, and others. According to İnceoren et al. (2023), chili has been found to possess preventive properties against cancer and diabetes.

The chili crop is susceptible to infection by various microorganisms such as fungi, bacteria, viruses, and nematodes, which can result in reduced yields (Shahbaz et al., 2015; Khan et al., 2018; Asghar et al., 2020; Tariq-Khan et al., 2020; Ali et al., 2022; Saba et al., 2022; Aslam and Mukhtar, 2023a, b; Zeeshan et al., 2023). Viral infections in plants are known to contribute to various factors (Ashfaq et al., 2014; Murtza et al., 2017; Ahmed et al., 2021; Naseer et al., 2021). The annual reduction of crop yields is caused by numerous viruses (Khan et al., 2018). To date, a total of 65 viruses have been documented, among them begomoviruses, which are responsible for the spread of chilli leaf curl virus (ChiLCV) disease in chili crops worldwide (Nigam et al., 2015). The ChiLCV has been identified as the most harmful virus in relation to both incidence and yield reduction. Studies have reported instances of complete loss of marketable fruit in severe cases (Senanayake et al., 2012).

Under severe disease conditions, ChiLCV can result in stunted growth, a lack of blooms or fruits, puckering, and upward curling (Thakur et al., 2018). The transmission of begomoviruses is facilitated by the whitefly *Bemisia tabaci*. These viruses possess circular single-stranded genomes ranging from 2.7 Kb (monopartite species) to 5.4 Kb (bipartite species) (Prasanna et al., 2010). India has reported the description of genomic sequences of four begomoviruses, namely ChiLCPV, ToLCJV, ToLCNDV, and ChiLCV, which are known to infect chili plants. According to Chavan et al. (2015), a strain of Tomato Leaf Curl Virus (ToLCV) was discovered in Maharashtra, which has been observed to decrease the yield of tomato and chili by 90-100%.

Whitefly has spread worldwide during the 1990s. This vector is controlled through the implementation of barriers, synthetic insecticides, and agronomic practices (Udiarto et al., 2023). Young whiteflies tend to hide themselves beneath leaves and within the lower regions of the plant canopy, thereby becoming challenges for chemical therapy. Polyphagous pests exhibit a preference for both cultivated and weed hosts. The organism rapidly acquires resistance to both synthetic and natural pesticides. The application of insect growth regulators and novel pyrethroid insecticides has the potential to effectively manage this particular pest (Roy et al., 2023). It is improbable for a single strategy to effectively ensure the absence of viruses in crops. Therefore, it is necessary to implement multiple economically justified preventive methods (Roy et al., 2023).

The management of the vector *B. tabaci*, employment of resistant germplasm, and implementation of other disease management measures has been identified as effective means of controlling plant pathogens including ChiLCV (Iqbal et al., 2020; Mukhtar and Kayani, 2020; Azeem et al., 2021; Mukhtar et al., 2021; Saeed et al., 2021; Mukhtar et al., 2023; Shahbaz et al., 2023). The utilization of resistant germplasm can effectively and sustainably manage plant viral infections over an extended period (Ahmad et al., 2021a). The effective management of diseases necessitates the identification of sources that are resistant to pathogens (Ashfaq et al., 2014; Aslam et al., 2017; Jafir et al., 2018; Asif et al., 2018; Ahmad et al., 2021b; Ahmed et al., 2021; Haq et al., 2022). The utilization of chemicals has been identified as an effective method for managing whiteflies and reducing the spread of viruses (Naseer et al., 2021; Ali et al., 2022). Before planting, it is essential to inspect transplants for viruses and vectors, just as one would do for other pests. Transplants have a higher chance of thriving and succeeding in virus-free environments. Consequently, the primary aim of this study was to assess the existing chili genotypes to identify sources of resistance and employ chemicals to control the population of whiteflies, which are potential carriers of the chili leaf curl virus disease.

MATERIALS AND METHODS

Several chili genotypes, namely Hot Queen, CH 108, Talhari, CH 109, Tarapuri, GSL-111, Hybrid-46, CH 107, and CBS1292, were sown in the Research Area of Vegetable Production at Ayub Agriculture Research Institute (AARI) Faisalabad during two consecutive years, 2019-2020 and 2020-2021. Throughout the experiment, no insecticides were used to control the whitefly population. The screening of varieties was carried out by observing symptoms in plants on a weekly basis throughout the growing season. The disease severity data for each genotype were evaluated using the disease rating scale described by Kumar et al. (2006). To determine disease incidence (DI), the total number of plants was counted, and the number of sick plants was divided by that total. Each line or variation was repeated three times using the Randomized Complete Block Design (RCBD) design.

