

Survey and detection of the diseases of Bhut Jolokia (*Capsicum chinense* Jacq.) in Assam

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Received:14-09-2014; Revised: 05-01-2015; Accepted:15-01-2015

ABSTRACT

Bhut Jolokia (Capsicum chinense Jacq.) a chilli pepper formerly recognized as the worlds's hottest chilli by Guinness book of World Records (Bosland and Baral, 2007). It is extensively grown in North Eastern region of India, predominantly in the states of Assam, Nagaland and Manipur. Field surveys were carried out in Bhut Jolokia growing district of Assam (Sivasagar, Jorhat, Golaghat). Among the diseases 10 per cent showed fungal infection, 3 per cent showed bacterial infection and 60 per cent showed viral infection. Five fungal diseases on Bhut Jolokia, Anthracnose / Fruit rot caused by Colletotrichum capsici, Die- back caused by Colletotrichum gloeosporoides, Stem rot and wilt caused by Sclerotinia sclerotiorum, Collar Rot caused by Rhizoctonia solani, leaf spot caused by Corynespora cassicola were identified. Bacterial wilt disease caused by R. solanacearum was observed on Bhut Jolokia at later stages of crop growth. The average virus disease incidence after 15-20 days after transplanting in the farmers field ranged from 8 to 78%. Four different viruses were identified based on symptomatology, transmission, host range and serological assays. These diseases were identified as Mosaic caused by Cucumber mosaic virus (CMV), Potato virus Y (PVY), Leaf and Stem necrosis disease caused by Tomato Spotted Wilt Virus (TSWV) and leaf curl disease caused by Chilli leaf curl virus (ChLCV). CMV, PVY and TSWV were easily transmitted by grafting and by sap inoculation to Bhut Jolokia. However, ChLCV is transmitted by grafting. Sap inoculation failed to produce any symptom. The presence of PVY, CMV and TSWV were confirmed using DAS-ELISA. The highest incidence of PVY (87.50 per cent) followed by CMV (75.00 per cent) and TSWV (62.50 per cent) were recorded in Golaghat district. Lowest infection was recorded at Sivasagar district for all the four viruses. However ChLCV incidence was low in the surveyed field.

Keywords: Bacterial wilt, bhut Jolokia, collar rot, DASELISA, die- back, pvy

Bhut Jolokia (*Capsicum chinense*) is a type of chilli found in all the North Eastern states of India with different local names. In Assam, it is called as Bhut Jolokia (Bhut means Ghost, Jolokia means chilli in Assamese which is probably due to its ghostly bite). It is recognized in the Guinness book of World Records as the world hottest chilli, having a rating of 1,001,304 Scoville heat units (SHU's) (Bosland and Baral, 2007). Bhut Jolokia is cash earning chilli variety and is gaining momentum in the international market for its high capsaicin content (3.0%), medicinal property (quick relief from heart pain, neuropathic pain and kills cancer cells) apart from its culinary uses and pickle preparations (Tewksbury and Nabhan, 2001). Bhut jolokia has disease genetic resources and wide variability exists in plant and fruit characters (Murmu *et al.* 2014). Assam is endowed with diverse genetic resources of this crop and wide variability exists in plant and fruit characters. However, the productivity of the crop is hindered due to attack of various diseases and pests. During the survey, several different type of virus disease symptom were observed in Bhut Jolokia fields.

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It was, therefore decided to identify these viruses causing different types of symptoms in Bhut Jolokia plants.

MATERIALS AND METHODS

During 2009-11, surveys were conducted for the presence of various diseases of 'Bhut Jolokia' growing areas of Golaghat, Sivasagar and Jorhat districts of Assam. The percentages of disease incidence with various symptoms were recorded. The different nature of symptoms on *Capsicum chinense* plant in the surveyed areas was grouped as isolates. Each isolates of different systemic symptoms of naturally infected Bhut Jolokia plants were collected separately for further confirmation through Kochs postulates, transmission and ELISA.

