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INTEGRATED MANAGEMENT OF VIRAL DISEASE COMPLEX INFECTING *BHUTJOLOKIA* (*CAPSICUM CHINENSE* JACQ.) IN ASSAM, INDIA

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ABSTRACT

BhutJolokia (*Capsicum chinense* Jacq.) also known as King chilli is one of the important spice crops mainly cultivated in the North Eastern Region of India. Owing to its traditional importance, pharmaceutical applications, and high commercial value, King chilli is gaining tremendous importance among the growers but the production of the crop has been hindering due the single and combine infection of a number of viruses specifically, Cucumber Mosaic Virus (CMV), Potato Virus Y (PVY) and Chilli Leaf Curl Virus (ChiLCV). A field experiment was conducted to evaluate the effect of integrated management practices in managing the viral disease complex infecting *Bhut jolokia*. The treatment combination comprising of net cover of seedlings + yellow Sticky trap + Benzothiadiazole @ 300 ppm at 2-3 leaf stage at 3 days interval for 3 times + Bio-Meta @ 5% at 60, 90 and 120 Days After Transplanting (DAT) + Foliar spray with neem oil @ 5ml/L at 10 days interval from 30 Days After Germination (DAG) for 5 times was found to be the most effective treatment resulted in delaying first appearance of disease symptom, lowest disease incidence, maximum yield, and highest benefit-cost ratio over control.

***Keywords*:** BhutJolokia, cucumber mosaic virus, potato virus Y, Chilli Leaf Curl Virus, disease, management.

Introduction

BhutJolokia (*Capsicum chinense* Jacq.), commonly known as '*Naga King Chilli*' is one of the important chilli landraces native to North Eastern Region of India. It is an inter specific hybrid between *Capsicum chinense* and *Capsicum frutescens* (Bosland and Baral, 2007). *Bhut Jolokia* is well known in the world as it was formerly recognized by the Guinness World Record as the world's hottest pepper in 2007 (Lopez, 2007) This chilli is widely cultivated in the North eastern states of India (Meetei *et al*., 2016), also in the Sylhet region of Bangladesh. Traditionally the fruits are used in various homeopathic preparations (Bhagowati and Changkija, 2009). Although there is vast scope for production of the crop, there still exists a few bottlenecks for its commercial cultivation. A number of viruses infecting the crop have been reported viz. *Chilli leaf Curl Virus* (ChiLCV) (Talukdar *et al*., 2015; Baruah *et al*., 2016), *Chilli Veinal Mottle Virus* (ChiVMV) (Routhu *et al*., 2022), *Cucumber Mosaic Virus* (CMV), *Potato Virus Y* (PVY) (Talukdar *et al*., 2015; Baruah *et al*., 2016), *Tomato Spotted Wilt Virus* (TSWV) (Kalita *et al*., 2014), *Groundnut Bud Necrosis Virus* (GBNV) (Baruah *et al*., 2016). At times, the crop has been reported to be infected by a single virus or collectively by more than one viruses and thereby forming a viral complex (Kalita *et al*., 2014) and causing severe reduction in the production and productivity of the crop. Realizing the importance and severity of the viral diseases, the present investigation was undertaken to evaluate the efficacy of combination of few management practices for Integrated management of viral disease complex in *bhutJolokia* in Assam.

Materials and Methods

Layout of the experiment

The experiment was conducted during 2020-21in *rabi* season in the Horticultural Experimental Farm, Assam Agricultural University, Jorhat, Assam, situated at 26° 47' N latitude, 94° 12' E longitude and 86.8 m above mean sea level. Healthy, uninfected seeds collected from fully ripe *bhutJolokia* fruits were used for raising seedling.

Treatment combinations used for disease management

The following four treatment combinations along with an untreated control were evaluated for the management of viral disease complex in *bhutJolokia*.

T1: Net cover of seedlings + Sticky trap + Benzothiadiazole @ 300 ppm at 2-3 leaf stage at 3 days interval for 3 times + Biopesticide (Bio-Meta) @ 5% at 60, 90 and 120 DAT + Foliar spray with neem oil @ 5ml/L at 10 days interval from 30 DAG for 5 times.

T2: Net cover of seedlings + Sticky trap + Salicylic acid @ 300 ppm at 2-3 leaf stage at 3 days interval for 3 times + Biopesticide (Bio-Meta) @ 5% at 60, 90 and 120 DAT + Foliar spray with neem oil @5ml/L at 10 days interval from 30 DAG for 5 times.

