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MOLECULAR CHARACTERIZATION OF WHITEFLY *BEMESIA TABACI* (GENN.) AND DEVELOPMENT OF IPM MODULE AGAINST CHILI LEAF CURL COMPLEX

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ABSTRACT

Molecular identification/ characterization of *Bemisia tabaci* (Genn.) collected across five agroecological zones of West Bengal, India revealed that it resembles genetic group Q. Seed treatment with thiamethoxam 70WS 3 g/ kg seed incorporated with seedling treatment with acetamiprid 20SP @ 1g/ l, seedling rising under insect proof net and border netting with insect proof net showed efficacy with reducing the occurrence and dispersal of thrips *Scirtothrips dorsalis* Hood, mite *Polyphagotersonemus latus* and whiteflies *B. tabaci;* while their least incidence was observed with IPM module (integration of seed treatment, seedling treatment, seedling raising under insect proof net, border netting technology, installation of yellow sticky trap and need based spot application of spiromesifen and diafenthiuron), with 92.97, 82.68 and 72.97% reduction, respectively; and 98.56% reduction of chilli leaf curl virus (CLCV) incidence could be obtained through IPM with maximum yield of green chili (1.66 t/ ha). Panchagavya, dasaparni and bhramvastra appeared as potent biopesticides in reducing the CLCV causative agents.

Key words: Chilli, CLCV, IPM, *Scirtothrips dorsalis*, *Polyphagotersonemus latus*, *Bemisia tabaci* Q genetic group, seed/ seedling treatment, insect proof net, norder netting, yellow sticky trap, spiromesifen, diafenthiuron, yield

Among the five domesticated cultivars of chili peppers, *Capsicum annum* is the most popular vegetable or spice native of Peru and Mexico. Chili crop is affected by biotic and abiotic factors of which losses due to insect pests and diseases are serious. It is often infested by a group of sucking and chewing insect pests of which thrips, yellow mites, whiteflies and borers are predominant (Hosmani, 1993). Many viral diseases also infect this crop and induce mild to severe mosaic, yellow mosaic, mosaic mottle, leaf curl, leaf roll, and bushy stunt and necrosis symptoms. Out of which chili leaf curl virus is considered as severe one. The yield losses range from 50-90% due to insect pests of chili (Kumar, 1995). Kandaswamy et al. (1990) estimated 50% yield losses solely due to thrips *Scirtothrips dorsalis* Hood. For the last decade wide spread of whitefly, *Bemisia tabaci* (Genn.), development of its different genetic groups with a high potency of virus transmission (Gemini/ begomovirus) are posing serious threat. In India, Senanayake et al. (2007) reported first time chili leaf curl virus on chili. Recently the crop has been suffering from heavy infestation of leaf curl virus vectored by *B. tabaci*; consequently, CLCV is becoming the major constraint (Senanayake et al., 2007). The genus *Bemisia* contains 37 species and is thought to have originated from Asia (Mound and Halsey 1978).

Bemisia tabaci, being possibly of Indian origin (Fishpool and Burban, 1994), was described under numerous names before its morphological variability was recognized. Originally, three distinct groups of *B. tabaci* were identified by comparing their mitochondrial 16S ribosomal subunits: New World; India/ Sudan; and remaining Old World (Frohlich and Brown, 1994). It has been accepted through mitochondrial cytochrome oxidase 1 (mtCO1) gene comparison that the *B. tabaci* is divided into 11 genetic groups instead of considering as one complex species and the genus *Bemisia* is divided into 34 morphologically indistinguishable species. (Dinsdale et al., 2010; Boykin and De Barro, 2014). The first reported genetic group B known as Middle East-Asia Minor 1 species (MEAM1) evolved in 1980s (Brown et al., 1995b), whereas several other 'genetic groups' (up to S) have now been described (Brown et al., 1995b; Boykin and De Barro, 2014). A distinctive, non-specific esterase banding pattern is also helpful in identification (Brown et al., 1995a) but is not foolproof still can be utilized as basic screening of the genetic

