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Molecular characterization of whitefly (*Bemesia tabaci* Genn.) and development of management module against chilli leaf curl complex

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ABSTRACT

Chilli leaf curl is the major constraint in chilli cultivation which is associated with the infestation of thrips, yellow mites and whitefly mediated leaf curl virus. Now a days chilli leaf curl virus is spotted as threat to chilli growers as most of the potent chemical in solo application found unsuitable for the management of whitefly biotype Q. Attempts have been made to address the issues of chilli leaf curl complex, molecular characterization of whitefly biotype and development of its effective management module. Experimental findings of molecular identification of different adult Bemisia tabaci samples collected across five agro-ecological zones of West Bengal, India revealed that out of 11 samples collected from different location samples from 8 locations resembles biotype Q based of amplicon pattern using Bem 23 microsatellite marker and samples from other 3 locations showed a little genetical diversity. Based on our present findings seed treatment with thiamethoxam 70 WS 3 g kg^{-1} seed incorporated with seedling treatment with acetamiprid 20 SP @ 1g l^{-1}, seedling raising under insect proof net of 80 mesh size and border netting with insect proof net showed significant impact in reducing the occurrence and dispersal of thrips (Scirtothrips dorsalis Hood), mite (Polyphagotersonemus latus) and whiteflies. Lowest population (per three leaves) of thrips (1.92), whiteflies (1.14) and mites (2.30) was recorded by 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%, 72.97% reduction, respectively. Reduction of chilli leaf curl virus (CLCV) incidence was also highest (98.56%) through IPM with maximum yield of green chilli (1.66 t ha^{-1}). Panchagavya, dasaparni and bhramvastra appeared as potent bio-pesticides in reducing the CLCV causative agents. The residue analysis result showed that the pesticides used were below the instrumental LOQ range.

Keywords: Chilli, chilli leaf curl virus, IPM, mite, thrips and whitefly

Capsicum annum is the most popular vegetable or spice native of Peru and Mexico. Chilli is frequently infested by a group of sucking and chewing insect pests of which thrips, yellow mites, whiteflies and borers are predominant (Hosmani, 1993). Kandaswamy *et al*. (1990) estimated 50% yield losses solely could be made due to thrips, *Scirtothrips dorsalis* Hood. Except insect attack, several viral diseases also infest this crop. Out of which chilli leaf curl virus (CLCV) is considered as severe one. Yield loss amounting 50-90 % may be incurred due to insect pests of chilli (Kumar, 1995). Moreover, for the last decade wide spread of whitefly, development of its different genetic groups and having a high potency of virus transmission (Gemini/ Begomo virus) are posing serious threat to the chilli growers. Senanayake *et al*. (2007) for the first time reported chilli leaf curl virus infestation from India. Recently the crop has been suffering from heavy infestation of leaf curl virus (CLCV) vectored by the tiny homopteran insect (*Bemisia tabaci* Genn.) in almost all chilli growing belts; consequently, CLCV is becoming the major constraint for successful chilli cultivation (Senanayake *et al*., 2007). 37 species have been described under the genus *Bemisia* and are assumed to have originated from Asia (Mound and Halsey, 1978). The species *Bemisia tabaci* (Gennadius), being perhaps of Indian origin (Fishpool and Burban, 1994), was illustrated under copious names before its morphological variability was recognized. Based on the study conducted by mitochondrial 16S ribosomal subunits, three divergent groups of *B. tabaci* were identified *viz*: New World, India/ Sudan and remaining Old World (Frohlich and Brown, 1994). *B. tabaci* is divided into 11 genetic groups instead of considering as one complex species and the genus *Bemisia* is divided into 34 morphologically identical 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*., 1995), whereas several other 'genetic groups' (up to S) have now been described (Brown *et al*., 1995; Boykin and De Barro, 2014). It has been found that the Mediterranean species (Q genetic group) is coexisted with the MEAM1 and exhibits a high level of resistance due to exposure to widespread injudicious insecticide applications and intensive agriculture (Dennehy *et al*.,

