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Management of Chili leaf curl disease (ChiLCD) through resistant germplasm and Nutrients in relation to Environmental Factors

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Abstract

The aim of present study was to evaluate the selected germplasm against chili leaf curl virus disease (ChiLCVD) and its management through nutrients. The chili varieties i.e., Zinia F1, Desi (Jalalpuri), Desi (Jalalpuri 2), Wonder Hot, Summer Queen F1 and Faisalabad Selection was evaluated against the ChiLCVD. After screening, Wonder Hot and Summer Queen F_1 showed moderately resistant (MR) response whereas, 3 varieties i.e., Zinia F_1, Desi (Jalalpuri) and Desi (Jalalpuri 2) had a moderately susceptible (MS) response and only a single variety (Faisalabad Selection) expressed resistant (R) response. Four varieties; Desi (Jalalpuri), Desi (Jalalpuri 2), Zinia F1 and Wonder Hot, were used for management experiment with 4 nutrients combinations, T_1 ($ZnSO_4$ + $CuSO_4$), T_2 ($MnSO_4$ + $CuSO_4$), T_3 (Boric Acid + $CuSO_4$) and T_4 ($ZnSO_4$ + $CuSO_4$ + $MnSO_4$ + Boric Acid). When compared to control (58.14%), only T_4 demonstrated minimum disease severity (11.63%). In case of disease incidence, T_4 gave the best results with minimum disease incidence (35.65%) as compared to control (92.59%). Treatments reduced disease progression even during favorable environmental conditions. There was significant ($p<0.05$) but positive correlation between wind speed and disease severity. Wind speed was highly correlated with the disease severity of variety Wonder Hot ($r=0.91$). It could be concluded that the application of nutrients activates the plant defense system and at suppress the vector infestation.

Key words: Management, Nutrients, Whitefly, Epidemiology

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1. INTRODUCTION

Chili (*Capsicum annuum* L.) is the most important vegetable cultivated worldwide in tropical and subtropical areas[1,2]. Chili contains a number of phytochemicals with antibacterial, anti-degenerative, and antioxidant activities along with vitamins (A, B_1, B_2, E and C) flavonoids, carotenoids and phenolics[3,4].

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Pakistan is the 4th largest chili producer's country in the world after India, China, and Taiwan [5]. Chili was cultivated on 65.3 thousand hectares in Pakistan with a production of 148.7 thousand tons in 2018 [6].

Chili crop is devastated by 83 diseases caused by bacteria, nematodes, fungi and viruses[7]. Viral diseases of chili are the major threat to yield losses in the world. Chili plant is attacked by *Chili leaf curl virus* (ChiLCV) that cause the chili leaf curl disease (ChiLCD)[8]. ChiLCV belongs to genus *Begomovirus* and family *Geminiviridae*[9,10].The most prevalent vector of the begomoviruses is the whitefly (*Bemisia tabaci*) [11]. The genome of ChiLCV consists of circular ssDNA with associated β-satellites that cause 100% yield losses in tomato, chili, cucurbits, cassava and beans[12]. ChiLCD exhibit upward curling, puckering, vein clearing, yellowing of leaves, stunted plant growth and reduced leaf size with shortened internodes[13]. In early phase of infection virus causes young leaves to become distorted and curled towards the midrib[14, 15].

It is challenging to manage ChiLCD in susceptible chili cultivars; application of insecticides and cultural practices to lower the disease incidence and whitefly populations, are the most promising strategies[16]. The injudicious usage of chemical pesticides not only increase input costs but also deteriorate environment [17, 18].The use of disease-resistant cultivars is the most effective method of viral disease management [19]. In past, different chili cultivars were grown in greenhouse to evaluate the level of resistance against ChiLCD aiming at onward recommendations to farmers [20]. ChiLCV could be managed by controlling its vector *B. tabaci* by using resistant germplasm, botanical sprays and nutrients. Resistant genotype provides a long-term and environmentally sustainable alternative for the management of ChiLCD[5]. The prevalence of ChiLCD depends upon the whitefly infestation that varies in different areas with respect to prevailing environmental conditions[21]. It was noted that whitefly population increased with increase in temperature and decreased with increase in relative humidity[22]. The intensity of disease was minimum during winter months because of lower whitefly population in low temperature[23]. It is a dire need to find out resistant sources against ChiLCD and its management through non-chemical means.

The present study was aimed at screening of chili germplasm against ChiLCD, its nutritional management and correlation of favourable environmental conditions with the disease.

2. MATERIALS AND METHODS

2.1 Experimental area and germplasm collection

A trial was conducted at research area of Plant Pathology, University of Agriculture, Faisalabad (UAF), Pakistan by sowing 06 commercial cultivars of chili i.e. Summer Queen F_1, Zinia F_1, Wonder Hot, Faisalabad, Desi (Jalalpuri) and Desi (Jalalpuri 2).

2.2 Establishment of disease screening nursery

A disease screening nursery was established to find a source of resistance to the ChiLCD during 2020. All the cultivars were sown on bed with 12 inches Plant x Plant and 30 inches Row x Row distance. The recommended agronomic activities such as irrigation, hoeing and, weeding were implemented at the appropriate times to ensure maximum growth.