Fungal pathogens were isolated from the infected Bhut Jolokia fruits, stems and leaves on potato dextrose agar (PDA) medium as per standard procedures. Pure cultures of the pathogens were stored in refrigerator at 4C and periodic sub culturing of the isolates were done throughout the study. From the pure culture the morphological features *viz.*, colour, shape and size of

these isolates were confirmed by observing under light microscope. Observations were made in respect to hyphal and conidiophores characteristics. The fungal cultures were inoculated to healthy pot-grown Bhut Jolokia plants. Fifteen plants were maintained in each pot, 10 plants were inoculated by making slight injury with needle and small piece of inoculum was poured to the injured portion and with cotton and 5 plants were kept as control without inoculation. The inoculated plants were observed periodically for symptoms expression. Re-isolation of the fungus was made and it was examined in details to identify the causal organism by comparing with the available literature.

For pathogenicity test of bacterial isolates, thin sections of the diseased plant were made on a drop of sterile water, placed over a slide and examined under microscope for bacterial ooze. One cm of stem pieces showing bacterial ooze was cut and surface sterilized with 0.1% mercuric chloride solution. The treated pieces were subsequently, washed in sterile water with the help of a forceps and scalpel and were cut into small bits (about 0.25 cm long). Five such bits were then transferred to a test tube containing 10 ml of sterile distilled water and allowed the bacteria to diffuse. Three further dilutions were made from the stock suspension, each time transferring 1 ml to tubes containing 9 ml of sterile distilled water. A loopfull of the bacterial suspension from the final dilution was streaked with the help of sterile inoculating needle on Triphenyl Tetrazolium Chloride (TTC) medium and incubated at $28 \pm 1^\circ\text{C}$ for 24 h. The virulent colonies on TTC, which were fluidal, dull white in colour, irregularly round with light pink centre were again streaked on TTC medium to get pure culture of the bacterium. Seedling of 'Bhut Jolokia' were raised in earthen pot (25cm) diam. 30 days old seedlings were transplanted in earthen pots and in each pot two seedlings were kept. Total 10 plants were inoculated with 10 ml suspension of *Ralstonia solanacearum* @ 1×10^8 cfu ml⁻¹ at the collar region of the plant (Kelman 1954). For sap inoculation of different virus isolates the infected plant specimens collected from the different localities were ground in a sterilized mortar and pestle with 0.1 M phosphate buffer, pH 7.0 at 1:1 ratio. A small amount of celite (540 mesh) were used as an abrasive during inoculation on hosts viz, *Capsicum chinense*, *Nicotiana tabacum*, *Lycopersicon esculentum*, *Datura stramonium* and *Chinopodium amaranticolor*. These hosts served as a differential hosts for suspected viruses (Tobias et al., 1982).

Two species of aphids (*Aphis gossypii* and *Myzus persicae*), thrips (*Scirtothrips dorsalis*) and whitefly (*Bemisia tabaci*) were used as insect vector for

transmission of different virus isolates. The standard procedure were followed on these insect transmission. After inoculation plants were sprayed with Dimethoate (0.1 per cent) to kill the viruliferous vector. The inoculated plants were kept in the net house for symptoms expression (Jeyaranjan and Ramakrishnan, 1969). The virus isolates of different typical symptoms were also inoculated by wedge grafting on (45 days old) healthy Bhut Jolokia plants for confirmation of virus etiology.

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) of virus infected samples were performed following methods as described by (Hobbs et al., 1987). The antibodies were obtained from Bioreba AG, CH4153, Reinach BL1, Switzerland. Optimum concentration of antigen (dilution 1:20), conjugate (anti-rabbit IgG labeled with alkaline phosphatase, Sigma) (dilution 1:10000), antisera (PVY, CMV TSWV and LCV) (dilution 1:1000) and substrate (PNPP, Sigma) (concentration 0.6 mg/ml) were used. The colour reaction was measured by the ELISA plate reader (Bio Rad) using 405 nm wavelength. Plants were considered infected if the ELISA reading was four times higher the average reading of the healthy samples (usually $e^{0.1}$) (Azzam et al. 2001).