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2010). Development of biotype Q and its wide dispersal pose a serious threat to manage the menace as most of the insecticides identified best for biotype B may not give that much of potency. Thus in our present study we have urged to study the whitefly population of different agro ecological zones of West Bengal through basic genetical tools for development of an effective management module. As insecticidal management is the most potent and acceptable tools over the farming community who are intensively using different insecticides; thus aggravating the problem. 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 and are moderate to highly susceptible to the attack of chilli leaf curl complex. To mitigate the problem chemical management is not the best suited option for long run; a holistic approach involving all potent management options may be required. Attempts have been made to develop an ecology based IPM package to address the issue.

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 4 different treatment modules comprised of T_1 (IPM): seed treatment with thiamethoxam 70 WS (Cruiser) @ 3g kg^{-1} seed + seedling raised under insect proof net (80 mesh) + seedling dipped in acetamiprid 20 SP + border cover with insect proof net (80 mesh) + installation of yellow sticky trap @18 ha^{-1} + need based application of spiromesifen 240 SC at 30 DAT @ 1.25ml l^{-1} and diafenthiuron 50WP @ 1.5g l^{-1} after seven days; T_2 (organic module): seed bed treatment with *Trichoderma viride* enriched cow dung (30 g *T. viride* with 7.5 kg cow dung) + application of panchagavya (cow dung: cow urine: milk: curd: ghee =5:3:2:2:1, 10 nos. ripe banana and 1 lt tender coconut water was mixed to enrich the culture) spray 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., Calotrophis sp. and Clerodendrum viscosum* leaves each 1 kg weight, mixed with 2 kg cow dung and 40 lt cow urine; incubated for 30 days) @ 100ml l^{-1}; T_3 (chemical module): rotational spray of flonicamid 50 WG at 30 DAT @ 0.4g l^{-1} and spiromesifen @1.25 ml l^{-1} after seven days; T_4 (integration of inorganic and organic amendments): seedling treatment with thiamethoxam @ 3g kg^{-1} seed + application of bhramvastra (paste of one kg each leaves of *Azadirachta indica, Datura sp., Calotropis sp., Annona reticulata, Psidium guajava*: cow urine: cow dung: chilli paste : allium paste:10:5:0.25:0.25) at 30 DAT @ 25ml l^{-1} and need based spot application of diafenthiuron after seven days @1.5g l^{-1} along with untreated check. Each treatment were replicated four times and randomized accordingly. Crop was raised with recommended package of practices in 3 x 3 m^2 plots at a spacing of 50 cm x 50 cm.

Population count of thrips, whitefly and mites was enumerated from three randomly selected leaves (upper, middle and lower) per plant from five randomly selected plants per plot before spray and after spray (very next day, third day and seventh day after spray). Observation was taken during early morning hours. Thrips population was counted using hand lens (10x magnification); whitefly population was counted by eye observation whereas, mite population was enumerated with the help of microscope (Magnus stereo zoom) from the leaf samples collected from field in PP bags after properly labeled. Disease incidence (per cent) of chilli leaf curl virus was enumerated on 3 days after spraying and 7 days after spraying from 5 selected plants.

Per cent CLCV disease incidence was calculated by following formula suggested by Nene (1972):

$$\text{Per cent disease incidence} = \frac{\text{No. of diseased units}}{\text{Total assessed units}} \times 100$$

Reduction in disease incidence: Percent disease reduction was calculated by following formula:

$$\text{Per cent disease reduction} = \frac{C - T}{C} \times 100$$

Where: C is percent disease incidence in untreated plants, T is percent disease incidence in treated plants.

The collected data of insect and mite population thus obtained were subjected to analysis of variance after making square root transformation, whereas in case CLCV % incidence was analyzed after angular transformation and the treatment means were compared by following the design of RBD at a probability level of p=0.05 (Gomez and Gomez, 1984). Corrected efficacy % on test insects 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 collected data was subjected to analysis using IBM SPSS statistics 21.