2.3 Data recording

The data of diseased plants was started after the appearance of characteristics symptoms soon after transplanting. The frequency of data recording was fortnight and disease incidence of each germplasm was categorized according to the rating scale[24]. Immune: absent of leaf curling, Resistant: 1-10% with very mild curling, Moderate resistant: 11-25% with puckering and curling, Moderate susceptible: 26-50% severe puckering and curling, Susceptible: 51-75% severe puckering and curling with stunting of plant growth and small leaf size, Highly susceptible: >75% with all ChiLCD reported symptoms.

2.3 *In-vivo* management of ChiLCD

In-vivo management of ChiLCD was done by sowing 4 lines i.e. Zinia F_1, Wonder Hot, Desi (Jalalpuri) and Desi (Jalalpuri 2) in 3 repetitions using RCBD (Randomized Complete Block Design). Each row consisted of 12 plants with 12 inches Plant x Plant and 30 inches Row x Row distance. The experiment contained 5 treatments T_1 (Zinc Sulphate + Copper Sulphate), T_2 (Manganese Sulphate + Copper Sulphate) and T_3 (Boric Acid + Copper Sulphate) at the concentration of 0.25g/500ml water was used. In treatment T_4 (Zinc

Sulphate + Manganese Sulphate + Boric Acid + Copper Sulphate) was used with the concentration of 0.13g/500ml water. All treatments were applied at 15 days interval and data was recorded by using following formulae

Disease incidence (%): No. of infected plants/Total No. of plants observed ×100

Disease severity: No. of infected leaves/ Total No. of leaves × 100.

2.4 Observation of Plant growth parameters

After the crop harvesting the agronomic data including Shoot length (cm), Root length (cm), Fresh weight (g) and Dry weight (g) was also recorded. From each replication, plants of each genotype were taken and root length was measured using a scale. Plants' fresh and dried weights were also assessed in grams (g) by using electric balance.

2.5 Effect of environmental factors on ChiLCD

The effect of weather variables i.e. maximum and minimum temperatures, wind speed and, relative humidity was studied on ChiLCVD development by using correlation analysis. The weather data was obtained from the World Weather Online (WWO) official website.

2.6 Statistical analysis

Statistix 8.1 software was used to analysis the data during the current research and assessment between treatments and genotypes was done according to Tukey (1949) at 5% level of significance.

3. RESULTS AND DISCUSSIONS

3.1 Response of chili germplasm against ChiLCD under field conditions

In screening experiment, no variety was highly resistant against ChiLCD; 2 varieties (Wonder Hot and Summer Queen F_1) showed disease severity 16.10% and 23.43%, respectively and regarded as moderately resistant (MR) with disease rating 3. There were 3 moderately susceptible (MS) cultivars i.e. Zinia F_1 (36.93%), Desi Jalalpuri (49.43%) and Desi Jalalpuri 2 (37.33%) disease severity with disease rating 5 and only a single variety (Faisalabad Selection) with 8.73% disease severity expressed resistant (R) response in disease rating 1. There was no susceptible and highly susceptible variety among screened entries shown in Fig 1. Ali et al. [22] stated that the infected chili plants showed crinkling and downward rolling of the leaves. In most acute conditions, leaves become twisted, narrowed, and plant showed stunted growth with bushy look. Jose *et al.* (2003) tested 37 chili cultivars against the ChiLCV under natural field conditions in Kerala and reveald that the genotypes Alampady local-1, Nayyattinkara local, Chandera local, Haripuram local, Mangalapuram local, Kottiyan local, KottiKulam local, and Pant C-1 were tolerant to ChiLCVD and 27 varieties showed susceptible response and 2 varieties were highly susceptible. Kumar et al. [23]tested 307 genotypes of Chili against ChiLCVD and varieties were ranked into resistant and susceptible under field trials based on co-efficient of infection (CI). Three lines GKC-29, EC-497636 and BS-35 showed resistance against the ChiLCVD.

3.2 Effect of nutrients on disease severity

After screening, effect of different nutrients on ChiLCD management was evaluated on 4 varieties (Zinia F1, Desi (Jalalpuri), Desi (Jalalpuri 2) and Wonder Hot). Zinia F1 shows maximum reduction (20.77%) in disease severity with the nutrient combination that gave the best results was Zinc Sulphate + Copper Sulphate (ZS+CS) followed by Desi Jalalpuri (20.59%) with the combination of (ZS+CS+MnS+BA). Maximum Increase in disease severity was recorded in Desi Jalalpuri 2 (29.01%) with untreated control (Table 1; Fig 2).

3.3 Effect of nutrients on disease incidence

Out of all varieties, Wonder Hot show minimum increase (11.10%) in disease incidence with the nutrient combination (Zinc Sulphate + Copper Sulphate (ZS+CS) followed by Wonder Hot (12.96%) with the combination of (BA+CS), Zinia F1 (12.96%) with the combination of (ZS+CS+MnS+BA), and Desi (Jalalpuri 2) (12.96%) with the combination of (ZS+CS). Maximum Increase in disease incidence was recorded in Desi (Jalalpuri 2) (70.73%) in untreated control (Table 2; Fig 3). Plants and microbes both require nutrients for