RESULTS AND DISCUSSION

During surveys the disease incidence and variable nature of symptoms on Bhut Jolokia plants were recorded (Table 1; Fig. 1,2). The farm survey results indicated that the prevalence of viral diseases (60%) were more than the fungal (10%) and bacterial diseases (3%) in the farmers' fields. During on farm surveys, five fungal diseases viz., anthracnose and fruit rot caused by *Colletotrichum capsici*, die-back caused by *Colletotrichum gloeosporioides*, stem rot and wilt caused by *Sclerotinia sclerotiorum*, collar rot caused by *Rhizoctonia solani* and leaf spot caused by *Corynespora cassicola* were detected in Bhut Jolokia fields (Fig. 1 a - m). However, only one bacterial disease i.e. bacterial wilt, caused by *Ralstonia solanacearum* was identified. Symptoms of leaf spot disease are quite common as compared to other diseases of Bhut Jolokia. The disease appears as small brown circular spots with light grey to white grey centers bearing dark brown halos. Sometimes with round necrotic lesion becomes dark or often with wavy border. Later on, the spot coalesce become chlorotic, blightend and eventually dried. Defoliation starts at the later part of the symptom affecting flowers and fruits. Severely infected plants were wilted under humid condition. Similar disease symptoms on chilli were reported by Jin-Hyeuk et al. (2006) Based on the morphological character of the fungus was identified as *Corynespora cassicola*. (Fig. 1. g & h)

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In Anthracnose and fruit rot diseases the initial symptoms developed as small grey spots on leaves. The spots increased in size and gradually developed dark brown colour at the centre. Later it causes necrosis of twigs; black dots (acervuli) were formed all over the necrotic surface of the affected twig. Both green and ripe fruits were also affected. The tissues shrink irregularly at different portion of the fruits which become rough and straw coloured whereas on green fruits, they become

pale and dirty green. Acervuli appear as minute grey to black dots on diseased tissues. The fruits get detached from the petiole and fall on the ground. These symptoms were similar to anthracnose disease of Chilli reported by Manandhar *et al.* (1995); Kim and Hong (2008). Based on the morphological character of the fungus, the causal organism identified as *Colletotrichum capsici* (Fig. 1. b, c & d).

Table 1: Percent disease incidence, symptoms and ELISA reaction of the leaf samples collected from Bhut Jolokia field during 2009-11 in three district of Assam

Location	Disease incidence (%)			Symptoms			ELISA reaction with antisera at OD 405nm			
	Fungal	Bacterial	Viral	Fungal	Bacterial	Viral	PVY	CMV	TSWV	ChLCV ***
Sivasagar	5.5-6.2	1.0	12-48	LS	Wilting	MM,SV,CR,RC, VC,VB,BA	0.63 0.78	-	-	-
	2.5-5.0	0.0	14-32	LS, FR, SR	-	VC,MM, Mo,ST,St	-	0.53 0.62	-	-
	0.0	1.0-2.5	8-15	-	Wilting	VC,Mo, NS,D,DB	-	-	0.38 0.56	-
	8.0-10	0.0	8-10	LS, CR, SR	-	UC,LR,BA, St,Mo	-	-	-	-
Golaghat	4.0-5.6	0.5-1.8	23-72	LS, SR, LS	Wilting	MM,SV,CR,RC, VC,VB,BA	0.57 0.91	-	-	-
	0.0	0.2-3.0	13-62	CR, LS	Wilting	VC,MM, Mo,ST,St	-	-	0.47 0.81	-
	0.0	1.0-2.1	26-48	LS	Wilting	VC,Mo, NS,D,DB	-	-	0.54 0.69	-
	0.0	0.0	10-12	-	-	UC,LR, BA,St,Mo	-	-	-	-
Jorhat	0.0	0.0	14-53	LS, SR, LS	-	MM,SV,CR,RC, VC,VB,BA	0.62 0.88	-	-	-
	0.0	1.5-2.6	12-48	CR, LS	-	VC,MM, Mo,ST,St	-	0.42 0.69	-	-
	8.0-9.2	0.0	10-24	LS	-	VC,Mo, NS,D,DB	-	-	0.32 0.57	-
	2.0-5.9	0.0	8-12	-	-	UC,LR, BA,St,Mo	-	-	-	-