Molecular characterization of whitefly

Adult whitefly samples were collected from 12 different places (Kalimpong, Nadia, Guskara, Danga,

Narendrapur, Sonarpur, Baruipur, Diamond Harbour, Kakdwip, Namkhana, Patharpratima) under 5 different agro-climatic zones of West Bengal (Hill zone, New alluvial zone, Old alluvial zone, Red and laterite zone, Coastal and saline zone) in 70% ethanol solution and carried over with ice bucket to laboratory. The collected samples were stored at -20^{0}C freezer (Blue Star) to avoid denaturation. Screening of the collected sample was done using microsatellite site "Bem 23 analysis: "Bem-23 F" (5′CGGAGCTTGCGCCTTAGTC3′) and "Bem-23-R" (5′CGGCTTTATCATAGCTCTCGT3′) illustrated by Bel-Kadhi *et al*. (2008). Following PCR amplification protocols were used:25 µl optimized polymerase chain reaction (PCR) mixture contained the following in specific amounts of Sterile double distilled water (Deionized): 15.7 µl, 10X PCR Buffer: 2.5 µl (1X), MgCl2 (25 mM): 1.5 µl (3 mM), dNTPs (2.5 mM): 1.5 µl (150 µM each), Primer (Forward) (10 pmol µl^{-1}): 1.25 µl(125 ng), Primer (Reverse) (10 pmol µl^{-1}): 1.25 µl(125 ng), Taq DNA Polymerase (5U µl^{-1}): 0.3 µl (1.5 U), DNA sample template^{-1} (10 ng µl^{-1}): 2.0 µl. PCR mixture was prepared in PCR tubes. The tubes were then placed in the thermocycler. The below mentioned thermal profile [95°C for 5 minutes (initial hot start) start cycle X 35, 95°C for 30 seconds (denaturation), 55°C for 45 seconds (annealing), 72°C for 1 minute (elongation), end cycle: 72°C for 10 minutes (single final elongation), store the solution at 4°C until electrophoresis] was set for 35 cycles for amplification.

Residue analysis for major pesticides in harvested chilli

The residue analysis were carried out to assess the concentration of different pesticides in harvested sample following the standard protocol. Five gram (5g) of green chilli 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 vortex 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 florisil and 25 mg PSA was added to it and vortex 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].

RESULTS AND DISCUSSION

The effect of different treatment modules on thrips, whiteflies, mites and chilli leaf curl virus (CLCV) incidence shows that seed treatment with thiamethoxam 70 WS 3 g kg^{-1} seed incorporated with seedling treatment with acetamiprid 20 SP @ 1g l^{-1} and seedling raising under insect proof net showed significant impact in reducing the thrips, whiteflies and CLCV incidence. Border netting with insect proof net has showed significant impact in reducing the dispersal of the said pests.

Efficacy of different treatment modules on thrips (Scirtothrips dorsalis Hood) population of green chilli

The population of thrips before spray showed significant variation and the population varied to the tune of 5.33 to 22.75 individuals per three leaves. Minimum population was recorded in IPM (5.33 thrips per three leaves), which may be correlated with the effect of seed treatment, seedling treatment and raising seedling under insect proof net. In respect of overall mean population lowest population was counted from IPM plots (T_1) (1.92 individuals per three leaves) with 92.97% population reduction over control closely followed by integration of inorganic and organic amendments (T_4) (77.70% population reduction over control), chemical management (T_3) (77.40% population reduction over control) and organic practices (T_2) (59.61% population reduction over control) (Table 1, 2).

