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Designing of an Innovative Multiplex Model to Control Insects/Pests (Whitefly) in Okra Crop Fields and Assessment for its Efficacy in Control Viral Diseases

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ABSTRACT

Whitefly (*Bemisia tabaci*) destroys a great proportion of crops in India. Whitefly is the carrier of begomovirus which causes okra yellow vein mosaic viral disease. Preventing the pest infestation without the use of chemical fertilizers and pesticides is the need of the hour. Integrated Pest Management (IPM) involves strategies that maintain pest population below Economic Injury Level (EIL). In the present study a multiplex insect catching trap was constructed with the aim of controlling pest infestation in a sustainable and eco-friendly manner. The multiplex trap was prepared using yellow sticky papers and Methyl Eugenol pheromone as an insect/pest attractant. The traps were placed in field and thereafter plants were tested for the presence of viral DNA using PCR. The fields in which traps were placed showed negligible or few instances of viral infection while the fields in which no traps were placed showed considerable virus infection. Additionally, the fields with the trap showed a higher yield percentage of crop compared to the field with no trap.

Keywords Whitefly, Methyl eugenol, Integrated Pest Management (IPM), Yellow trap, Okra yellow vein mosaic.

INTRODUCTION

Problem of insect pest is huge in India and there is a loss of up to 40% every year world wide and cost up to $220 billion of losses as per the latest estimates by food and agriculture organization of the United Nations (FAO 2020). According to a study in India crops worth Rs 50000 crores are lost due to pests and disease attack every year. About 30–35% of the annual crops are lost to pests in India (thehindu.com 2017). Farmers, growers and people from agriculture sector rely heavily on agrochemicals and pesticides to save these crops from insects/pests. Apart from damaging the crops by eating them or sucking them, these insects' pests are also vectors of viruses, which cause severe viral diseases and for which we have no remedy what so ever till date (Tudi *et al.* 2021). To manage pest menace farmers resort to use commercially available pesticides.

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Recent studies have established linkages between aerial spraying of the pesticide and the growing health disorders in Kasaragod district in Kerala (due to endosulfan). Some of the major issues with endosulphan usage were development of physical deformities, mental retardation, poisoning and neurotoxicity (Kucuker *et al.* 2009, Amizadeh and Askari Saryazdi 2011, Milesi *et al*. 2021). Endosulfan pesticide was used widely on crops like cashew, cotton, tea, paddy, fruits and others until 2011, when the Supreme Court banned its production and distribution. Over 20 years of aerial spraying on cashew plantations in Kerala and other states has left many with mental and physical disorders. Interestingly after the ban such cases of mental and physical deformities are not anymore, establishing a very strong association of such traits with the use of endosulfan. Over the years, other studies confirmed these findings, and the health hazards associated with pesticides are now widely known and accepted. The Malwa region of Punjab, India, also is facing an unprecedented crisis of environmental health linked to indiscriminate, excessive, and unsafe use of pesticides, fertilizers and poor groundwater quality. The region has been described as India's "cancer capital" due to abnormally high number of cancer cases, which have increased 3-fold in the last 10 years. Studies of this region have also highlighted a sharp increase in many other pesticide-related diseases, such as mental retardation and reproductive disorders. The most affected individuals are the agricultural workers who are directly exposed to pesticides (Mittal *et al.* 2014). The situation is so grave that from Bathinda which is in the Malwa region, a special train 'cancer express' runs and carries all cancer patients to a hospital in Rajasthan.

The indiscriminate use agrochemicals (fertilizers and pesticides) resulted in their incorporation in the food chain. In India hazardous chemicals like endosulfan, chlorpyrifos, DDT, glyphosate, monocrotophos and many more are routinely and excessively used by farmers all over India on crops like wheat, rice, chilli, brinjal, tomato, okra, cabbage i.e., on all green vegetables (Devi *et al.* 2022). The nourishing and health benefit properties of green vegetables are marred due to problems associated with pesticide abuse which cause several mental and physical disorders (Głąbska *et al.* 2020).

Okra a member of Malvaceae is a nutritive fruit rich in vitamin, proteins, calcium and minerals. India is one of the largest producers of okra. Other than fungal diseases, sucking insect cause huge loss of crop productivity and results in various symptoms e.g. loss of vigor, speckled pale yellow shrivelled leaves (Chauhan 1972, IBPGR 1990). A whitefly pest acts as a vector to transmit yellow vein mosaic virus disease caused by begomoviruses. Genus *Begomovirus* are single stranded DNA virus belongs to family Geminiviridae, they can be monopartite or bipartite belongs to old and new world, respectively (Briddon *et al.* 2010). The viral disease cause formation of network of yellow veins and ultimately results in stunted growth and small sized yellowish okra fruits with tough texture due to which the crop yield drops to 80-90% (Mubeen *et al.* 2017, Mohanta *et al.* 2020). Generally to tackle white fly okra crop is sprayed 4 to 5 times with pesticide solution of malathion/ monocrotophos/dimethoate (Boopathi *et al.* 2010).