T3: Net cover of seedlings + Sticky trap + Biopesticide (Bio-Meta) @ 5% at 60, 90 and 120 DAT + Foliar spray with neem oil @5ml/L at 10 days interval from 30 DAG for 5 times.

T4: Net cover of seedlings + Sticky trap + Biopesticide (Bio-Meta) @ 5 at 60, 90 and 120 DAT + Nuvan 76% E.C. @1ml/L at 7 days interval from 30 DAT for 3 times.

T5: Control.

Application of treatments

Foliar application of BTH and SA @ 300 ppm was given at 3-4 leaf stage of the seedlings for 3 times at 3 days interval before transplanting. Yellow sticky trap of size 0.5 m x 0.5 m was installed at the center of each plot.

Recording of observations

Symptomatology and disease incidence

The *BhutJolokia* plants were observed regularly by inspecting each plant individually and appearance of symptom of the viral diseases were recorded. The viral infection was confirmed by performing PCR of the symptomatic plant samples. The Per cent disease incidence was calculated by using the following formula:

$$\text{Percent disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants examined}} \times 100$$

Detection of viruses in *bhutJolokia* plants

Detection of CMV, PVY and ChiLMV

BhutJolokia plant samples, both symptomatic and asymptomatic were collected from the experimental field for RT-PCR detection of CMV, PVY and ChiLMV using specific primers (Table1). Gel electrophoresis was performed for the amplified PCR product to examine the expected band size.

Table 1 : Specific primers for detection of CMV, PVY and ChiLCV

Specifications	Primer name	Sequence	Product size (bp)	GC content (%)	Temp. (°C)	Reference sequence ID
CMV- CP	IK-4F	AGTGCTGGTCGTAACCGTC	633	58	60	NC_001440
	IK-6R	GACTGGGAGCACTCCAGATG		60	60	
PVY-CP	IK-1F	TGGAACCTCGCCAAATGTCA	386	50	60	NC_001616
	IK-2R	TGGTGTGCCTCTCTGTGTTC		55	60	
	CPR	TTTTTTTTTTTTTTTAACGCCAACTATTG		-	59	
ChiLCV-CP	IK-3F	AACTTCGACAGCCCTTATGC	524	50	58	NC_004628
	IK-4R	TGTTCCTTCGAAGCGTACTG		50	58	

Agarose gel electrophoresis

The PCR products were resolved on 1.2% agarose gel in TBE containing 0.5 µg/ml of ethidium bromide. The gel was visualized on an UV- transilluminator Gel-doc to observe the amplified bands.

Effect of viral disease of *BhutJolokia* on plant growth and yield parameters

Assessment was done to study the effect of viral diseases in relation to different treatments on plant growth and yield parameters of *BhutJolokia* , viz., plant height, number of leaves/plant, number of fruits/plant, fruit weight, fruit length and fruit girth. Plant height were recorded at 120 DAT. Data on fruit weight, fruit length and fruit girth were recorded from 10 fruits per plant from both healthy and virus infected plants, and percent reduction over healthy was calculated by using the following formula.

$$\text{Per cent reduction in growth and yield parameters} = \frac{\text{Parameters of healthy plants} - \text{Parameters of diseased plants}}{\text{Parameters of healthy plants}} \times 100$$

Effect of viral diseases of *BhutJolokia* on yield

The fruits were harvested at ripe stage. For recording of yield of each plant in each treatment, fresh weight fruits of four pickings were taken. The total yield of ten randomly selected plants was recorded and average was calculated and finally converted to yield/hectare by using the formula given by Nchang *et al*., 2018.