Efficacy of different treatment modules on whitefly (Bemisia tabaci Guen.) population

All the treatments showed positive impact in reducing the whitefly population. At 7th day after first spray, IPM treated plot recorded lowest population (1.33 whiteflies per three leaves) with 60.04% mortality followed by T_3 (2 whiteflies per three leaves and 88.21% mortality), T_4 (4.87 whiteflies per three leaves and 43.27% mortality) and T_2 (7.33 whiteflies per three leaves and 59.50% mortality). Data recorded on 7th day after second spray showed that need based spot application of insecticides exerted good impact on whitefly population and thus recorded lowest population (0.33 whiteflies, with 82.68% mortality) in T_1. In respect of mean population recorded at the end of the experiment showed that only 1.14 whitefly individuals per three leaves was recorded from IPM plot followed by T_4 (3.39 whitefly individuals per three leaves), T_3 (3.60 whitefly individuals per three leaves) and T_2 (6.87 whitefly individuals per three leaves) (Table 3, 4).

Table 1: Efficacy of different treatment modules on thrips (*Scirtothrips dorsalis* Hood) population

Treatments	Before spray	Mean thrips population per 3 leaves						Mean	Reduction over control (%)
		After first spray			After second spray				
		1 Day	3 Days	7 Days	1Day	3 Days	7 Days		
T_1	5.33	2.75	2.33	1.33	0.67	0.33	0.67	1.92	92.97
	(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
	(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
	(4.66)	(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
	(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**	**-**	**-**
LSD (0.05)	**1.62**	**1.46**	**0.89**	**1.12**	**0.81**	**0.62**	**1.07**	**-**	**-**

*Figure in the parentheses is square root transformed value

Table 2: Per cent (%) reduction of thrips population over control in different days of interval

Treatments	Before spray	Mean thrips population reduction after spraying (%)						Mean
		After first spray			After second spray			
		1 Day	3 Days	7 Days	1 Day	3 Days	7 Days	
T_1	76.57	48.68	57.59	79.23	49.25	78.59	61.4	64.47
T_2	14.29	35.37	54.98	50.18	14.8	43.28	56.21	38.44
T_3	4.4	61.9	79.17	80.67	53.52	88.55	94.99	66.17
T_4	63.74	7.64	63.66	29.57	32.6	35.09	26.78	37.01

Table 3: Efficacy of different treatment modules on whitefly (*Bemisia tabaci* Genn.) population

Treatments	Before Spray	Mean whitefly population per 3 leaves						Mean	Reduction over control (%)
		After first spray			After second spray				
		1Day	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
	(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
	(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
	(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
	(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**	**-**	**-**
LSD (0.05)	**2.03**	**1.33**	**1.07**	**1.19**	**0.91**	**0.70**	**0.49**	**-**	**-**

*Figure in the parentheses is square root transformed value

Efficacy of different treatment modules on yellow mite (Polyphagotersonemus latus Banks) population

After imposing the management modules significant decrease of mite population has been noted in subsequent count. On 1st day after first application lowest population was recorded in T_1 (3.33 mites per three leaves). At 7th day after first spray, 84.38% mortality of mite was achieved and recorded as most potent treatment module followed by T_2 (68.95%), T_4 (63.94%) and T_3 (26.28%). Following application of second spray the

Table 4: Per cent (%) reduction of whitefly population over control in different days of interval

Treatments	Before spray	Mean whitefly population reduction (%) after spraying						Mean
		After first spray			After second spray			
		1 Day	3 Days	7 Days	1 Day	3 Days	7 Days	
T_1	78.76	41.31	36.85	60.04	79.78	60.27	82.68	62.81
T_2	+15.50	24.29	52.22	59.50	59.20	58.36	58.77	42.41
T_3	+8.30	51.80	86.13	88.21	19.74	62.27	62.66	51.79
T_4	45.21	58.43	65.84	43.27	35.08	62.27	73.20	54.76

+ denotes more insect than control

Table 5: Efficacy of different treatment modules on yellow mite (*Polyphagotersonemus latus* Banks) population