For farmers and amateur gardeners who are interested in growing vegetables and develop kitchen garden/terrace garden purely on organic basis without using pesticide, we have developed a pest trap model to simultaneously trap a group of insects which target the vegetables. The proposed model will be an alternative environment friendly, non pesticide management tool which will work on selective targeted pests.

MATERIALS AND METHODS

Material required

Plastic bottle, cotton wick, electrical wire, yellow bulb, bulb holder, bendable wire, paper cup, eppendorf, sticky traps, Methyl Eugenol and cellophane tape.

Designing and working of multiplex trap

To design a multiplex insect trap, a plastic bottle was taken and cut from the bottom. Eight holes were made on it, two holes opposite to each other. A bulb was taken and fixed in a holder. A small hole at the center of the cap of the bottle was made and four small holes surrounding the cap were made. A paper cup was taken and hung with the help of bendable wires

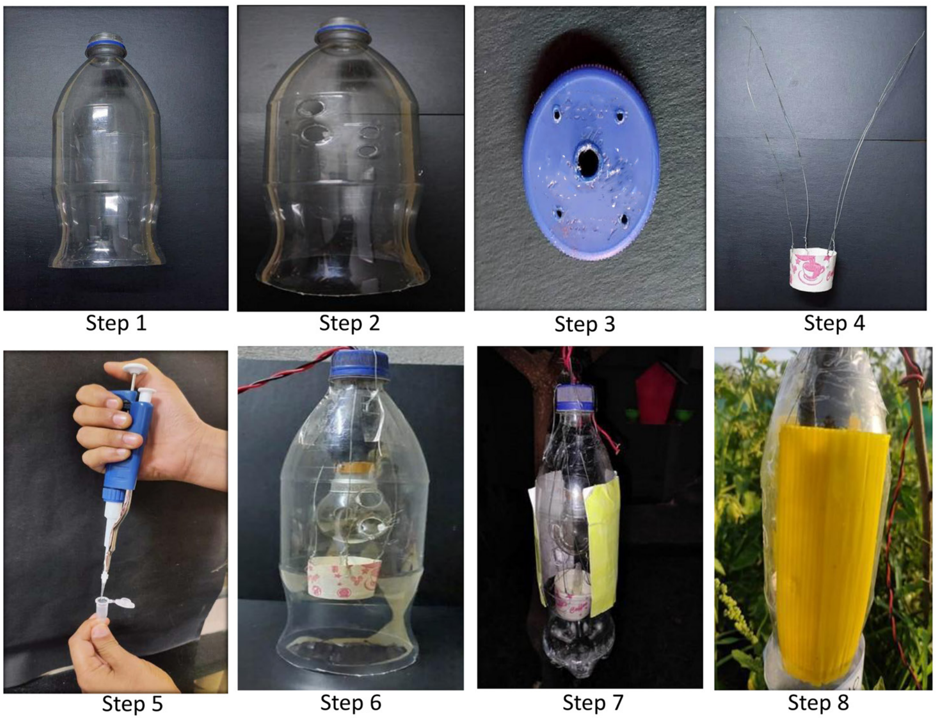

Fig. 1. Figure showing steps (1-8) for the construction of multiplex trap.

by passing through the cap. The bulb was hung with holder inside the bottle. 1 ml of methyl eugenol was taken and filled in the 1.5 ml eppendorf tube. A small hole at the base of eppendorf tube was made and a thread was passed through it to make a wick. Then eppendorf tube was fixed onto the paper cup. Sticky traps were stuck onto sides of the bottle. The bottom of the bottle was filled with water and attached with the help of cellophane tape. The trap was hung in the field at different places at a height of around 2 feet and bulb was switched on in night to attract more insect vector whitefly (Fig. 1).

Table 1. Primer sequence used for the detection of begomoviruses infection in okra leaves.

Sl. No.	Primer name	Nucleotide sequence (5'-3')	Reference
1	PAL1V1978 PAR1c715	Forward– 5'GATTTCTGCAGTT-DATRTTYTCRTCCAA 3' Reverse- 5'GCATCTGCAGGCC-CACATYGTCTTYCCN 3'	Rojas *et al.* (1993)

Field trial to check the efficacy of designed multiplex trap for whitefly vector of begomovirus

To check the efficacy of the designed trap, okra fields in different fields of vegetable gardens were setup in the BBA university campus from seedling stage to fruiting stage during three different seasons. First season is summer from March 2022 to May 2022, second season is winter season from August 2022 to November 2022 and third season is also summer from March 2023 to May 2023. The area of the trial field taken was around 360 m^2. In each season, four okra fields was set up, in three of the okra field, multiplex trap hung at a distance of ten rows in each direction in the field designated as test fields, while the fourth field had no trap designated as control field. After 24-48 hrs of setting the trap in fields, random sampling of okra leaves preferably with or even without symptoms of