Note: *** = Not performed; LS = leaf spot, FR = fruit rot, SR = stem rot, CR=collar, SM = Severe Mosaic, MM =Mild Mosaic, NS = Necrotic streak on petiole, BA= Bushy appearance, LR=Leafroll, UC= Upward curling, ST= Shoe string, VB= Vein banding, RL= Reduce leaf size, St = Stunting, VC =Vein Clearing, Mo = Mottling, DB=Die-back, CR=Crinkling, D=Defoliation

Table 2: Per cent diseases incidence of CMV, PVY and TSWV in Bhut Jolokia in three districts of Assam using DAS-ELISA

Districts	Disease incidence (%)			Average incidence (%)
	PVY	CMV	TSWV	
Sivasagar	33.33	33.33	31.25	30.36
Jorhat	37.50	50.00	37.50	41.67
Golaghat	87.50	75.00	62.50	75.00
Total	52.00	52.0	42.00	48.67

In die-back disease, the symptoms developed as necrosis of tender twigs from the tip to downwards. The entire plant or branch may wither away. Black dots (acervuli) were formed all over the necrotic surface of the affected twig. The twigs become straw coloured in advanced stage of the disease. Large numbers of black dots were seen on the necrotic parts of the plants. The fungus also attacked the stem. The disease was caused by *Colletotrichum gloeosporioides* (Fig. 1. k, l & m)

In stem rot and wilt disease of Bhut Jolokia, light brown water soaked lesions develop in patches in any

portion of the stem and branches which decay in wet weather. The plants wilt at decaying stage and ultimately dies, White cottony growth of mycelium develops on the affected portions on which black, sclerotia of variable sizes up to 4 mm diameter develop. When these mycelia come in contact with the green parts of the plants that also gets infected and rots. These symptoms are similar to earlier reported by Roy (1973); Saikia (1986) Based on symptom, morphological character of the fungus and pathogenicity test the causal organism was identified as *Sclerotinia sclerotiorum*.



Fig. 1: Different fungal and bacterial diseases of Bhut Jolokia, a: Bhut Jolokia plants, b: fruit rot, c & d: acervuli and conidia of *Colletotrichum capsici*, e: collar rot, f: mycelium of *Rhizoctonia solani*, g: leaf spot, h: Conidia of *Corynespora cassicola*, i: stem rot and wilt, j: Sclerotia of *Sclerotinia sclerotiorum*, k: Die-back, l & m: acervuli and conidia of *Colletotrichum gloeosporioides*, n & o : bacterial wilt caused by *Ralstonia solanacearum*



Fig. 2: Symptoms of CMV, PVY, TSWV and LCV on Bhut Jolokia plants along with healthy plant

In Collar rot disease of Bhut Jolokia, the stem of the infected plant exhibits brownish black discolouration at collar region, shriveled near the soil and the affected plant gets wilted and later dried up completely. Similar,

symptom on chilli was earlier reported by Singh (1987); Chupp and Sherf (1960). Based on symptom, morphological character of the fungus and pathogenicity test the causal organism of collar rot