Treatments	Before spray	Mean mite population per 3 leaves						Mean	Reduction over control (%)
		After first spray			After second spray				
		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
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
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
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
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**	**-**	**-**
LSD (0.05)	**NS**	**0.69**	**1.08**	**1.20**	**0.73**	**0.97**	**1.29**	**-**	**-**

*Figure in the parentheses is square root transformed value

Table 6: Per cent (%) reduction of mite population over control in different days of interval

Treatments	Before spray	Mean mite population reduction (%) after spraying						Mean
		After first spray			After second spray			
		1 Day	3 Days	7 Days	1 Day	3 Days	7 Days	
T_1	45.66	48.93	63.14	84.38	75.84	52.87	20.64	55.92
T_2	+11.79	38.50	57.52	68.95	58.29	50.64	41.79	43.41
T_3	+6.74	11.54	15.66	26.28	86.81	94.92	91.24	45.67
T_4	+3.88	38.47	56.67	63.94	55.70	46.93	49.24	43.87

*'+' denotes insects more than control

data recorded on one day after, lowest population was also recorded by T_1 (0.33 mites per three leaves with 75.84% mortality) followed by T_3 (1.67 mites per three leaves with 86.81% mortality). 94.92% and 91.24% mortality was achieved by imposing spiromesifen as an component of chemical management. At the end of the experiment 72.97% reduction of mite population over control in respect of mean population was recorded by T_1 followed by T_4 (30.18%), T_2 (28.76%) and T_3 (12.61%) (Table 5, 6).

Efficacy of different treatment modules on chilli leaf curl virus incidence

The per cent disease incidence counted on before treatment showed no infestation in IPM plot, whereas 18.67% incidence of CLCV was noted 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 which may be reflected in our present observation. 98.56% reduction of leaf

Table 7: Efficacy of different treatment modules on chilli leaf curl virus incidence

Treatments	CLCV disease incidence (%)					Mean	Overall reduction over control (%)
	Before spray	After first spray		After second spray			
		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
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
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
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
T_5	13.50 (21.56)	22.45 (28.25)	32.67 (34.82)	39.33 (38..82)	53.33 (46.89)	32.26	-
SEm(±)	**1.02**	**0.90**	**1.16**	**1.09**	**1.59**	**-**	**-**
LSD (0.05)	**3.21**	**2.81**	**3.42**	**3.33**	**4.92**		

*Figure in the parentheses is angular transformed value

Table 8: Disease incidence reduction/ increase (%) after imposing treatments

Treatments	Disease progress reduction/ increase (%) after spraying				Mean
	After first spray		After second spray		
	3 Days	7 Days	3 Days	7 Days	
T_1	-	-	-	2.33	0.58
T_2	3.54	25.44	0.00	7.81	9.20
T_3	57.35	72.80	7.16	2.86	35.05
T_4	29.04	39.88	10.03	8.18	21.78
T_5	66.30	142.00	20.39	35.60	66.07

Table 9: Per cent (%) disease reduction over untreated check at different days of interval after imposing the treatments

Treatments	% disease reduction over untreated check				Mean
	After first spray		After second spray		
	3 Days	7 Days	3 Days	7 Days	
T_1	100.00	100.00	100.00	95.63	98.91
T_2	13.90	28.31	40.45	52.65	33.83
T_3	33.76	50.02	55.50	66.25	51.38
T_4	40.62	55.77	59.57	67.75	55.93

Table 10: Effect of treatment modules on yield

Treatments	Yield (t ha^{-1})	Increased yield over control (%)
T_1	1.66	71.13
T_2	1.19	22.68
T_3	1.38	42.26
T_4	1.49	53.60
T_5	0.97	-