groups (Byrne et al., 1995). It has been found that the Mediterranean species (Q genetic group) coexisted with the MEAM1 and over recent years, exposure to extensive insecticide applications and within areas of intensive agriculture exhibits a high level of resistance (Dennehy et al., 2010). Tejaswini, Bullet and locally selected high yielding varieties like Iret and Sonirag are the main cultivars widely grown in the South 24 parganas district of West Bengal, showing moderate to highly susceptible to CLCV. To mitigate the problem faced by the farmers, who are accustomed to spray the crop on daily basis aggravating the problem day by day, development of an ecology based IPM packages is necessary and hence this study.

MATERIALS AND METHODS

Field experiments were conducted in the Instructional Farm of Sasya Shaymala Krishi Vigyan Kendra situated at Arapanch, Sonarpur, West Bengal, India (22º4 N, 88.2ºE). The experiments were laid in randomized block design (RBD) during pre-kharif of 2017 and 2018 with four treatment modules. These comprises- T_1 (IPM): Seed treatment with thiamethoxam + seedling raised under insect proof net + seedling dipped in acetamiprid + border cover with insect proof net + installation of yellow sticky trap @18/ ha + need based application of spiromesifen at 30 DAT @ 1.25ml/ l and diafenthiuron @ 1.5gm/ l after seven days; T_2 (organic practices): seed bed treatment with *Trichoderma* enriched cow dung+ application of panchagavya (cow dung: cow urine: milk: curd: ghee =5:3:2:2:1, ripe banana and coconut water was mixed to enrich the culture) at 30 DAT @ 5% and seven days after dasaparni spray (fermented product of *Azadirachta indica, Carica papaya, Ficus hispida, Annona reticulata, Psidium guajava, Datura* sp*., Calotropis* sp. and *Clerodendrum viscosum* leaves each 1 kg, mixed with 2 kg cow dung and cow urine; incubated for 30 days) @ 100ml/ l; T_3 (chemical management): rotational spray of flonicamid at 30 DAT @ 0.4g/ l and spiromesifen @1.25ml/ l after seven days; T_4 (integration of inorganic and organic amendments): seedling treatment with thiamethoxam+ application of bhramvastra (paste of one kg each leaves of *A. indica, Datura* sp*., Calotropis* sp*., A. reticulata, P. guajava*: cow urine: cow dung: chili paste: allium paste: 10:5:0.25:0.25) at 30 DAT @ 25ml/ l and need based spot application of diafenthiuron after seven days @1.5g/ l along with untreated check. Each treatment was replicated four times and randomized, with crop raised with recommended package of practices in 3x 3 m^2 plots at a spacing of 50x 50 cm.

Counts of *S. dorsalis*, *P. latus, B. tabaci* were done from three randomly selected leaves (upper, middle and lower)/ plant from five randomly selected plants/ plot before and after spray (very next day, third day and seventh day after spray). Observation was taken during early morning hours. Thrips incidence was counted using hand lens (10x); whitefly by eye observation whereas, mite was enumerated with a microscope (Magnus stereozoom); and % disease incidence of chili leaf curl virus was enumerated 3 and 7 days after spraying. The collected data on incidence were subjected to ANOVA after square root transformation, whereas in case of CLCV, % incidence was subjected to angular transformation. The treatment means were compared following the design of RBD (p=0.05) (Gomez and Gomez, 1984). Corrected efficacy % was calculated using Abbott's formula as on before spraying data (Abbott, 1925) and the post treatment data was corrected using Henderson-Tilton's formula (Henderson and Tilton, 1955). The data were subjected to analysis using IBM SPSS statistics 21.