Results and Discussion

Symptomatology

In the experimental field, different types of symptoms typical to infection by viruses like CMV, PVY and ChiLCV and also their mixed infection were observed on the *BhutJolokia* plants. It was observed that each virus produced distinct and multiple symptoms. Infection by CMV and PVY produced symptoms like, mosaic (Plate1 A,B), puckering of leaves (Plate1C,D); downward and upward curling of leaves for ChiLCV (Plate1E,F), stunted growth (Plate1G), yellowing and leaf size reduction (Plate1H), bushy appearance of the plants with small leaves (Plate1I), reduced plant growth (Plate1J,K) and deformed fruits (Plate1L). Infection at the earlier stages of plant growth, mild mosaic accompanied with reduced leaf size was observed in the infected plants. At later stage of growth, severe mosaic of the leaves was observed with highly reduced leaf size and leaf filiformity (Plate1M). Mixed infection by all the three viruses resulted in stunted growth of the plants (Plate1N), puckering and crinkling of leaves (Plate1O) with fewer flowers and fruits and deformed fruits. The symptoms caused by CMV + PVY recorded in the present study were similar to those reported by earlier workers in Bhut jolokia, commercial chilli and capsicum crops (Talukdar *et al*., 2015; Chanu *et al*., 2017; Borah *et al*., 2019). Symptoms observed in mixed infections were found to be more severe than those in single viral infections. Syller (2012) reported synergistic interaction between two different viruses in mixed infected chilli plants which resulted in the severity of the symptoms in case of mixed infection as compared to infection by a single virus.

Incidence of the Viral diseases on *bhutJolokia*

The recording of data for incidence and symptomatology of viral disease complex of *BhutJolokia* were monitored at 20 days interval starting from 10 days after transplanting and final disease incidence at 150DAT. The first disease incidence of CMV and PVY were observed at 31 Days after transplanting (DAT) of seedlings, while ChLCV was recorded at 29 DAT. The incidence of the diseases was presented in Table 2.

Incidence of CMV + PVY

From the data presented in table 2, it is evident that the disease incidence due to CMV + PVY infection varied from 22.88 to 31.23 per cent in the treated plots under the four different treatments. The lowest disease incidence (22.88%) was recorded in treatment T_1 which was followed by T_2 with 24.98 per cent incidence. The highest disease incidence was recorded in T_5 (control) with 33.33 per cent disease incidence. Various other workers also reported CMV and PVY infection in *BhutJolokia* with disease incidence of 75 per cent (CMV infection) and 87.50 per cent (PVY infection) from *BhutJolokia* growing areas of Assam (Talukdar *et al*., 2015), 37.97 per cent from king chilli growing areas of Manipur and its neighboring states (Chanu *et al*., 2017).

Incidence of ChiLCV

ChiLCV disease incidence ranged from 12.45 to 16.67 per cent in different treatments (Table2). Highest disease incidence of 19.44 per cent was observed in the control plots (T_5). Similar to the present observation, incidence of ChiLCV in *BhutJolokia* have been reported by other workers from various regions (Adluri *et al*., 2016). ChiLCV infection were also reported in chilli and pepper growing areas of India (Senanayake *et al*., 2007; Kumar *et al*., 2011).

Combined infection of CMV +PVY and ChiLCV

The result of the combined infection of CMV+PVY and ChLCV in the experimental plot is presented in Table2. From the table it is evident that the combined infection of the viruses was recorded highest in T_5 (47.23%) and lowest disease incidence was recorded in T_1 (Net cover of seedlings + Sticky trap + Benzothiadiazole + Bio-Meta + Neem oil) with 20.80 per cent incidence of the disease

RT-PCR detection of CMV, PVY and ChiLCV

The amplified product of *BhutJolokia* plant samples, both symptomatic and asymptomatic collected from the experimental plot for RT-PCR detection of CMV, PVY and ChiLCV using specific primers yielded band size of 633bp, 386bp and 524 bp respectively on gel electrophoresis.

Effect of CMV, PVY, ChiLCV and their mixed infection in Bhut jolokia on growth attributing and yield attributing characters

Effect on Growth attributing characters

The observations made on the effect of viral disease incidence on growth attributing characters of *bhutJolokia* are presented in Table 2. The maximum plant height (89.58 cm) was observed in T_1, followed by 89.03 cm inT_2 against the lowest (71.03 cm) in control (T_5) plants. The maximum number of leaves per plant was recorded in T_2 with an average of 207.3 leaves per plant. This was followed by T_1 with 205.6 leaves per plant. The lowest number of leaves per plant (181.62 leaves) was recorded in T5 (control).

Effect on yield attributing characters

The observations made on the effect of viral disease incidence on yield attributing characters were presented in Table2.

Number of fruits per plant

The highest number of fruits per plant (44.13 fruits) was recorded in T_1. This was followed by T_2 with 39.85 fruits and the lowest number of 8.35 fruits per plant was observed in T_5 (control). All the mean values on number of fruits per plant were found to be statistically significant among themselves.

