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Validation of IPM Modules for the Management of Whitefly, *Bemisia tabaci* and *Mungbean Yellow Mosaic Virus* Disease in Greengram

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ABSTRACT

Greengram is known to be infected by several viral diseases. Among them, *Mungbean Yellow Mosaic Virus* (MYMV) is the most destructive in India, and it is popularly known as the yellow plague. MYMV transmitted exclusively through plant-to-plant by whitefly, *Bemisia tabaci* (Gennadius). For the management of MYMV, vector control is the only possible option. Hence, the attempt was made to evaluate IPM modules against whiteflies by reducing their population or disrupt MYMV transmission. The field experiment was conducted at Agricultural and Horticultural Research Station, Bhavikere, during *the* Kharif seasons of 2018 and 2019. The results of two seasons indicated that, the lowest whitefly population (1.70 per trifoliate leaf) and significantly lowest MYMV incidence was recorded in module-3 (Maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of fish oil rosin soap @ 5ml/liter at 40 DAS). The highest grain yield (9.64 q/ha) and cost: benefit ratio (1: 3.23) was recorded in this module, followed by module-1 (Maize as a border crop + Seed treatment with imidacloprid 600 FS @ 5 ml per kg of seeds). For the management of MYMV and its vector whitefly, *B. tabaci* module-3 was effective compared to the standard check (Dimethoate 30 EC at the rate of 1.75 ml per liter). The importance of IPM in whitefly management to reduce virus incidence is discussed.

Keywords: Asia-I cryptic species, Border crop, C: B ratio, maize, seed treatment, vector, virus incidence, grain yield.

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INTRODUCTION

Greengram, [*Vigna radiata* L.] is a minor pulse crop grown worldwide, but in India, it is one of the major crop (Chatterjee & Randhawa, 1952). India is the largest producer of greengram and accounts for 54 per cent of world production, and covers 65 per cent of the world acreage (https://www.indiastat.com). Short duration and nitrogen fixation make the crop ideal for catch cropping, intercropping and relay cropping system (Lindeman & Glover, 2003). The worldwide average yield of greengram is low (389 kg/ha), and its production has not increased over the years. The main reason for the low yield is the susceptibility of the crop to biotic and abiotic stresses. Among the biotic factors, viruses are the most important group of plant pathogens affecting crop production (Sharma, Yadav, Thareja, Kaushik, & Sharma, 1993). Among the viral diseases, *Mungbean Yellow Mosaic Virus* (MYMV) causes economic losses in greengram and reducing seed yield and quality (Kang, Yeam, & Jahn, 2005). Whitefly is an important sap sucking pest that causes heavy damage to the crop through direct loss of plant vitality by feeding cells sap and by transmitting the MYMV (Banks et al, 2001). The economic losses due to this virus account for up to 85 per cent in greengram, which is spreading faster towards newer areas (Karthikeyan et al, 2014).

For the management of MYMV, farmers are mostly resorting to synthetic insecticides because of their quick knock-down effect with or without knowing the adverse effects. To minimize the use of hazardous chemicals, IPM strategies need to be evolved to avoid toxicity to human beings, the environment and non-target organisms. Keeping the above points in view, the present investigation was undertaken to manage whitefly vector and MYMV disease by identifying an effective IPM module.

MATERIALS AND METHODS

Experimental site and raising of a crop

Experiments were conducted at Agricultural and Horticultural Research Station (AHRS) Bhavikere (13˚14′679″N 75˚43′525″E, 567m) Tarikere, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India. The widely grown greengram variety in Karnataka, KKM-3 (Karnataka Kattalagere Mungbean-3), was sown on *Kharif* seasons of 2018 and 2019. The crop was raised by following all standard procedures according to the package of practices of University of Agricultural and Horticultural Sciences, Shivamogga, except for the plant protection measures for sucking insect pests.