Adult *B. tabaci* samples were collected from five agroclimatic zones of West Bengal (Kalimpong, Nadia, Guskara, Danga, Narendrapur, Sonarpur, Baruipur, Diamond Harbour, Kakdwip, Namkhana, Patharpratima) in 70% ethanol and carried in ice bucket. The locations selected covered costal saline ecosystem. The samples after morphological analysis were stored at -20ºC (Blue Star). Screening of the collected sample was done using microsatellite site "Bem 23 analysis: "Bem-23 F" (5'CGGAGC TTGCGCC TTA GTC) and "Bem-23-R" (5'CGGCT TTATCA TAGCTCT CGT) illustrated by Bel-Kadhi et al. (2008); 5g of chilli sample was taken in a 50 ml centrifuge tube and 10 ml (ethyl acetate: cyclohexane) mixture was added and subjected to vortex for 2 min. After that adding 5 gm of activated Na_2SO_4, the sample was again vortexed for 3 min. Then the sample was centrifuged for 15 min at 10,000 rpm and then 5 ml supernatant liquid was taken in 10 ml centrifuge tube. Afterwards 25 mg each of florisil and PSA was added to it and vortexed for 2 min and the sample was again centrifuged for 10 min at 5000 rpm. Then 3 ml supernatant liquid was collected from it and evaporated to dryness in N_2 evaporator at 25°C. The residue was then reconstituted in 3 ml of ethyl acetate. The sample was then filtered through 0.2µ membrane filter and taken for final analysis in GC/MS [Varian (Walnut Creek, CA) Saturn 2200 mass spectrometer coupled to a Model 3800 gas chromatograph. The mass spectrometer was used single ion scan (SIS) mode with electron impact (EI) ionization].

Table 1. Efficacy of IPM modules *S. dorsalis*

Treatments	Mean incidence/ 3 leaves							Mean	% reduction over control	Mean reduction (%) after spraying							Mean
	Before spray	After first spray			After second spray					Before spray	After I spray			After II spray			
		1 Day	3 Days	7 Days	1 Day	3 Days	7 Days				1 Day	3 Days	7 Days	1 Day	3 Days	7 Days	
T_1	5.33	2.75	2.33	1.33	0.67	0.33	0.67	1.92	92.97	76.57	48.68	57.59	79.23	49.25	78.59	61.4	64.47
	(2.31)	(1.66)	(1.53)	(1.15)	(0.82)	(0.57)	(0.82)										
T_2	19.50	12.67	9.05	11.67	9.87	7.67	6.67	11.01	59.61	14.29	35.37	54.98	50.18	14.8	43.28	56.21	38.44
	(4.42)	(3.56)	(3.01)	(3.42)	(3.14)	(2.77)	(2.58)										
T_3	21.75	8.33	4.67	5.05	2.33	0.67	0.33	6.16	77.40	4.4	61.9	79.17	80.67	53.52	88.55	94.99	66.17
	(4.66)	(2.89)	(2.16)	(2.25)	(1.53)	(0.82)	(0.57)										
T_4	8.25	7.66	3.09	6.98	4.67	5.25	6.67	6.08	77.70	63.74	7.64	63.66	29.57	32.6	35.09	26.78	37.01
	(2.87)	(2.77)	(1.76)	(2.64)	(2.16)	(2.29)	(2.58)										
T_5	22.75	22.87	23.45	27.33	27.13	31.67	35.67	27.27									
	(4.77)	(4.78)	(4.84)	(5.23)	(5.21)	(5.63)	(5.97)		-								
SEm (±)	0.51	0.48	0.29	0.34	0.27	0.19	0.33	-	-								
CD (0.5)	1.62	1.46	0.89	1.12	0.81	0.62	1.07	-	-								