disease identified as *Rhizoctonia solani* Kuhn In bacterial wilt disease, the initial symptom includes sudden wilting of foliage on the youngest leaves, which may recover during night. With the progress of the disease, the initial wilt is proceed to a sudden and permanent wilt of entire plants with slight or no leaf yellowing. Young infected Bhut Jolokia plants die immediately while older plants first show leaf drooping and discolouration on one side or part of the plant. Finally the entire plant wilts and dies. The vascular tissues are discoloured pale yellow to brown in colour. On the basis of symptoms and pathogenicity studies the bacterial isolate was identified as *Ralstonia solanacearum* which cause bacterial wilt. Similar Bacterial wilt disease on chilli and bell pepper were reported by Biswas and Singh (2007), Sharma and Kumar (2009). Bacterial wilt disease caused by *R. solanacearum* was observed on ‘Bhut Jolokia’ at later

stages of crop growth. The initial symptom of the viral infected Bhut Jolokia plants were clearing of veins of the apical leaves, followed by dark green to light green mottling ,mild to severe mosaic, necrotic streaks on the veins and petioles. In severe infection plants were stunted and bushy appearance with smaller deformed mottled leaves. The infected plants bears few flowers and fruits.

The percent disease incidence based on the number of plants found positive in DAS ELISA in different fields were shown in table- 2. The highest incidence of PVY infection (87.50 per cent) followed by CMV infection (75.00 per cent) TSWV (62.50 per cent) infection were recorded at Golaghat district. Based on symptomatology (Table1), transmission and host range studies (Table 3) and ELISA test (Table 2) the virus isolates were identified. The plants of isolate-1 showed vein clearing followed by mild mosaic. Later leaves

Table 3: Various transmission assays of different virus isolates of Bhut Jolokia

Virus Isolates	Type of inoculation	Host plants	% infection	Symptom produced	Virus
Isolate 1	Graft	<i>Capsicum chinense</i>	100.00	Mosaic	CMV
		<i>Datura stramonium</i>	80.00	Local lesion	
	Mechanical	<i>Chenopodium amaranticolor</i>	60.00	Local lesion	
		<i>Nicotiana tabacum</i>	60.00	Local lesion	
	<i>Aphis gossypii</i>	<i>Lycopersicon esculentum</i>	0.00	No symptom	
		<i>Capsicum chinense</i>	60.00	Mosaic	
		<i>Capsicum chinense</i>	100.00	Mosaic	
		<i>Myzus persicae</i>	100.00	Mosaic	
		<i>Capsicum chinense</i>	100.00	Mosaic	
		<i>Capsicum chinense</i>	100.00	Mosaic	
Isolate 2	Graft	<i>Capsicum chinense</i>	100.00	Chlorotic	PVY
		<i>Datura stramonium</i>	0.00	No symptom	
	Mechanical	<i>Chenopodium amaranticolor</i>	60.00	Local lesion	
		<i>Nicotiana tabacum</i>	0.00	No symptom	
	<i>Aphis gossypii</i>	<i>Lycopersicon esculentum</i>	0.00	No symptom	
		<i>Capsicum chinense</i>	0.00	No symptom	
		<i>Capsicum chinense</i>	100.00	Chlorotic	
		<i>Myzus persicae</i>	100.00	Chlorotic	
		<i>Capsicum chinense</i>	100.00	Chlorotic	
		<i>Capsicum chinense</i>	100.00	Chlorotic	
Isolate 3	Graft	<i>Capsicum chinense</i>	100.00	Necrosis	TSWV
		<i>Datura stramonium</i>	40.00	Chlorotic lesion	
	Mechanical	<i>Chenopodium amaranticolor</i>	30.00	Necrotic spot	
		<i>Nicotiana tabacum</i>	0.00	No symptom	
	<i>Scirtothrips dorsalis</i>	<i>Lycopersicon esculentum</i>	0.00	No symptom	
		<i>Capsicum chinense</i>	40.00	Necrotic spot	
		<i>Capsicum chinense</i>	30.00	Necrosis	
		<i>Capsicum chinense</i>	100.00	Leaf curl	
		<i>Datura stramonium</i>	0.00	No symptom	
		<i>Chenopodium amaranticolor</i>	0.00	No symptom	
Isolate 4	Graft	<i>Nicotiana tabacum</i>	0.00	No symptom	ChLCV
		<i>Lycopersicon esculentum</i>	0.00	No symptom	
	Mechanical	<i>Capsicum chinense</i>	0.00	No symptom	
		<i>Capsicum chinense</i>	0.00	No symptom	
	<i>Bemisia tabaci</i>	<i>Capsicum chinense</i>	0.00	No symptom	
		<i>Capsicum annuum</i>	100.00	Leaf curl	
		<i>Myzus persicae</i>	20.00	Leaf curl	
		<i>Aphis gossypii</i>	40.00	Leaf curl	
		<i>Scirtothrips dorsalis</i>	10.00	Leaf curl	
		<i>Nicotiana tabacum</i>	10.00	Leaf curl	