Experimental design and treatments imposition

The experiment was laid under field conditions in a Randomized Complete Block Design (RCBD) with five modules and replicated four times. The sprays of Neem Seed Kernel Extract (NSKE 5%), Fish Oil Rosin Soap [(FORS), Meenark L®, West Coast Herbochem Limited)] and Dimethoate (Tafgor® 30 EC, Rallis India) were given according to the treatment wise when the crop was 20 and 40 days old. The spraying was done manually using a hand-operated knapsack sprayer with a standard volume of water (500 litres/ha). The treatments were implemented, as mentioned in Table 1. Greengram seeds were treated with imidacloprid (Gaucho® 600 FS, Bayers Crop Science Limited) at 5 ml per kg. The seeds were spread on the polythene sheet, and the chemical was sprinkled uniformly on seeds, mixed thoroughly by hands and shade dried for 30 minutes. Border crop maize (Hybrid: Pioneer-P3551) was selected, which was sown fifteen days before sowing of greengram.

Table 1. Details of the IPM modules evaluated against *Bemisia tabaci* and MYMV disease incidence in greengram.

Modules	Details
Module-1	Maize as a border crop + Seed treatment with imidacloprid 600 FS at the rate of 5 ml per kg of seeds
Module-2	Maize as a border crop + Seed treatment with imidacloprid 600 FS at the rate of 5 ml per kg of seeds + Reflective ribbons
Module-3	Maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of Fish Oil Rosin Soap (FORS) at the rate of 5ml per liter at 40 days after sowing
Module-4	Two sprays of Dimethoate 30 EC at the rate of 1.7 ml per liter at 20 and 40 days after sowing (Standard Check)
Module-5	Untreated Control

NSKE preparation (5%)

Neem fruits were collected at UAHS, Shivamogga campus during the bearing season, and the fruits were shade dried under laboratory conditions. Five kg of neem seed kernels were grounded and powdered. The powder was soaked in 10 litres of water overnight, and then the solution was filtered through muslin cloth, and the volume was adjusted to 100 litres by adding water. Freshly prepared kernel extract was used for spraying by adding 1 per cent detergent.

Observations on whitefly population and MYMV incidence

Whiteflies were counted in the early morning on ten randomly selected tagged plants in each treatment. The number of adult whiteflies per trifoliate leaf at 15, 30, 45 and 60 days after sowing was recorded (Ambarish, Kalleshwaraswamy, & Venkataravanappa, 2020). For effective interpretation of data, the average was calculated from all the observations of whiteflies. Similarly, MYMV disease incidence was recorded at 15 days interval by counting the number of plants showing distinct MYMV symptoms and the total number of plants in an experimental plot (for each treatment), and Per cent Disease Incidence (PDI) was worked out. To know the impact of IPM modules on MYMV per cent disease incidence, 60 days after sowing data was used for the explanation of results. Further, the data of both seasons were pooled and analyzed to bring out valuable conclusions.

Identification of *B. tabaci* cryptic species

For identification of *B. tabaci* cryptic species, total nucleic acid from the adult *B. tabaci* collected from the experimental plot was isolated by following the method described by De Barro, & Driver, 1997. Fifty nano grams of total nucleic acid of *B. tabaci* was subjected to PCR amplification using mtCOI gene specific forward primer (C1-J-2195-TTGATTTTTTGGTCATCCAGAAGT) and reverse primer (L2-N-3014-TCCAATGCACTAATCTGCCATATTA) (Simon, Frati, Beckembach, Crespi, Liu, & Flook 1994). Negative control was used without DNA condition in each amplification. The amplified products were cloned into the pTZ57R/T vector (Fermentas, Germany) and sequenced. The nucleotide sequence identity of mtCOI gene similarity was checked by using BLAST program available at the NCBI (National Center for Biotechnology Information, San Diego, CA, USA). Further, the mtCOI gene sequences of *B. tabaci* cryptic species were compared with sequences generated by Kankala, & Ghanim, (2019) to determine whether or not they diverged by more than 3.5%, the value these authors have reported to identify putative species genetic boundaries. The mtCOI gene sequences analysis indicated of five *B. tabaci* samples showed 98% identity among them. Hence, only one accession number (ON385112) submitted was included in phylogenetic analysis (Fig. 1). The phylogenetic analysis of mtCOI gene from the *B. tabaci* was closely clustered with *B. tabaci* cryptic species belongs to the Asia-I group.

Confirmation of MYMV through PCR

Mungbean yellow mosaic virus was confirmed by visual viral symptoms and PCR using a degenerative primer to specific to begomovirus. The DNA isolated from greengram samples was amplified through PCR using primers pairs AV494/AC1048, (MYMVF-ATGGGKTCCGTTGTATGCTTG and MYMVR-GGCGTCATTAGCATAG GCAAT) as described by (Naimuddin, Akram, & Aditya, 2011). A band of approximately 1.4 kb was amplified from DNA extracted from virus infected greengram plants.