*Figure in parentheses square root transformed value

Table 2. Efficacy of IPM modules on *B. tabaci*

Treatments	Mean incidence/ 3 leaves							Mean	% reduction over control	Mean reduction (%) after spray							Mean
	Before spray	After first spray			After second spray					Before spray	After I spray			After II spray			
		1 Day	3 Days	7 Days	1 Day	3 Days	7 Days				1 Day	3 Days	7 Days	1 Day	3 Days	7 Days	
T_1	2.33	1.33	1.67	1.33	0.33	0.67	0.33	1.14	92.82	78.76	41.31	36.85	60.04	79.78	60.27	82.68	62.81
	(1.53)	(1.15)	(1.29)	(1.15)	(0.57)	(0.82)	(0.57)										
T_2	12.67	9.33	6.87	7.33	3.67	3.87	4.33	6.87	56.81	+15.50	24.29	52.22	59.50	59.20	58.36	58.77	42.41
	(3.56)	(3.05)	(2.62)	(2.71)	(1.92)	(1.97)	(2.08)										
T_3	11.88	5.57	1.87	2.00	1.97	0.87	1.07	3.60	77.33	+8.30	51.80	86.13	88.21	19.74	62.27	62.66	51.79
	(3.45)	(2.36)	(1.37)	(1.41)	(1.40)	(0.93)	(1.03)										
T_4	6.01	2.43	2.33	4.87	3.88	2.33	1.87	3.39	78.69	45.21	58.43	65.84	43.27	35.08	62.27	73.20	54.76
	(2.45)	(1.56)	(1.53)	(2.21)	(1.97)	(1.53)	(1.37)										
T_5	10.97	10.67	12.45	15.67	19.23	19.87	22.45	-	-								
	(3.31)	(3.27)	(3.53)	(3.96)	(4.39)	(4.46)	(4.74)										
SEm(±)	0.67	0.44	0.33	0.38	0.29	0.21	0.19	-	-								
CD (0.5)	2.03	1.33	1.07	1.19	0.91	0.70	0.49	-	-								

*Figure in parenthesessquare root transformed value; + denotes more insects than control

Table 3. Efficacy of IPM modules on *P. latus*

Treatments	Mean incidence/ 3 leaves							Mean	% reduction over control	Mean reduction (%) after spray							Mean
	Before spray	After first spray			After second spray					Before spray	After I spray			After II spray			
		1 Day	3 Days	7 Days	1 Day	3 Days	7 Days				1 Day	3 Days	7 Days	1 Day	3 Days	7 Days	
T_1	6.45 (2.54)	3.33 (1.82)	2.67 (1.63)	1.33 (1.15)	0.33 (0.57)	0.67 (0.82)	1.30 (1.14)	2.30	72.97	45.66	48.93	63.14	84.38	75.84	52.87	20.64	55.92
T_2	13.27 (3.64)	8.25 (2.87)	6.33 (2.52)	5.44 (2.33)	2.33 (1.53)	2.87 (1.69)	3.90 (1.97)	6.06	28.76	+11.79	38.50	57.52	68.95	58.29	50.64	41.79	43.41
T_3	12.67 (3.56)	11.33 (3.37)	12.00 (3.46)	12.33 (3.51)	1.67 (1.29)	0.67 (0.82)	1.33 (1.15)	7.43	12.61	+6.74	11.54	15.66	26.28	86.81	94.92	91.24	45.67
T_4	12.33 (3.51)	7.67 (2.77)	6.00 2.45	5.87 2.42	2.67 1.63	3.33 (1.82)	3.67 (1.92)	5.93	30.18	+3.88	38.47	56.67	63.94	55.70	46.93	49.24	43.87
T_5	11.87 (3.93)	12.00 (3.42)	13.33 (3.27)	15.67 (3.37)	16.09 (1.83	16.75 (1.82)	19.30 (1.92)	15.00	-								
SEm(±)	0.99	0.22	0.34	0.37	0.22	0.31	0.42	-	-								
CD (0.5)	NS	0.69	1.08	1.20	0.73	0.97	1.29	-	-								

*Figure in parentheses square root transformed value; *'+' denotes insects more than control