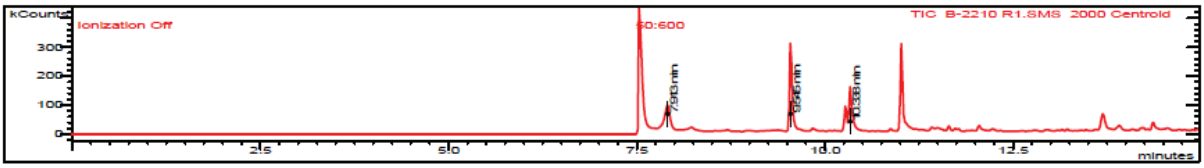

Fig. 1. Residue analysis performed by GC/MS

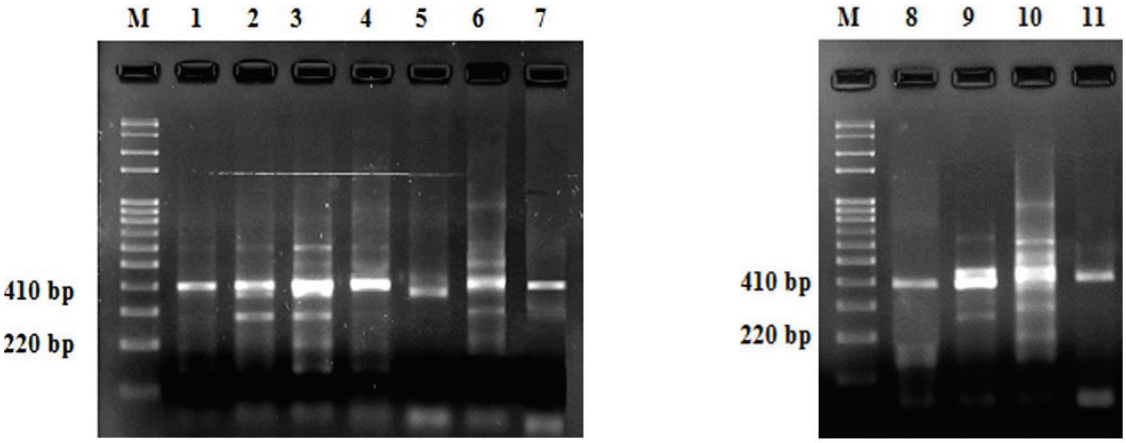

Fig. 2: A representative RAPD reaction set for differentiation of whitefly samples

RAPD profile of 1= Baruipur2, 2= Narendrapur6, 3= Namkhana6, 4= Patharpratima7, 5= Kakdwip5, 6= Diamond Harbour 4, 7= Arapanch2, 8= Guskara2, 9= Kalimpong1, 10= Nadia3, 11= Danga9 in respect of M= 100bp plus DNA marker

curl virus incidence over control was recorded in T_1 followed by T_4(55.85%), T_3 (52.78%) and T_2 (31.74%). In comparison with mean data related to progress of disease incidence recorded maximum increase in control plot (66.07%) followed by T_3 (35.05%), T_4 (21.78%) and T_2 (9.2%). Only 0.58% disease progress was observed in T_1 (Table 7, 8, 9).

Efficacy of different treatment modules on yield

Maximum yield of green chilli (1.66 t ha^{-1}) with 71.13% increased yield over control was achieved in T_1 followed by T_4 (1.49 t ha^{-1} and 53.6% increased yield over control), T_3 (1.38 t ha^{-1} and 42.26% increased yield over control) and T_2 (1.19 t ha^{-1} and 22.68% increased yield over control). Only 0.97 t ha^{-1} green chilli was recorded in untreated check where no intervention related to pest management was imposed (Table 10).

In our present experimental result it is prominent that integrated pest management comprised of seed treatment with thiamethoxam 70 WS @ 3 g kg^{-1} seed, seedling treatment with acetamiprid 20 SP @ 1g l^{-1}, seedling raising under insect proof net, installation of yellow sticky trap and need based spot application of spiromesifen 240 SC and diafenthiuron 50 WP recorded superlative result in managing the leaf curl causative agents over the other treatments. It was found that the pesticides used as control agents present in the sample in relatively small or moderate amount (Below the Instrumental LOQ range) (Fig. 1).