Grain yield and cost economics

Grain yield of both the years of 2018 and 2019 were recorded on each treatment and computed to a hectare basis. The Cost: Benefit ratio was calculated based on the current price available in the market based on inputs and outputs.

Statistical analysis

All the experimental data were transformed with the square root of $\sqrt{x+0.5}$ before analysis. Data were then subjected to analysis of variance (ANOVA), and treatments were compared by Tukey's test ($p=0.05$). The statistical analysis was performed in SPSS software (Version 16.0).

RESULTS

Whitefly population as influenced by different IPM modules

During *Kharif* 2018, the mean number of whitefly population varied significantly among the different IPM modules evaluated ($p<0.05$). The lowest number of whiteflies (1.80) per trifoliate leaf was recorded in module-3, which was found to be on par with module-2 (2.21) and module- 4 (2.30). The maximum number of whiteflies (3.89) per trifoliate leaf was noticed in the untreated control, and it is found to be on par with module-1 (2.68) (Table 2).

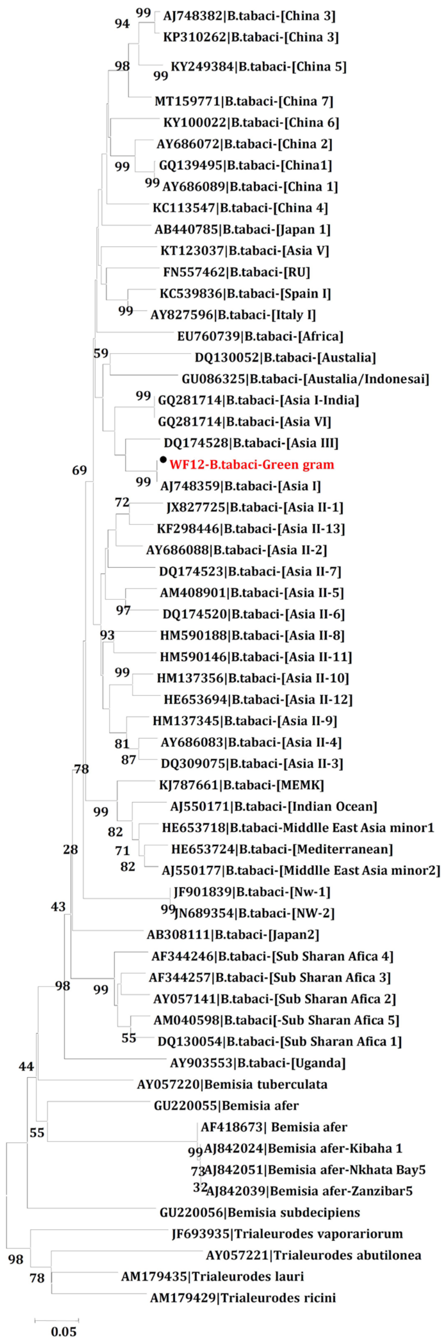

Fig. 1. Phylogenetic analysis of *B. tabaci* for confirmation of cryptic species.

Table 2. Evaluation of IPM modules against *Bemisia tabaci* on greengram during Kharif 2018.

Modules	Mean number of whiteflies per trifoliate leaf				
	15 DAS	30 DAS	45 DAS	60 DAS	Total mean
Module-1	1.80 (1.52)^{a}	2.25 (1.66)^{b}	3.10 (1.90)^{b}	3.55 (2.01)^{c}	2.68 (1.78)^{ab}
Module-2	1.60 (1.45)^{a}	1.85 (1.53)^{ab}	2.50 (1.73)^{b}	2.90 (1.84)^{bc}	2.21 (1.65)^{a}
Module-3	2.13 (1.61)^{a}	1.74 (1.50)^{a}	1.45 (1.40)^{a}	1.90 (1.55)^{a}	1.80 (1.52)^{a}
Module-4	2.90 (1.84)^{b}	2.20 (1.64)^{b}	1.75 (1.50)^{a}	2.35 (1.69)^{ab}	2.30 (1.67)^{a}
Module-5	2.85 (1.83)^{b}	3.55 (2.01)^{c}	4.25 (2.18)^{c}	4.90 (2.32)^{d}	3.89 (2.09)^{b}
F	16.74	37.57	64.02	54.40	6.03
P	<0.05	<0.05	<0.05	<0.05	<0.05