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growth and development, and the nutrients particularly play crucial role in preventing disease. Numerous critical nutrients are engaged in many processes that can alter a plant's response to pathogens; hence their effect on decreasing disease severity can be related to their involvement in plant physiology and biochemistry [24]. Nutrients can also have an indirect impact on disease resistance, as nutrient-deficient plants not only have a weakened defense response, but many metabolites such as reducing sugars and amino acids leak outside the plant cell, making them more accessible for feeding. Plant diseases may be suppressed by nutrients due to activation of systemic acquired resistance (SAR) [25]. A single foliar treatment of $CuSO_4$, $KMnO_4$, H_3BO_3, and MnCl2 which gave systemic defense against powdery mildew of cucumber, has also been found to reduce disease severity in other crops [26].Chili plants were treated with naphthalene acetic acid, NPK solution, Zinc solution and Humic Acid. Significant reduction in disease incidence was observed due to different treatments as compared to control in all the varieties. Minimum disease incidence (9.04%) was recorded due to naphthalene acetic acid as compared to control followed by NPK solution, humic acid and Zinc solution, respectively. Sujay et al. [27]used NAA along with other treatments against sucking pests of chili and observed that the population of target pests was significantly reduced and the incidence of respective viral diseases was subsequently decreased. Bio-regulators such as NAA regulate the plants to withstand the attack of whitefly and leaf curl virus. This was the second most effective strategy for the management of above mentioned menaces among the applied treatments. The reduction in disease incidence by NPK application might be attributed to nutrition-induced resistance in plants against insect pests [28].

3.4 Effect of nutrients on plant growth parameters

3.4.1 Shoot Length

Desi Jalalpuri (30.78%) showed maximum shoot length with the nutrient combination Zinc Sulphate + Copper Sulphate + Manganese Sulphate + Boric Acid (ZS+CS+MnS+BA) followed by Desi Jalalpuri 2 (26.37%) with the combination of (ZS+CS+MnS+BA). Minimum shoot length was recorded in Zinia F1 (8.28%) with untreated control (Table 3).

3.4.2 Root Length

Desi Jalalpuri 2 (17.93%) showed maximum root length with the nutrient combination i.e. Zinc Sulphate + Copper Sulphate + Manganese Sulphate + Boric Acid (ZS+CS+MnS+BA) followed by Zinia F1 (14.23%) with the combination of (ZS+CS+MnS+BA). Minimum root length was recorded in Wonder Hot (4.57%) with untreated control (Table 4).

3.4.3 Fresh Weight

Desi Jalalpuri (11.40%) showed maximum fresh weight with the nutrient combination i.e. Zinc Sulphate + Copper Sulphate + Manganese Sulphate + Boric Acid (ZS+CS+MnS+BA) followed by Desi Jalalpuri 2 (9.77%) with the combination of (ZS+CS+MnS+BA). Minimum fresh weight was recorded in Zinia F1 (2.66%) with untreated control (Table 5).

3.4.4 Dry Weight

Desi Jalalpuri (3.27%) showed maximum dry weight with the nutrient combination i.e. Zinc Sulphate + Copper Sulphate + Manganese Sulphate + Boric Acid (ZS+CS+MnS+BA) followed by Zinia F1 (2.99%) with the combination of (ZS+CS+MnS+BA). Minimum dry weight was recorded in Desi Jalalpuri 2 (0.51%) with untreated control (Table 6).

3.5 Effect of environmental factors on disease severity in absence of any nutrients

Effect of environmental conditions (minimum and maximum temperature, wind speed and relative humidity) on ChiLCD development was studied. The correlation coefficient between disease severity and environmental conditions was also determined. There was a negative correlation between the maximum temperature and disease severity of all the four varieties. Negative correlation indicated that as the maximum temperature increases then the disease severity decreases. The relationship was found significant at $p<0.05$ and the value of correlation coefficients of the varieties were Zinia F_1 (-0.73%) followed by Desi Jalalpuri (-0.74%), Wonder Hot (-0.79%) and Desi Jalalpuri 2 (-0.81%). Hussain and Atiq [5]tested 4

Chili lines/varieties in field conditions and studied the disease development in relation to environmental conditions (Fig 4).

Negative correlation was found between minimum temperature and disease severity of all the four varieties. Negative correlation indicated that as the minimum temperature increases then the disease severity decreases. The relationship was found significant at $p<0.05$ and the value of correlation coefficients of the varieties were Zinia F_1 (-0.69%) followed by Desi Jalalpuri (-0.71%), Wonder Hot (-0.76%) and Desi Jalalpuri 2 (-0.79%) (Fig 5).

Relative humidity was negatively correlated with the disease severity of all the four varieties. Negative correlation indicated that any increase in relative humidity decreases the disease severity. The relationship was found significant at $p<0.05$ and the value of correlation coefficients of the varieties were Zinia F_1 (-0.53%) followed by Wonder Hot (-0.59%), Desi Jalalpuri (-0.66%) and Desi Jalalpuri 2 (-0.71%) shown in Fig 6.