Table 4. Efficacy of IPM modules on chili leaf curl virus incidence and yield

Treatments	CLCV disease incidence (%)					Mean	% overall Reduction over control	Disease reduction/ increase (%) after spray				Mean	% disease reduction over untreated check				Mean	Yield (t/ ha)	Increased yield over control (%)
	Before spray	After first spray		After second spray				After I spray		After II spray			After I spray		After II spray				
		3 Days	7 Days	3 Days	7 Days			3 Days	7 Days	3 Days	7 Days		3 Days	7 Days	3 Days	7 Days			
T_1	0.00	0.00	0.00	0.00	2.33 (8.72)	0.47	98.56	-	-	-	2.33	0.58	100.00	100.00	100.00	95.63	98.91	1.66	71.13
T_2	18.67 (25.55)	19.33 (26.06)	23.42 (28.93)	23.42 (28.93)	25.25 (30.13)	22.02	31.74	3.54	25.44	0.00	7.81	9.20	13.90	28.31	40.45	52.65	33.83	1.19	22.68
T_3	9.45 (17.85)	14.87 (22.63)	16.33 (23.81)	17.50 (24.73)	18.00 (25.1)	15.23	52.78	57.35	72.80	7.16	2.86	35.05	33.76	50.02	55.50	66.25	51.38	1.38	42.26
T_4	10.33 (18.72)	13.33 (21.39)	14.45 (22.30)	15.90 (23.50)	17.20 (24.50)	14.24	55.85	29.04	39.88	10.03	8.18	21.78	40.62	55.77	59.57	67.75	55.93	1.49	53.60
T_5	13.50 (21.56)	22.45 (28.25)	32.67 (34.82)	39.33 (38..82)	53.33 (46.89)	32.26	-	66.30	142.00	20.39	35.60	66.07						0.97	-
SEm(±)	1.02	0.90	1.16	1.09	1.59	-	-												
CD (0.5)	3.21	2.81	3.42	3.33	4.92														

*Figure in parentheses angular transformed value

RESULTS AND DISCUSSION

The effect of IPM treatment modules on *S. dorsalis*, *P. latus* and *B. tabaci* and CLCV incidence show that seed treatment with thiamethoxam 70WS @ 3 g/ kg seed incorporated with seedling treatment with acetamiprid 20SP @ 1g/ l and seedling raising under insect proof net showed significant superiority. Border netting with insect proof net has showed significant impact in reducing their dispersal. Against thrips *S. dorsalis*, before spray incidence showed significant variation (5.33 to 22.75/ 3 leaves, and the least incidence was in IPM (5.33/ 3 leaves), which may be correlated with the effect of seed treatment, seedling treatment and raising seedling under insect proof net; overall mean was observed to be the least from IPM plots (T_1) (1.92/ 3 leaves) with 92.97% reduction over control. This is closely followed by integration of inorganic and organic amendments (T_4) (77.70% reduction), chemical management (T_3) (77.40% reduction) and organic practices (T_2) (59.61% reduction) (Table 1). As regards *B. tabaci*, all the treatments showed efficacy; on seven days after first spray IPM treated plot showed the least incidence (1.33/ 3 leaves) with 60.04% reduction; seven days after second spray showed that need based spot application of insecticides was effective (0.33/ 3 leaves- 82.68% reduction) in T_1; overall only 1.14 whiteflies/ 3 leaves was observed from IPM. (Table 2). With *P. latus s*ignificant decrease in incidence was observed with treatments- one day after first application least incidence was observed with T_1 (3.33/ 3 leaves), and 7 days after first spray 84.38% reduction was observed; likewise second spray with T_1 (0.33/ 3 leaves- 75.84% reduction) was the best, and overall it led to 72.97% reduction of mite population over control in respect of mean population was recorded by T_1 reduction (Table 3). The CLCV incidence was nil in the IPM plot initially, but with 18.67% in T_2 followed by control plot (13.5%).