Numbers in the parenthesis are $\sqrt{(x+0.5)}$ transformed values

DAS- Days after sowing

Mean values followed by the different letters are significantly different by Tukey's test ($p \leq 0.05$)

During 2019, a similar trend was noticed in different modules evaluated. Significantly, a lower mean number of whiteflies per trifoliate leaf (1.60) was recorded in module-3, which was found to be on par with module-4 (2.21) and module-2 (2.29). The control plot recorded the 3.63 whiteflies per trifoliate leaf, and it was found to be on par with module-1 (2.65) (Table 3).

Table 3. Evaluation of IPM modules against *Bemisia tabaci* on greengram during Kharif 2019.

Modules	Mean number of whiteflies per trifoliate leaf				
	15 DAS	30 DAS	45 DAS	60 DAS	Total mean
Module-1	1.75 (1.50)^{ab}	2.2 (1.64)^{a}	3.08 (1.89)^{c}	3.58 (2.02)^{cd}	2.65 (1.77)^{ab}
Module-2	1.65 (1.47)^{a}	2.0 (1.58)^{a}	2.70 (1.79)^{bc}	2.83 (1.82)^{bc}	2.29 (1.67)^{a}
Module-3	1.95 (1.57)^{abc}	1.45 (1.40)^{a}	1.35 (1.36)^{a}	1.65 (1.47)^{a}	1.60 (1.45)^{a}
Module-4	2.7 (1.79)^{b}	2.05 (1.60)^{a}	1.75 (1.50)^{ab}	2.35 (1.69)^{ab}	2.21 (1.65)^{a}
Module-5	2.5 (1.73)^{ab}	3.4 (1.97)^{b}	4.08 (2.14)^{c}	4.55 (2.25)^{d}	3.63 (2.03)^{b}
F	6.47	9.267	12.86	30.97	7.11
P	<0.05	<0.05	<0.05	<0.05	<0.05

Numbers in the parenthesis are $\sqrt{(x+0.5)}$ transformed values

DAS- Days after sowing

Mean values followed by the different letters are significantly different by Tukey's test ($p \leq 0.05$)

Pooled data of two seasons indicated that the lowest number of whiteflies (1.70) per trifoliate leaf was recorded in module-3, which was found to be on par with module-2 (2.25) and module-4 (2.26). In module-1 recorded 2.66 whiteflies per trifoliate leaf. The untreated control plot recorded the 3.76 whiteflies per trifoliate leaf, and it was found to be a statistically significant difference over the other modules evaluated ($p<0.05$) (Table 4).

Table 4. Evaluation of IPM modules against *Bemisia tabaci* on greengram (pooled data of 2018 and 2019).

Modules	Mean number of whiteflies per trifoliate leaf				
	15 DAS	30 DAS	45 DAS	60 DAS	Total mean
Module-1	1.78 (1.51)[a]	2.23 (1.65)[b]	3.09 (1.89)[b]	3.56 (2.02)[c]	2.66 (1.78)[ab]
Module-2	1.63 (1.46)[a]	1.93 (1.56)[ab]	2.60 (1.76)[b]	2.86 (1.83)[b]	2.25 (1.66)[a]
Module-3	2.04 (1.59)[a]	1.59 (1.45)[a]	1.40 (1.38)[a]	1.78 (1.51)[a]	1.70 (1.48)[a]
Module-4	2.80 (1.82)[b]	2.13 (1.62)[b]	1.75 (1.50)[a]	2.35 (1.69)[b]	2.26 (1.66)[a]
Module-5	2.68 (1.78)[b]	3.48 (1.99)[c]	4.16 (2.16)[c]	4.73 (2.29)[d]	3.76 (2.06)[b]
F	17.03	30.43	42.13	64.79	5.70
P	<0.05	<0.05	<0.05	<0.05	<0.05

Numbers in the parenthesis are √(x+0.5) transformed values

DAS- Days after sowing

Mean values followed by the different letters are significantly different by Tukey's test (p≤0.05)