Wind speed was positively correlated with the disease severity of all the four varieties. Positive correlation indicated that as the wind speed increases then the disease severity also increases. The relationship was found significant at $p<0.05$ and the value of correlation coefficients of the varieties were Desi Jalalpuri (0.87%) followed by Zinia F_1 (0.88%), Desi Jalalpuri 2 (0.90%) and Wonder Hot (0.91%) shown in Fig 3.8. Senanayake *et al.* [35]have firstly reported the ChiLCV on chilli crop in India. Due to variation in the environmental factors of different areas insects show various responses in their incidence in degree and nature of damage to the crop. Along with biotic factors, abiotic factors also play an important role in determining the incidence and dominance of a particular pest or pest complex. Hussain and Naveed [36]tested 4 chilli lines/varieties in field conditions and studied the disease development in relation to environmental conditions. Effect of environmental conditions on disease development and whitefly population was observed. Their epidemiological data indicated that minimum and maximum temperature was positively correlated with whitefly population whereas negatively correlated with the development of disease. Relative humidity was negatively correlated with whitefly population indicated that due to any increase in relative humidity, whitefly population was decreased. Relative humidity and disease severity was positively correlated. Rainfall and whitefly population was negatively correlated whereas the correlation between rainfall and disease development was positive.

Sitaramaju *et al.* [37]narrated that there was a positive correlation between the population of whitefly and mean temperature. But correlation between whitefly population and relative humidity was negative. It indicated that rain may suppress the oviposition and increase the mortality rate of nymphs adults or emigration. The negative correlation between the population of whitefly with relative humidity and rainfall was due to control of *Bemisia tabaci* adults by rains and strong winds. Morales and Jones [38]studied that the diseases caused by numerous geminiviruses were more prevalent in humid and wet climatic conditions as compared to dry condition.

4. CONCLUSIONS

Use of resistant germplasm and nutrients are the most sustainable and environment friendly way to cope with ChiLCD. The correlation of environmental conditions with disease development indicated that disease can be avoided by adjusting date of sowing so that crop may escape from the favourable environment for vector.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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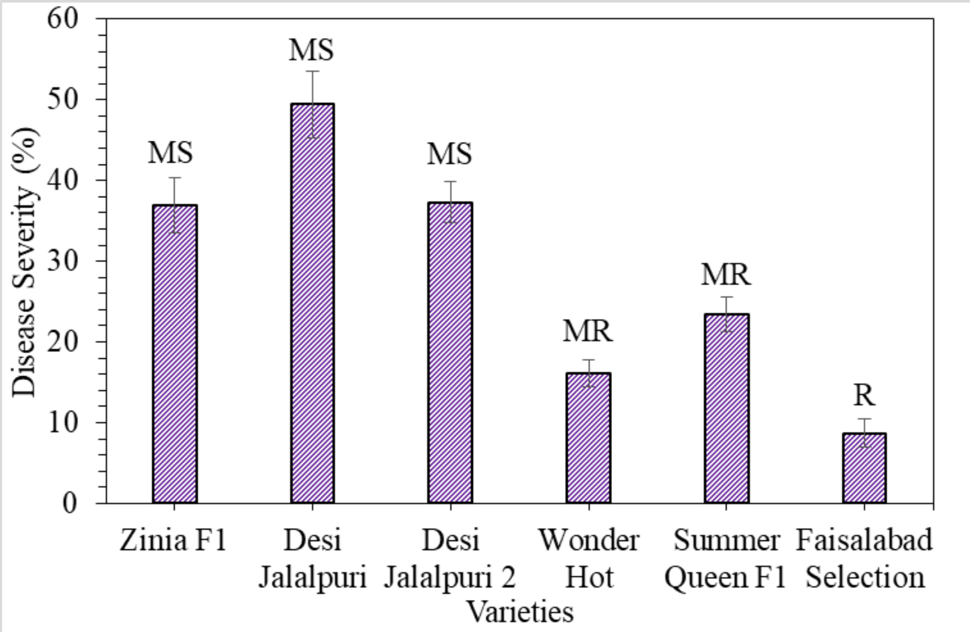