Weight of fruit

Maximum fruit weight was observed in T_1 (6.09 g/fruit), followed by T_2 (5.93 g/fruit), T_4 (5.83 g/fruit) and T_3 (5.85 g/fruit). The weight of fruit in control plot was recorded to be 4.17g/ fruit. The per cent increase of fruit weight over control was found to be 31.53, 29.68, 28.47 and 28.72 per cent in treatments T_1, T_2, T_4 and T_3 respectively.

Length of fruit

The result of effect of viral disease incidence in fruit length of *BhutJolokia* was presented in Table2. The maximum fruit length (7.12 cm) was recorded in fruits produced in treatment T_1, followed by treatment T_2 (6.9 cm); treatment T_4 (6.85 cm) and treatment T_3 (6.81 cm). The minimum fruit length of 4.17 cm was observed in T_5 (control).

Fruit yield

The effect of different treatments on fruit yield of *BhutJolokia* was presented in Table2. The highest fruit yield was recorded in treatment $T_1$with 3.22 kg/ plot of 4.5m^2. This was followed by T_2 (2.69 kg), T_4 (2.39 kg) and T_3(2.36 kg) per plot. The lowest yield was recorded in the control plot (T_5) amounting 0.418 kg/ plot.

Economics of production

Among the treatments used to manage the viral diseases of *BhutJolokia*, the highest benefit cost ratio was calculated in T_1 (8.56:1). This was followed by T_4 (7.82:1), T_2 (7.65:1), T_3 (6.56:1) and lowest benefit cost ratio of 3.64:1 was recorded in T_5 (control).

The results elucidated that the disease incidence was lowest in treatment T_1 which may be due to the combined effect of BTH, Bio- meta and neem oil. Neem oil, extracted from neem tree, *Azadirachta indica* Juss., which is originated from the Indian Subcontinent, has been an important source of phytocheimcals used for pest control (Mordue *et al.*,2000). Use of sticky traps were also reported to be effective in controlling PVY in pepper (Budnik *et al.*, 1996). Bio-meta, the talc based biopesticide contains the entomopathogenic fungi, *Metarhizium anisopliae* ($1x10^9$cfu/ml) along with a standard osmoticant, adhesive and protein source is effective against insects. Dichlorvos is an organophosphate insecticide which acts both as a contact and ingested poison against insects.

Conclusion

In the present investigation, the ttreatment combination comprising of Net cover of seedlings + Sticky trap + Benzothiadiazole @ 300 ppm at 2-3 leaf stage at 3 days interval for 3 times + Biopesticide (Bio-Meta) @ 5 per cent at an interval of 60, 90 and 120 DAG + Foliar spray with neem oil @5ml/L at 10 days interval from 30 DAG for 5 times) which resulted in the least disease incidence and highest B:C ratio may be used for managing the viral disease complex of *BhutJolokia.*