Border netting technology with insect proof net prevents the dispersal of whiteflies, whereas installation of yellow sticky trap within the netted plot allows attracting those whiteflies entered somehow within the netted plot as reflected in the present data; 98.56% reduction of CLCV over control was recorded in T_1 followed by T_4 (55.85%), T_3 (52.78%) and T_2 (31.74%), as against 66.07% in control; only 0.58% incidence was observed in T_1. Maximum yield of green chili (1.66 t/ ha) with 71.13% increase was obtained with T_1 followed by T_4 (1.49 q/ ha), T_3 (1.38 t/ ha) and T_2 (1.19 t/ ha) (Table 4). Thus, IPM treatment comprising of seed treatment with thiamethoxam 70WS 3 g/ kg seed, seedling treatment with acetamiprid 20SP @ 1g/ l, seedling raising under insect proof net, installation of yellow sticky trap and need based spot application of spiromesifen 240SC and diafenthiuron 50WP was observed to be the best in managing the CLCV and its causative agents. The pesticides used were observed for their residues and results revealed only small or moderate amounts (below the instrumental LOQ range) (Fig. 1).

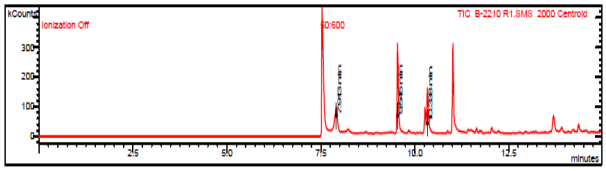

Fig. 1. Residue analysis performed by GC/ MS

Neonicotinoids as seed treatment are effective against sap feeders as observed in this study. Agreeing with the results of Kannan et al. (2004); imidacloprid @ 5 g/ kg of seeds was more effective against *B. tabaci* up to 40 days after sowing. Thiamethoxam exhibits systemic action and provides excellent control of sap feeders (Maienfisch et al., 2001). Spiromesifen is a potent insecticide/ acaricide in cotton and tomato (Ghosal and Chatterjee, 2018). The present results on acetamiprid and spiromesifen against CLCV causative agents agrees with those of Kontsedalov et al. (2009) on thrips; and spiromesifen was found safe to predatory mites, coccinellid beetles, spiders (Varghese and Mathew, 2013). Diafenthiuron was found to be efficient against chili thrips and whiteflies. Ishaaya et al. (1993) observed that diafenthiuron was effective against B. tabaci, on cotton. Vanisree et al. (2017) Chakrabarti and Sarkar (2014) and Dennehy et al. (2010) also revealed similar results. Fig. 2 shows the isolated DNA of samples collected from eleven locations of West Bengal resulted the same banding patterns of 410 bp using Bem 23 primer pairs. These observations

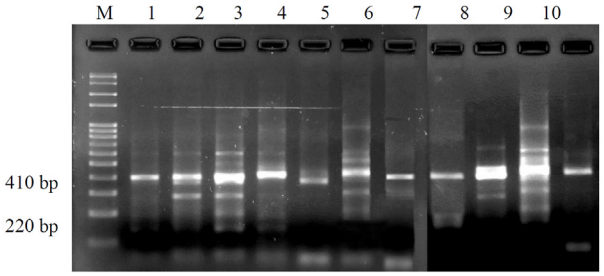

Fig. 2. Gel electrophoresis of RAPD: RAPD profile of 1= Baruipur2; 2= Narendrapur6; 3= Namkhana6; 4= Patharpratima7; 5= Kakdwip5; 6= Diamond Harbour4; 7= Arapanch2; 8= Guskara2; 9= Kalimpong1; 10= Nadia3; 11= Danga9 in respect of M= 100bp plus DNA marker

corroborate with those of Bel-Kahdi et al. (2008) and Mukherjee et al. (2016) that bands produced at 410 bp is characteristic of the genetic group Q, and 41 distinct populations including 24 genetic groups are known (Perring, 2001). Previously, it has been reported that RAPD primers (Bem-23 microsatellite marker) such as Bem 23F and Bem 23R, can easily differentiate the two genetic groups B and Q of *B. tabaci* (McKenzie et al., 2009). Genetic variability in *B. tabaci* has been studied using mtCOI and ITS1 marker genes (Boykin et al., 2007; Dinsdale et al., 2010).

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