Fig. 1. Response of chili varieties against chili leaf curl disease

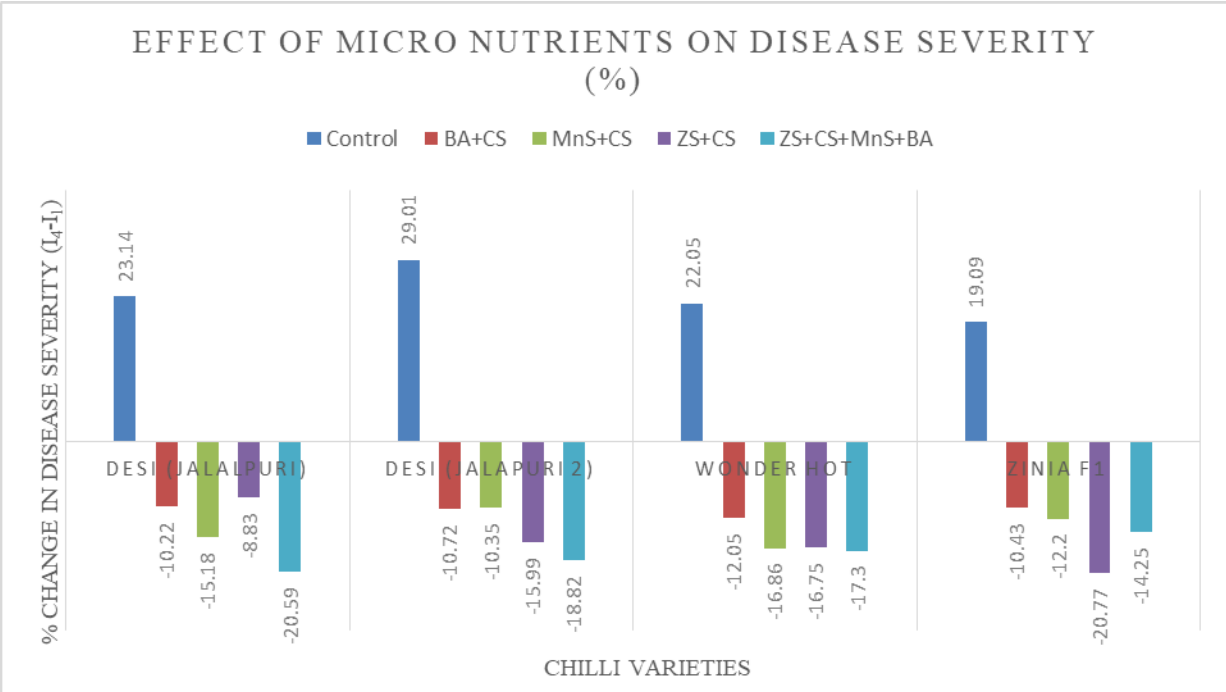

Fig. 2. Effect of nutrients on disease severity in 4 cultivars

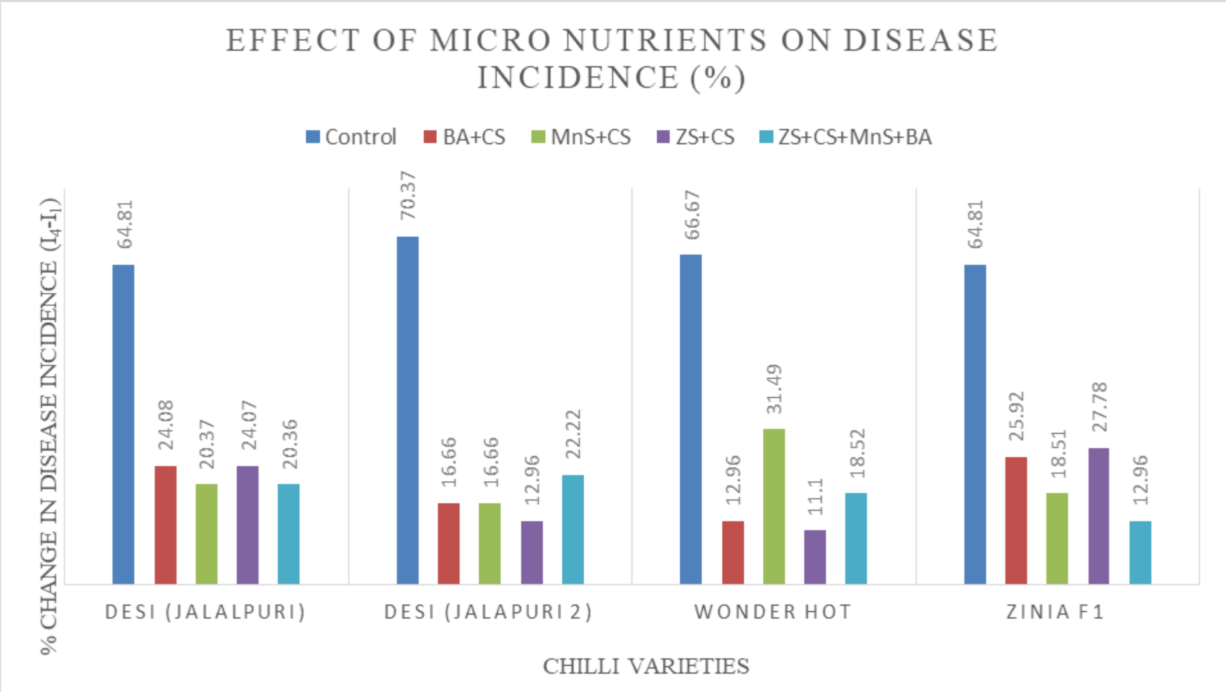

Fig. 3. Effect of nutrients on disease incidence in 4 cultivars