became filiformed with inward rolling and elongated tips (Fig.1). The virus was transmitted by *M. persicae* and *A. Gossypii* and easily by graft transmission to healthy Bhut Jolokia. In sap inoculation of the isolate 1 produced necrotic local lesion on *D. stramonium*, *C. amaranticolor* and *Nicotiana tabacum* whereas on *Capsicum chinense* produced typical mosaic symptom. The virus isolate-1 was identified as Cucumber mosaic virus (CMV). The infection of CMV on chilli were reported by Dossa and Mungur, (1982); Conti and Masenga (1977).

In isolate 2, the initial symptoms were in form of crinkling of young leaves, the midrib became wavy resulting in upward curling, crinkling and the leaf area is reduced considerably. Later on entire leaf developed a dark, green mottling with wavy margin followed by vein clearing mosaic mottling, vein banding with or without deformation of leaves (Fig.1). The virus isolate was transmitted by *M. persicae*, *A. Gossypii* and easily graft transmitted to healthy Bhut Jolokia. The sap of this virus isolate produced necrotic local lesion. Based on these results the isolate-2 was identified as Potato virus Y (PVY). The ELISA results show the mixed infection of CMV and PVY in 10 disease isolates. Nagaraju and Reddy (1981) reported the infection PVY on chilli. The mixed infection of CMV and PVY showed dark green vein banding and malformation of leaves with stunted growth of the chilli plants were reported by (Dossa and Mungur, 1982).

The diseased Bhut Jolokia plants of isolate 3 produced leaf and stem necrotic lesion at early stage of infection. Apical portion of twigs became naked due to dropping of leaves (Fig. 1). The symptom resembled the die back phase of anthracnose disease caused by *C. gleosporides*, but there were no acervuli of the fungus on the necrotic portion of the plants. In sap inoculation of the isolate 3 produced chlorotic lesion on *Datura stramonium* and necrotic spot on *Chenopodium amaranticolor* and *Capsicum chinense* (Table 3). In vector transmission, both Aphid species and *Bemisia tabacci* failed to transmit the virus. The isolate 3 was easily transmitted by thrips (*Scirtothrips dorsalis*) up to the extent of 80 percent in Bhut Jolokia plants (Table 2). The natural infection of TSWV on chilli crop were reported by Goldbach and Peter, 1996; Prins and Goldbach, 1998.

The symptom shown by the isolate 4 on Bhut Jolokia was characterized by upward curling and rolling of leaves, followed by crinkling, thickening of veins and bushy appearance due to presence of short side branches (Fig. 1). The virus isolate was easily transmitted by

grafting and vector white fly (*Bemisia tabacci*). The isolates also produced typical leaf curl symptom on chilli, tomato and tobacco seedlings when inoculated by grafting and *Bemisia tabacci*. The isolate was identified as Leaf curl virus (ChLCV), Leaf curl disease on chilli was reported by Ray et al. (2009).

On the basis of above results the viruses infecting Bhut Jolokia has been identified. Out of 150 samples studied, 67 were infected with PVY, 35 with CMV, 27 with TSWV and 15 with ChLCV. Six disease samples could not be identified as not transmitted by various means. Thus, the findings would be helpful in initiating research on disease management of Bhut Jolokia in Assam and elsewhere.

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