Effect of neonicotinoids as seed treatment agent in reducing the sap feeders is proved in our present investigation is in line with the conclusion of Kannan *et al*. (2004), who reported that seed treatment with imidacloprid @ 5 g per kg of seeds was superior in keeping the populations of *Bemisia tabaci* below economic threshold level up to 40 days after sowing. Thiamethoxam shows excellent control of a broad range of sap feeders as foliar spray and seed treatment also because of its exceptional systemic characteristics (Maienfisch *et al*., 2001). The tetronic acid derivative spiromesifen (lipid biosynthesis inhibitor) was proved as potent insecticides and acaricides shows high potency in checking the whitefly in cotton and tomato (Ghosal and Chatterjee, 2018) thus reduction of the CLCV incidence can be compared as a member of Gemini viruses. Our experimental result regarding activity of acetamiprid and spiromesifen against leaf curl of chilli causative agents is in agreement with the findings of Kontsedalov *et al*. (2009) who reported that acetamiprid 20 SP at 20 g a.i. ha^{-1} along with spiromesifen were found to be effective against chilli thrips. Spiromesifen was found to be the safest insecticide against different natural enemies like lady bugs, predatory mites, spiders and other non target insects thus it can be a good chemical component for IPM (Varghese and Mathew,

2013). Diafenthiuron, the ATP biosynthesis blocker was found as efficient molecule against chilli thrips and whiteflies. Ishaaya *et al*. (1993) reported that diafenthiuron was efficient on the different stages of *Bemisia tabaci*, on cotton seedlings with 90% mortality. Findings of Vanisree *et al*. (2017), Chakrabarti and Sarkar (2014) and Dennehy *et al*. (2010) is parallel with the outcomes of the present results. In our present observation organic bio-stimulant like panchagavya, dasaparni and bhramvastra were evolved as cogent bio-pesticide against leaf curl causative agents with potent anti-feedant and repellent action. Extraction of chemical composition of different leaves (neem, custard apple, guava, datura, papaya, bhat, hairy figs etc.) with cow urine exerted good effect on thrips, mite and whiteflies. Our experimental result is supported by the findings of Swain *et al.* (2014) and Ali *et al.* (2011).

Here in this present study Fig. 2 shows the isolated DNA of whitefly samples collected from 11 locations of West Bengal resulted that Lane 1, 2,3,4,6,7,8,9 showed amplicon size of <"410 bp and lane 5, 10 and 11 showed very minute genetical diversity as per the amplicon pattern using Bem 23 microsatellite marker, which may need further studeis to get an precise conclusion. Based on the findings of Bel-Kahdi *et al*. (2008) and Mukherjee *et al*. (2016). We may conclude that the population of whitefly collected from Baruipur, Narendrapur, Namkhana, Patharpratima, Diamond Harbour, Arapanch of South 24 Parganas district, Guskara of Burdwan district and Kalimpong resembles biotye Q. *B. tabaci* is known for its wide genetic diversity expressed as genetic groups or a complex of distinct cryptic species which are designated as morphologically identical but different in specific habit or abiotic and biotic characteristics such as geographical distribution, host specificity, resistance, virus transmitting capacity or ability to produce feeding disorders, feeding habit etc. 41 distinct population including 24 different genetic groups have been identified (Perring, 2001). Genotyping at two microsatellite markers identified by De Barro *et al.* (2003), BEM6-$(CA)_8$imp, and BEM23-$(GAA)_{31}$imp, was found to be diagnostic for B and Q biotypes based on a polymorphic amplicon size (McKenzie *et al*., 2009) are in further conformity with the present study. It has been shown that Bem 23 primer-pair can reproducibly amplify a 410bp band for subtype Q, whereas it was 220 bp for B genetic group. Genetic variability within this insect was achieved in real insight using mtCOI and ITS1 marker genes (Boykin *et al*., 2007; Dinsdale *et al*., 2010) which may be followed to get a specific conclusion. explain.)

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