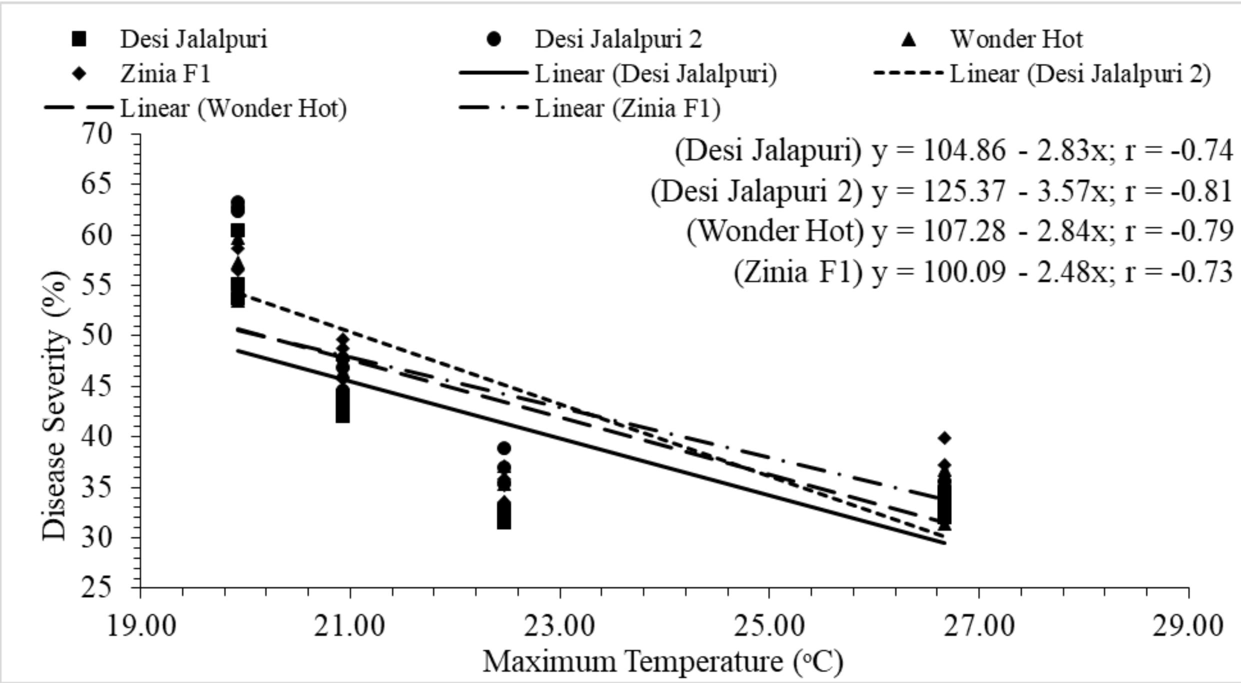

Fig. 4. Effect of maximum temperature on disease severity

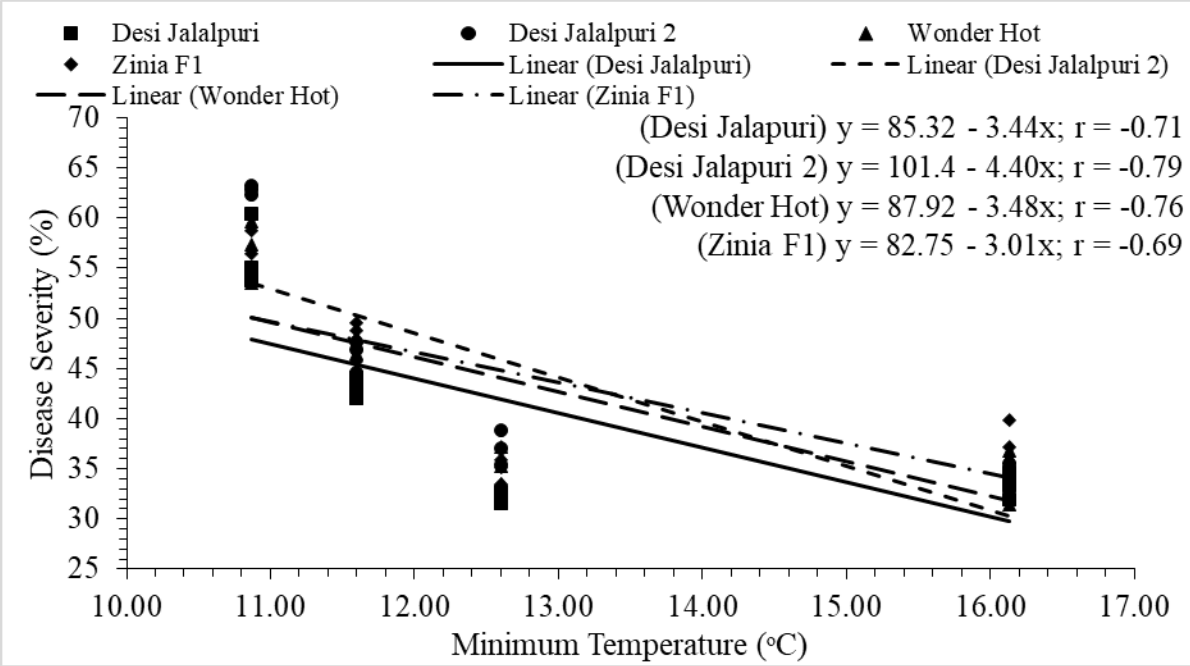

Fig. 5. Effect of maximum temperature on disease severity

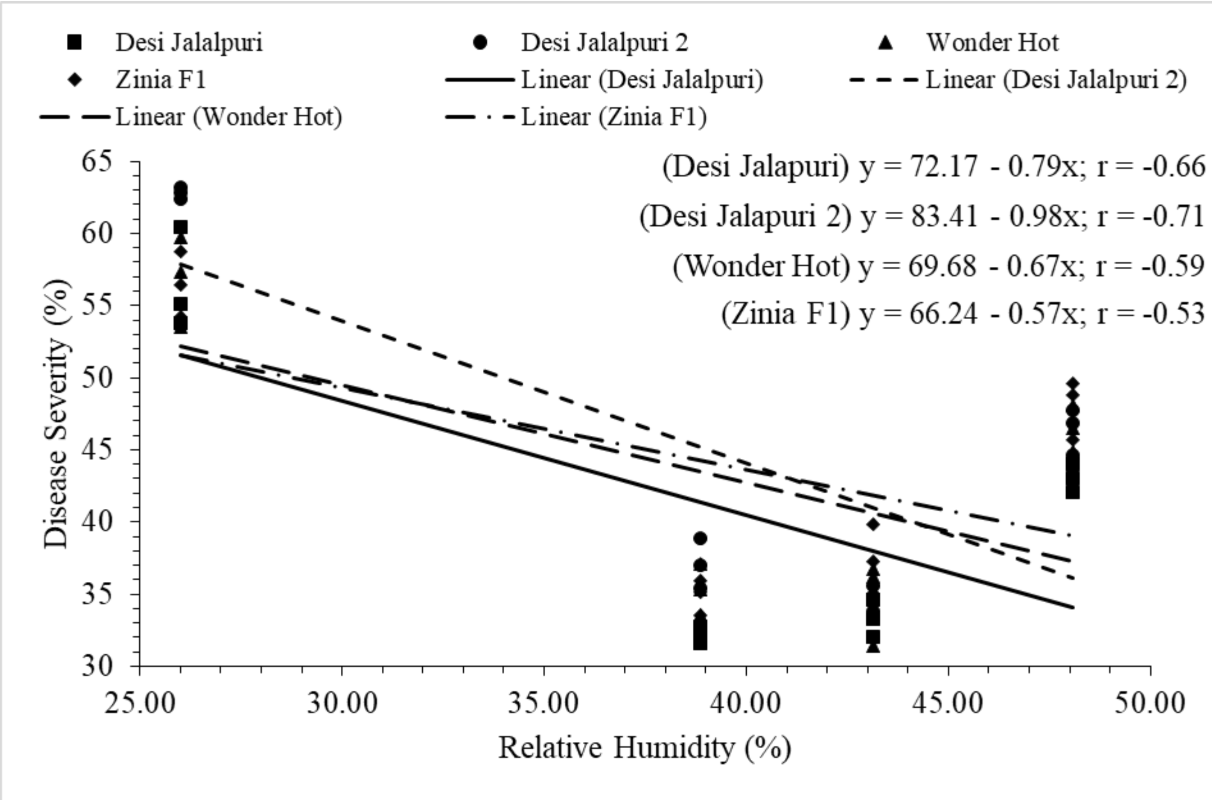