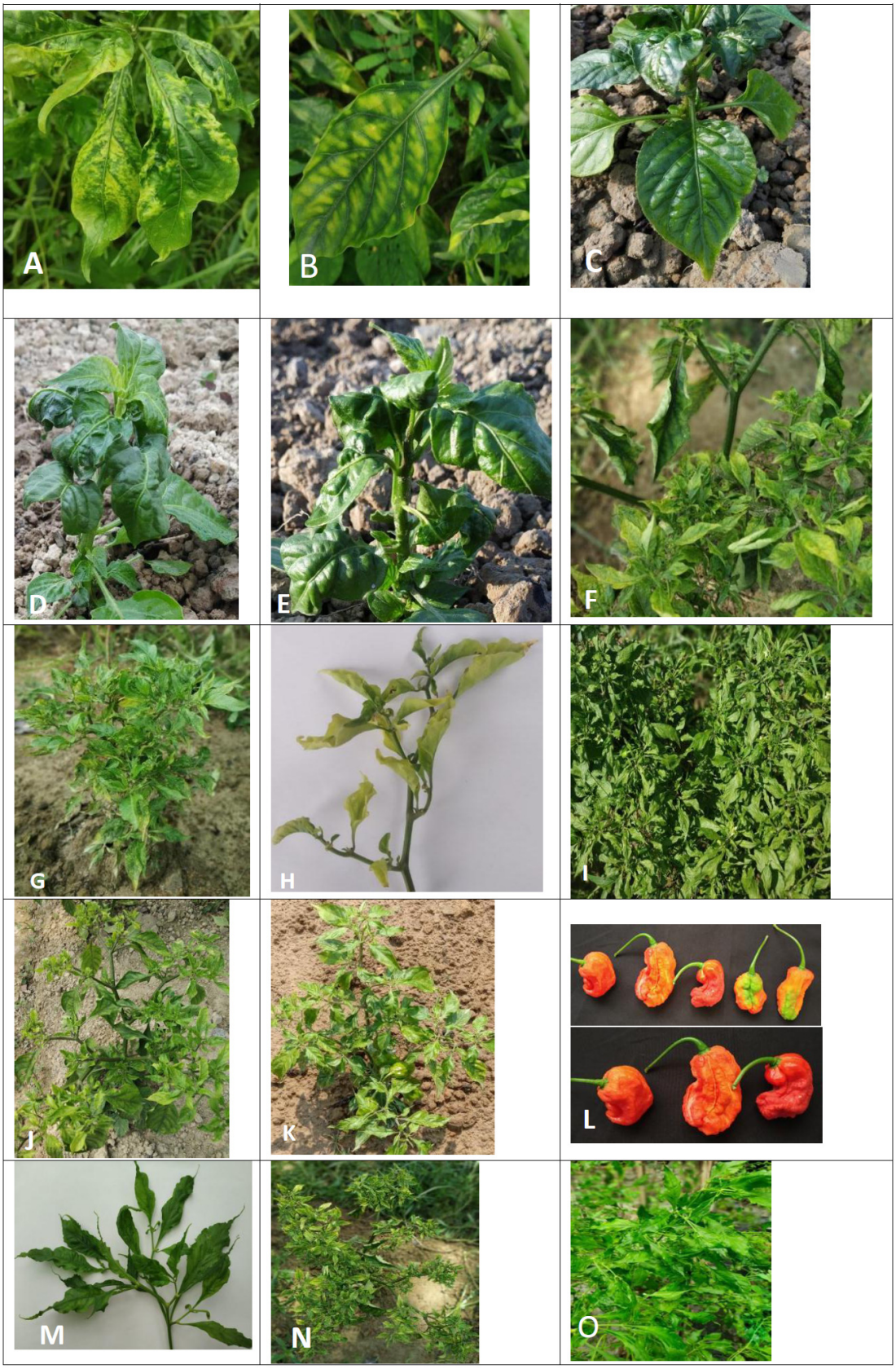

Plate 1: Symptoms of CMV, PVY, ChiLCV and their mixed infection in *BhutJolokia*

Table 2 : Effect of different management treatments on incidence of CMV+PVY, ChLCV and combine their infection in *BhutJolokia* during 2020-21 and effect on growth and yield.

Treatments	Disease Incidence			Growth and yield parameters						B:C ratio
	CMV + PVY	ChLCV	Combined infection of PVY, CMV and ChLCV	Plant height (cm)	No of leaves/ plant	No. of fruit/ plant	Fruit length (cm)	Fruit weight (g)	Fruit yield (kg/ 4.5 m^2)	
T1: Net cover + Sticky trap + Benzothiadiazole + Bio-Meta + Neem oil	22.88 (28.59)	12.45 (20.67)	20.80 (27.15)	89.58	205.6	44.13	7.12	6.09	3.22	8.56
T2: Net cover + Sticky trap + Salicylic acid + Bio-Meta + Neem oil	24.98 (30.00)	14.53 (22.42)	22.90 (28.60)	89.03	207.3	39.85	6.90	5.93	2.69	7.65
T3: Net cover + Sticky trap + Bio-Meta + Neem oil	31.23 (33.99)	16.67 (24.11)	31.23 (33.99)	80.28	186.97	33.64	6.81	5.85	2.36	6.56
T4: Net cover + Sticky trap + Bio-Meta + Nuvan	29.15 (32.69)	16.67 (24.11)	29.15 (32.69)	84.43	187.4	34.20	6.85	5.83	2.39	7.82
T5: Control	33.33 (35.28)	19.44 (26.17)	47.23 (43.43)	71.03	181.62	8.35	3.96	4.17	0.418	3.54
SEd	0.065	0.04	0.083	1.573	4.34	0.570	0.055	0.016	0.025	-
CD (P=0.05)	0.143	0.087	0.182	3.465	9.568	1.255	0.122	0.034	0.056	-

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