MYMV disease incidence as influenced by different IPM modules

At 60 days after sowing (pooled data of two years), there was a significant difference was observed among the different modules tested (p<0.05). The lowest per cent disease incidence was documented in module-3 (7.75 %), followed by 10.65 per cent in module-2, which was found to be on par with module-1 (11.26 %). However, in standard check (module-4) recorded 14.26 per cent disease incidence. The maximum MYMV disease incidence was noticed in the untreated control plot (26.80 %) (Fig. 2). Up to 15 days of sowing, there was no disease incidence observed in module-1 and module-2 because of the effect of seed treatment (imidacloprid) and border crop (maize) effect. The overall results indicated that IPM modules had reduced the MYMV incidence in greengram.

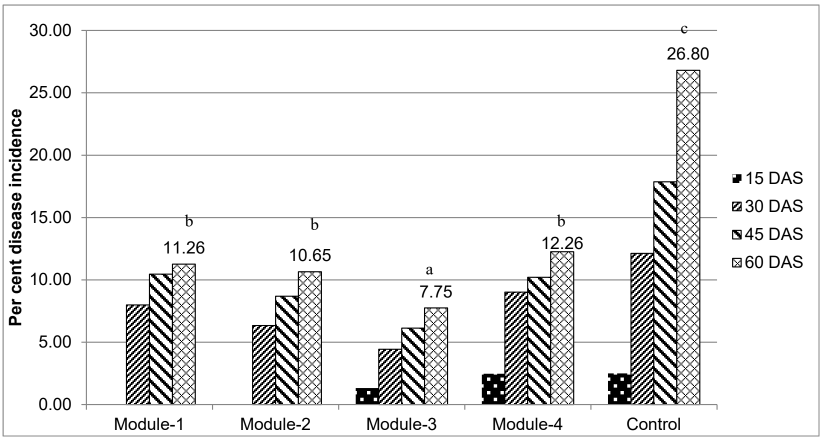

Fig. 2. MYMV disease incidence as influenced by different IPM modules in greengram (pooled data of 2018 and 2019).

Yield and economics of greengram as influenced by different IPM modules

Pooled data of two years (2018 and 2019) revealed that module-3 was superior among all the other treatments by documenting the highest grain yield of 9.64 q/ha. This was found to be on par with module-4 (8.93 q/ha) and module-2 (8.81 q/ha). The next best treatment with respect to yield was Module-1 (8.12 q/ha), the lowest grain yield was documented in the untreated control (6.08 q/ha) (Table 5).

Similarly, Module-3 documented the highest C: B ratio (1:3.23) and was superior when compared to other treatments, followed by module-1 (1:3.12), module-4 (1:2.94), module-2 (1: 2.89). Untreated control recorded the lowest C: B ratio (1:2.14 (Table 5).

Table 5. Impact of IPM modules on grain yield and cost economics of greengram (pooled data).

Modules	Grain yield (q/ha)	Yield of border crop (Maize) (q/ha)	Cost of treatments for plant protection (Rs./ha)	Total Cost of cultivation (Rs./ha)	Gross income (Rs./ha)	Net income (Rs./ha)	C:B
Module-1	8.12[b]	3.50	664	21 153.32	65 892.68	44 739.36	1: 3.12
Module-2	8.81[ab]	3.50	4 064	24 553.32	70 922.09	46 368.77	1: 2.89
Module-3	9.64[a]	3.50	3 355	23 844.32	76 971.96	53 127.64	1: 3.23
Module-4	8.93[ab]	--	1 642.6	22 131.92	65 090.77	42 958.85	1: 2.94
Module-5	6.08[c]	--	--	20 489.32	43 879.78	23 390.46	1: 2.14
F	19.65	--	--	--	--	--	--
P	<0.05	--	--	--	--	--	--

(Market price of greengram= Rs. 7313/quintal, Variety-KKM-3): (Market price of Maize= Rs. 1916/quintal)

Mean values followed by the different letters are significantly different by Tukey's test ($P \leq 0.05$)

Cost of production for all the modules: 20489.32 Rs./ha.

From the above results, it indicated that the module-3 (maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of FORS @ 5ml/liter at 40 days after sowing) was found to be superior in reducing the whitefly population and MYMV incidence. The grain yield (9.64 q/ha) and cost economics (1: 3.23) also superior when compared to the standard check and other modules evaluated.