Fig. 6. Effect of relative humidity on disease severity

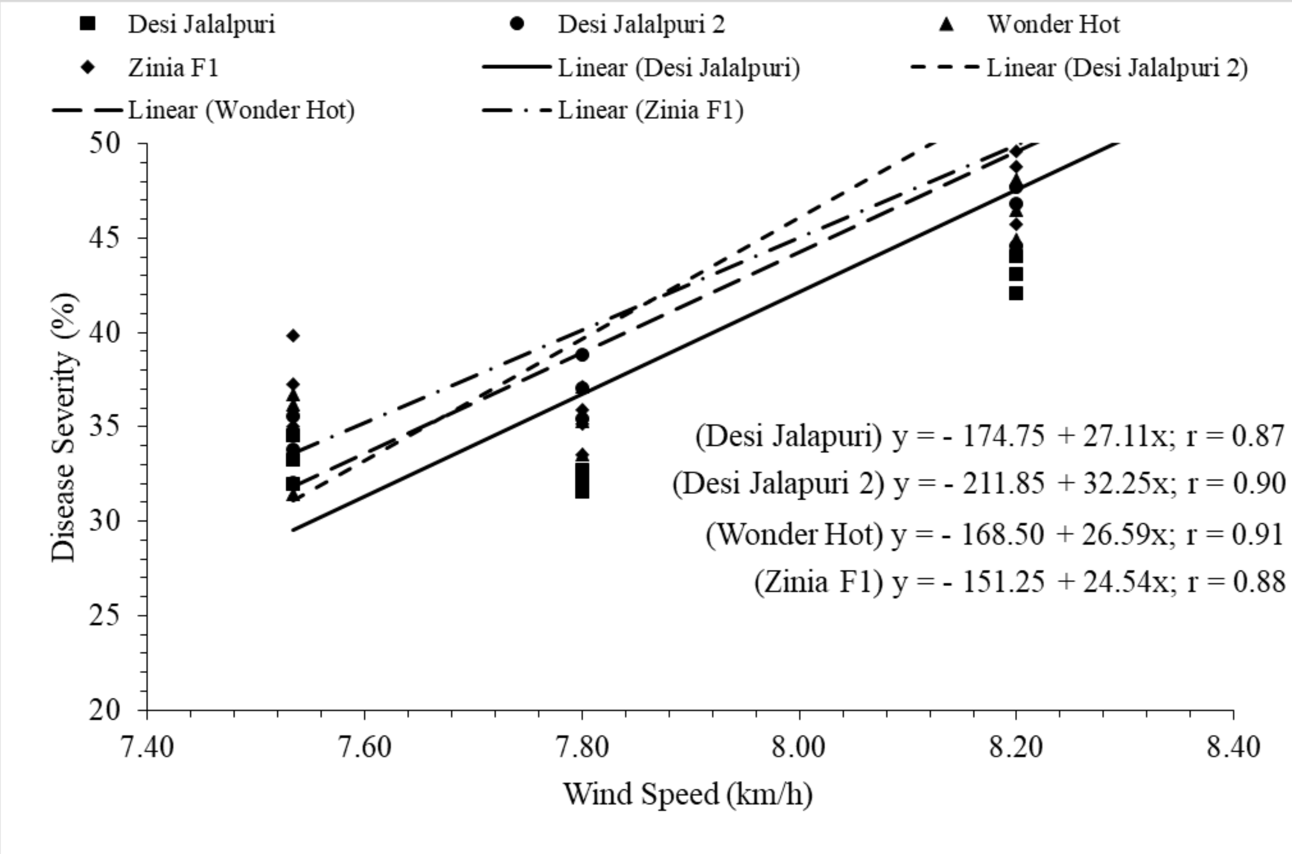

Fig. 7. Effect of wind speed on disease severity

Table 1. All-pairwise comparisons of disease severity from 12-12-2020 to 27-1-2021 for treatments x varieties