Data recording

After transplantation, disease severity and incidence data were recorded using the disease assessment scale proposed by Kumar et al. (2006b) and Devi et al. (2023). The scale used to assess the level of susceptibility of a plant to a particular disease is as follows: 0% indicates an absence of symptoms, signifying immunity; 1-5% is characterized by clearing and curling of the upper leaves, indicating high resistance (HR); 6-25% is marked by curling of leaves and swelling of veins, indicating resistance (R); 26-50% is characterized by puckering, leaf curling, yellowing, and vein swelling, indicating moderate susceptibility (MS); 51-75% is marked by leaf curling, stunted growth, and blistering of internodes, indicating susceptibility (S); and greater than 75% is characterized by leaf curling, deformed leaves, stunted growth, small flowers, and little fruit set, indicating high susceptibility (HS).

Following a period of fifty days, a disease severity grade was assigned to each genotype. The investigation of disease progression in various genotypes was conducted by using data on the percentage of leaf curling that was collected at weekly intervals. The whitefly population was quantified at dawn using a suction pump and magnifying lens. The population was determined through random sampling of the upper, lower, and middle sections of each individual plant. The confirmation of ChiLCV's pathogenicity was achieved by means of grafting infected chili scions into both healthy chili and datura plants.

In vivo evaluation of insecticides

Six different treatments were applied at weekly intervals to determine the most appropriate method of controlling whitefly populations under field conditions. The experimental treatments employed in this study were as follows:

T1 = Spray of Acephate 75 SP @ 1.5 g/L
T2 = Spray of Emamectin 200 ml / 100 L of water
T3 = Spray of Imidacloprid 250 ml / 100 L of water per acre
T4 = Spray of Diafenthiuron 200 ml / 100 L of water
T5 = Spray of a rotation of T1 + T2 + T3 + T4
T6 = Control

The incidence of leaf curl and the number of whiteflies were observed and recorded at seven-day intervals, starting from seven days after transplanting (DAT) and continuing until fruit formation. These observations were subjected to statistical analysis. The cumulative yield of chili fruit, as recorded during each picking, was converted into quintals per hectare and subjected to statistical analysis. The disease incidence was calculated using the expression given below:

$$Disease\ incidence\ (\%) = \frac{Number\ of\ diseased\ plants}{Total\ no.\ of\ plants\ observed} \times 100$$

Statistical analysis

The data pertaining to disease incidence, white fly populations, and green fruit yield were subjected to analysis of variance (ANOVA). In cases where a significant difference was observed among the treatments, means were determined using Duncan's multiple test ($P < 0.05$) through using of SPSS 2020 version 27.0.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

During the crop growing seasons of 2019-2020 and 2020-2021, it was observed that genotypes, including Tarapuri, CH 107, Talhari, CH 109, CH 108, and GSL-111, exhibited a susceptible response to ChiLCV, with varying degrees of disease severity. The CBS1292 variety showed a moderately susceptible response to disease severity, while the Hybrid-46 and Hot Queen varieties exhibited a moderately resistant response (Table 1).

Table 1. Response of different chili genotypes to ChiLCV disease severity (%) during the crop growing seasons of 2019-2020 and 2020-2021.

Genotypes	Disease severity (%)	Reaction	Genotypes	Disease severity (%)	Reaction
	2019-2020			2020-2021	
Tarapuri	78.0 A	S	Tarapuri	76.9 A	S
CH 107	67.8 AB	S	CH 107	63.7 B	S
Talhari	65.7 ABC	S	Talhari	56.8 C	S
CH 109	52.7 BCD	S	CH 109	54.4 D	S
CH 108	50.8 B CD	S	CH 108	51.1 E	S
GSL-111	42.6 CD	S	GSL-111	39.5 F	S
CBS1292	40.7 D	MS	CBS1292	39.1 G	MS
Hybrid-46	17.7 E	MR	Hybrid-46	18.5 H	MR
Hot Queen	15.5 E	MR	Hot Queen	13.4 I	MR

Means that do not share a common letter are significantly different.