The next best module with respect to cost economics was module-1 (Maize as a border crop + seed treatment with imidacloprid) (1:3.12). In this module, the grain yield was low when compared to the other modules. The C: B ratio was high because the cost incurred in this module was low, and also due to the addition of border crop yield (maize yield 3.50 q/ha). Hence, module-3 and module-1 were cost-effective in managing whitefly vector and MYMV disease incidence in greengram.

DISCUSSION

MYMV is one of the major bottle neck to greengram cultivation and production in Asia, including India. The management of this disease remains a major challenge. Currently, the outbreak of whitefly and the resistance development to most of insecticides (Ahmad, Arif, & Naveed, 2010), and incidence of MYMV was increased in many leguminous crops in Indian conditions (Karthikeyan, Shobhana, Sudha, Raveendran, Senthil, Pandiyan, & Nagarajan, 2014). Farmers spraying three rounds of insecticides for management of MYMV hence, the IPM modules play an important role in the ecofriendly management. In the present study, different combination of components was tested against whitefly and MYMV in greengram. Among the different combinations, maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of FORS @ 5ml/liter at 40 days after sowing was found to be effective. Because maize is a tall-growing plant and acts as a barrier crop that helps prevent the movement of small insect pests from one field to another, which are generally carried by the wind. Earlier reports also indicated maize as a border crop is known to reduce the whitefly and virus incidence in soybean (Raghupathi & Sabitha, 1994). Similarly, the treatment with border crop of African tall maize + seed treatment with imidacloprid 70 WS + reflective mulch + spraying of triazophos 40 EC at 0.175 % at 30 days after sowing + spraying with thiamethoxam 25 WDG at 0.05 % at 45 DAS recorded the lowest whitefly population, yellow mosaic virus incidence and highest yield in pole bean (Jyothi, Nagaraju, Padmaja, & Rangaswamy, 2013). The effectiveness of border crop and seed treatment for the management of insect pests in different pulse crops has been reported (Kumar et al, 2014; Anusha, Balikai, & Patil, 2016; Swathi & Gaur, 2017; Radhika, Reddy, Anitha, Vidhyasagar, & Ramesh, 2018).

Fish oil kills insects on contact by disrupting gas exchange (respiration), cell membrane function or structure. They also kill them by disrupting their feeding on oil-covered surfaces. The toxic action is more physical than chemical (Bogran, Ludwig, & Metz, 2011). The FORS was effective in many insect pests, as reported by Rahman & Batra (1945), who proved effective in controlling onion thrips. Natrajan, Sundramurthy, & Basu (1991) found that FORS was superior in controlling *Thrips tabaci* in cotton. Jha & Manoj Kumar (2018) reported that NSKE 5 % recorded the 76.02 per cent reduction in whiteflies. Similarly, Jayraj et al (1986) recorded 93.7 per cent mortality of whiteflies in NSKE treatment. Natarajan et al (1986) reported that neem oil was effective in suppressing the whitefly population. Hence, the combination of border crop and one spray of NSKE 5% and FORS has effectively reduced the deadly MYMV and its vector whitefly population in greengram. The earlier researchers also proved that IPM modules were effective in the reduction of the pest population, good grain yield and higher benefits in various pulse crops such as urdbean (Singh, Tomar, & Rikhari, 2018), french bean (Sharmah & Rahman, 2017) and pigeonpea (Gajendran, Chandrasekaran, & Jebaraj, 2006; Srinivasan & Sridhar, 2008). Various explanations have been given for the dynamics of insect pest populations under various cropping systems. Insect population reductions have been generally attributed to disruption of the insects visual and olfactory responses (Giga & Munetsi, 1989; Rizk, 2000). The companion and neighbouring plants can reduce pest pressure by providing habitat for the natural enemies, preventing entry into the field, dispersal and confuse pests (Wallace, 2013).

From the present investigation, it was revealed that IPM module consisting of maize as a border crop + one spray of NSKE 5 % at 20 days after sowing + one spray of FORS @ 5ml/liter at 40 days after sowing was found to be effective in the management of whitefly vector and MYMV disease incidence in greengram. This module recorded the highest grain yield and C: B ratio when compared to the standard check and other modules.

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