Disease Severity (%)						
Varieties	Intervals (15 Days)	Control	BA+CS	MnS+CS	ZS+CS	ZS+CS+MnS+BA
Desi (Jalalpuri)	I_1	33.29	29.48	33.45	30.04	30.78
	I_2	32.17	32.36	31.97	35.51	25.49
	I_3	43.05	25.26	24.77	28.21	17.89
	I_4	56.43	19.26	18.27	21.21	10.19
Desi (Jalalpuri 2)	I_1	33.78	37.30	31.62	32.42	31.34
	I_2	37.10	39.68	34.97	30.73	27.82
	I_3	46.38	32.58	27.77	23.43	20.22
	I_4	62.79	26.58	21.27	16.43	12.52
Wonder Hot	I_1	34.79	31.78	31.82	31.71	27.29
	I_2	35.32	30.23	26.06	24.64	22.69
	I_3	46.52	25.73	21.46	19.94	17.79
	I_4	56.84	19.73	14.96	12.94	9.99

Zinia F1	I_1	37.39	30.31	29.03	34.97	28.08
	I_2	34.87	31.68	29.23	27.20	27.83
	I_3	48.05	25.88	23.33	21.20	21.53
	I_4	56.48	19.88	16.83	14.20	13.83

*BA(Boric acid), CS(Copper sulphate), MnS(Manganese sulphate), ZS(Zink sulphate)

Table 2. All-pairwise comparisons of disease incidence from 12-12-2020 to 27-1-2021 for treatments x varieties

		Disease Incidence (%)				
Varieties	Intervals (15 Days)	Control	BA+CS	MnS+CS	ZS+CS	ZS+CS+MnS+BA
Desi (Jalalpuri)	I_1	33.34	29.63	33.34	18.52	20.38
	I_2	59.27	42.59	42.59	31.48	29.63
	I_3	77.78	55.56	51.86	38.89	33.33
	I_4	98.15	53.71	53.71	42.59	40.74
Desi (Jalalpuri 2)	I_1	20.37	18.52	29.63	20.37	12.96
	I_2	44.44	27.77	48.15	35.18	25.92
	I_3	74.07	44.44	53.70	37.04	28.22
	I_4	90.74	35.18	46.29	33.33	35.18
Wonder Hot	I_1	24.07	25.93	22.22	24.08	12.96
	I_2	50.00	40.74	37.04	30.55	22.22
	I_3	72.22	48.15	53.70	35.18	27.22
	I_4	90.74	38.89	53.71	35.18	31.48
Zinia F1	I_1	25.93	20.37	16.67	24.07	22.22
	I_2	51.85	35.18	33.33	25.91	29.63
	I_3	72.22	51.85	37.04	40.74	25.92
	I_4	90.74	46.29	35.18	51.85	35.18

Table 3. All-pairwise comparisons of shoot length for treatments x varieties

	Shoot length (cm)				
Varieties	BA+CS	MnS+CS	ZS+CS	ZS+CS+MnS+BA	Control
Desi Jalalpuri	10.08	11.70	13.05	30.78	10.89
Desi Jalalpuri 2	11.61	12.15	10.35	26.37	8.55
Wonder Hot	12.33	15.75	8.73	25.20	8.64
Zinia F1	15.75	18.18	14.13	25.65	8.28

Table 4. All-pairwise comparisons of root length for treatments x varieties

	Root length (cm)				
Varieties	BA+CS	MnS+CS	ZS+CS	ZS+CS+MnS+BA	Control
Desi Jalalpuri	8.77	5.63	6.3	12.833	4.90

Desi Jalalpuri 2	7.40	6.80	5.73	17.93	5.00
Wonder Hot	6.90	5.57	6.23	11.53	4.57
Zinia F1	9.23	6.93	6.17	14.23	5.10

Table 5. All-pairwise comparisons of fresh weight for treatments x varieties

	Fresh weight (g)				
Varieties	BA+CS	MnS+CS	ZS+CS	ZS+CS+MnS+BA	Control
Desi Jalalpuri	6.47	7.04	4.83	11.40	3.50
Desi Jalalpuri 2	7.00	7.19	3.83	9.77	2.75
Wonder Hot	7.25	8.43	3.23	9.33	2.78
Zinia F1	8.43	9.27	5.23	9.50	2.66

Table 6. All-pairwise comparisons of dry weight for treatments x varieties

	Dry weight (g)				
Varieties	BA+CS	MnS+CS	ZS+CS	ZS+CS+MnS+BA	Control
Desi Jalalpuri	1.47	1.39	1.03	3.27	0.55
Desi Jalalpuri 2	1.87	2.11	0.82	2.38	0.51
Wonder Hot	1.52	1.29	0.71	2.53	0.65
Zinia F1	2.98	1.96	1.44	2.99	0.82