The results of the present study were in line with the findings of Ashfaq et al. (2007), who evaluated 87 urdbean genotypes for Urdbean leaf crinkle virus in 2005-06. They found that nine genotypes were highly resistant, 19 were moderately resistant, and 29 showed a resistant response against chili leaf curl disease severity (%). Similarly, Daunde and Khandare (2020) tested fourteen chili varieties for ChiLCV resistance in open field trials. The two genotypes, CH111 and Tarapuri, were the most susceptible to chili leaf curl disease. Eight genotypes, namely GSL111, CH109, CH 106, CH 99, CH108, CH103, Talhari, and CH107, were vulnerable to the disease, whereas one genotype, CBS1292, exhibited a susceptible response. Only Hybrid-46 and Hot Queen were found to be moderately resistant against ChiLCV disease severity (%). The findings of the current investigation indicated that the application of treatments prior to the onset of infection during the season effectively controlled the percentage of chili leaf curl virus disease incidence (Roy et al., 2019). In order to protect the plants from whitefly infestation, it is recommended to utilize Diafenthiuron and a combination of Acephate + Emamectin + Imidacloprid + Diafenthiuron during the early and late stages of plant growth (Daunde and Khandare, 2020).

In vivo evaluation of insecticides

Effect of insecticides on % ChiLCV incidence

The application of all the treatments significantly reduced the ChiLCV disease incidence (%) during rating seasons of 2019-2020 and 2020-2021 (Table 2).

Table 2. Effect of insecticides on the disease incidence of ChiLCV during the crop growing seasons of 2019-2020 and 2020-2021.

Treatments	Disease incidence (%)	Disease incidence (%)
	2019-2020	2020-2021
T_1 = Spray of Acephate 75 SP @ 1.5 g/L	22.70 B	24.70 B
T_2 = spray of Emamectin 200 ml / 100 L	14.80 C	15.90 C
T_3 = spray of Imidacloprid 250ml / 100 L	12.01 D	13.67 D
T_4 = spray of Diafenthiuron 200 ml / 100 L	7.70 F	6.66 F
T_5 = spray of rotation of T_1 + T_2 + T_3 +T_4	10.70 E	12.83 E
T_6 = Control	57.73 A	53.73 A

Means that do not share a common letter are significantly different.

The application of Diafenthiuron spray at a concentration of 200 ml per 100 liters of water per acre was observed to be the most effective treatment in reducing disease severity. This treatment resulted in a reduction of disease incidence by 7.70% during the first rating season and 6.66% during the second crop season, demonstrating its superiority over other treatments. In the 2019-2020 and 2020-2021 crop seasons, the application of a spray consisting of the rotation of T1, T2, T3, and T4 was found to be the second most efficacious treatment, resulting in a reduction of disease incidence by 10.70% and 12.83%, respectively, followed by Imidacloprid (12.01 and 13.67%), Emamectin (14.80 and 15.90%), and Acephate (22.70 and 24.70%). The maximum disease incidence was observed under control conditions, as indicated by the values of 57.73 and 53.73% in the first and second crop growing seasons, respectively (Table 2).

Effect of treatments on number of whitefly population

The implementation of all treatments resulted in a significant reduction in whitefly populations during both the rating seasons of 2019-2020 and 2020-2021 (Table 3). The most effective treatment in reducing the population of whiteflies was observed to be the application of Diafenthiuron spray at a concentration of 200 ml per 100 liters of water per acre. This particular treatment resulted in a decrease in the whitefly population to 1.80/leaf during the initial assessment period of 2018-2019, and 1.56/leaf during the subsequent crop season of 2019-2020. The application of a spray solution involving the rotation of T1, T2, T3, and T4 was found to be the second most effective treatment, resulting in a decrease in the whitefly population by 2.90 and 2.50 during the first and second crop seasons, respectively, followed by Imidacloprid (5.00, 4.67), Emamectin (4.04, 4.02), and Acephate (4.40, 4.10). The maximum numbers of whiteflies were observed in the control group, with values of 10.50 and 10.33 recorded during the initial 2019-2020 and subsequent crop growing season 2020-2021, respectively (Table 3).

Table 3. Effect of treatments on the reduction of number of whitefly populations during the crop growing seasons of 2019-2020 and 2020-2021.

Treatments	Number of whitefly/leaf	Number of whitefly/leaf
	2019-2020	2020-2021
T_1 = Spray of Acephate 75 SP @ 1.5 g/L	4.40 C	4.10 C
T_2 = Spray of Emamectin 200 ml / 100 L	4.04 D	4.02 C
T_3 = Spray of Imidacloprid 250ml / 100 L	5.00 B	4.67 B
T_4 = Spray of Diafenthiuron 200 ml / 100 L	1.80 F	1.56 E
T_5 = Spray of rotation of T_1 + T_2 + T_3 +T_4	2.90 E	2.50 D
T_6 = Control	10.50 A	10.33 A

Means that do not share a common letter are significantly different.

Effect of insecticides on green fruit yield

The application of all treatments resulted in a statistically significant increase in the production of green fruit yield during the rating seasons of 2019-2020 and 2020-2021 (Table 4). The application of Diafenthiuron was found to be the most effective treatment for enhancing the green fruit yield of chili. This treatment resulted in a significant increase in the green fruit yield, with a recorded yield of 120.71 q/ha during the first crop season of 2018-2019, and 132.52 q/ha during the subsequent rating season of 2019-2020. The application of a spray solution that involves the rotation of T1, T2, T3, and T4 was determined to be the second most effective treatment, leading to an increase in green fruit yield by 115.52 q/ha in the initial crop season and 117.93 q/ha during the subsequent crop seasons, respectively, as compared to the yields obtained with Imidacloprid, Emamectin, and Acephate. The minimum green fruit yield was recorded under control conditions (Table 4).

The findings presented in the present study are consistent with the research conducted by Talukder et al. (2012), who examined the effectiveness of various chemicals and barrier crops in controlling the spread of chilli leaf curl disease. Several chemical compounds were assessed over a period of three consecutive years, including milk (10%), Furadan 5G (30kg/ha), Omite (0.1%), Admire (0.05%), and a barrier of foxtail millet arranged in a staggered pattern. The execution of a barrier of foxtail millet in a row-by-row design resulted in superior disease control and yielded the highest crop production, surpassing the effects of Admire and

Furadan. Conversely, the untreated control group exhibited the highest proportion of diseased plants. Hussain and Atiq (2017) conducted a study to evaluate the efficacy of pesticides in controlling Chilli leaf curl virus and its whitefly vector, with their investigation yielding similar outcomes. According to their study, the application of Disulfotan or Carbofuran at a rate of 1.5 kg a.i. /ha as a soil treatment or the implementation of four sprays of power oil (1%) at 10-day intervals effectively managed whitefly and mitigated leaf curl disease. This treatment resulted in a significant increase in crop yield. The disease was also effectively managed by the application of insecticides containing Oxydemeton methyl (0.05%) and Dimethoate (0.05%). Based on the findings of the study, the management of chilli leaf curl in the field can be achieved through the use of Diafenthiuron, Emamectin, Imidacloprid, and their combination (Daunde and Khandare, 2020). Timely application of pesticides has the potential to enhance crop productivity and reduce the incidence of diseases.

Table 4. Effect of treatments on the green fruit yield of chilli during the crop growing seasons of 2019-2020 and 2020-2021.

Treatments	Green fruit yield q/ha	Green fruit yield q/ha
	2019-2020	2020-2021
T_1 = Spray of Acephate 75 SP @ 1.5 g/L	74.73 E	77.94 E
T_2 = Spray of Emamectin 200 ml / 100 L	78.85 D	89.77 D
T_3 = Spray of Imidacloprid 250ml / 100 L	81.34 C	94.56 C
T_4 = Spray of Diafenthiuron 200 ml / 100 L	120.71 A	132.52 A
T_5 = Spray of rotation of $T_1 + T_2 + T_3 + T_4$	115.52 B	117.93 B
T_6 = Control	60.9 F	68.37 F

Means that do not share a common letter are significantly different.

CONCLUSION

It was concluded that two genotypes, Hybrid-46 (17.7%, 18.5%) and Hot Queen (15.5%, 13.4%), were moderately resistant to ChiLCV, while one, CBS1292 (40.7%, 39.1%), was moderately susceptible during both rating seasons. The screening experiment showed that most chili cultivars are susceptible to chili leaf curl disease incidence. Diafenthiuron significantly reduced the disease incidence (7.70%, 6.66%), white fly population (1.80, 1.56), and enhanced the green fruit yield (120.71, 132.52q/ha) as compared to Imidacloprid, Emamectin, and Acephate. This method proved safe, eco-friendly, and most effective for ChiLCV disease and its vector management.

AUTHORS' CONTRIBUTION

AM and SN designed, formulated and laid out the study; AM, SN, SA and HMA conducted the experiments; AAK, ZH and CMSH collected, arranged and analyzed the data; SS provided technical assistance; JAS supervised the work; YA and SN wrote the manuscript; AM and SS proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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