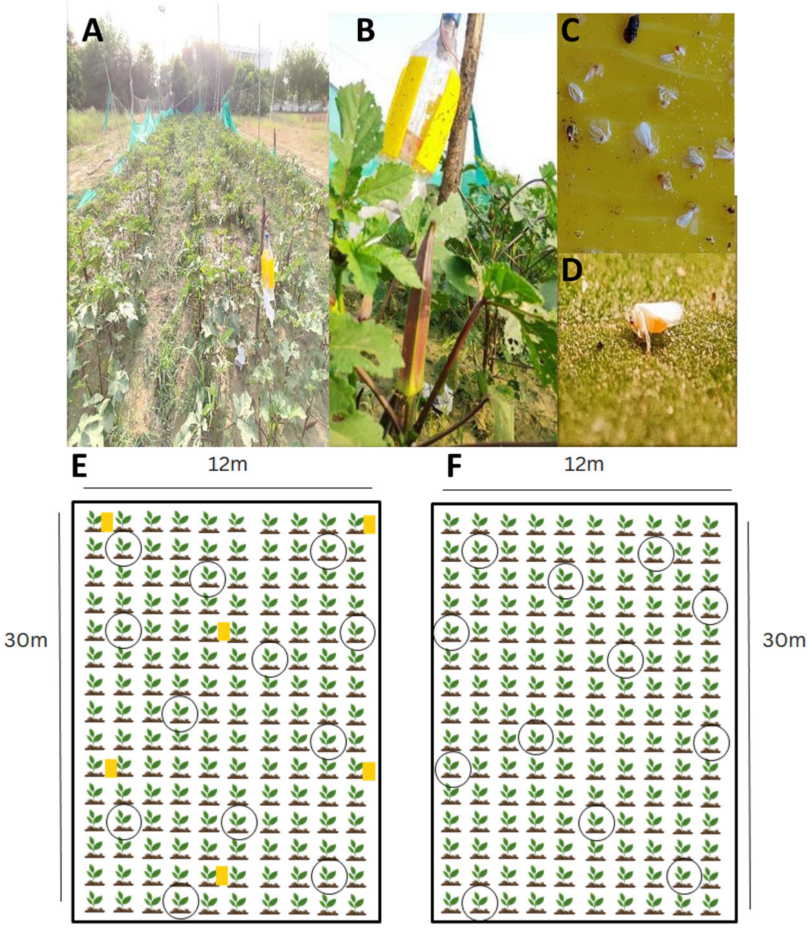

Fig. 2. Figures showing (A) Field trial of okra having multiplex trap, (B) Zoomed image of trap in the field, (C) Whitefly sticks on yellow sticky traps, (D) Presence of whitefly on okra leaves which is a potent host plant for causing okra yellow vein mosaic virus, (E) Multiplex trap position in test field, (F) Control field.

okra yellow vein mosaic disease was done from all the fields in every season (Fig. 2).

Detection of the incidence of okra yellow vein mosaic viruses caused by begomoviruses

To check the effectiveness of the designed trap, total genomic DNA extraction of collected leaves was done using CTAB method (Murray and Thompson 1980). Induction or reduction in the disease incidence before and after setting up the multiplex trap was analyzed by polymerase chain reaction (PCR) mediated detection of begomoviruses causing okra yellow vein mosaic disease. To confirm the presence of begomovirus, degenerate primers (PAL1v1978 and PAR1c715) for begomovirus detection was used (Rojas *et al.* 1993) (Table 1). The standardized PCR conditions are as follows: 94°C- 5 min, 94°C- 50 sec, 58°C- 45 sec, 72°C-90 sec (35 cycles), 72°C- 5 min.

RESULTS

Working and efficacy of multiplex trap

The working of multiplex insect trap includes three ways to catch insects and whitefly i.e., physical,

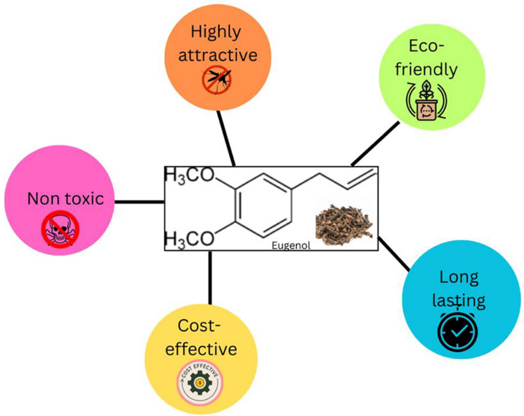

Fig. 3. Diagram depicting the benefits of Eugenol as a insect and pest attractant in multiplex trap.

mechanical and chemical. The physical trapping part consists of a light source-an incandescent bulb which attracts insects. The mechanical trap includes the yellow sticky trap which attracts the insects and renders them stationary on sticking to it. The chemical part of the trap involves the use of chemical- methyl eugenol as an insect attractant. Methyl eugenol is a natural constituent of over 450 species of plants from 80 families of gymnosperms and angiosperms (Tan and Nishida 2012). It is a potent attractant of pollinators. In many plants, floral fragrance is due to methyl eugenol. It also has antifungal activity and may have evolved in response to pathogens. Many fruits consumed by humans and animals contain methyl eugenol as a natural constituent for example-bananas, anise, black pepper, nutmeg, walnut, citrus. Methyl eugenol have various benefits as attractant for pests and insects in comparison of other chemical attractants used in various other traps as illustrated in Fig. 3. Methyl Eugenol is mostly used in the fields of Solanaceous scrubs like tomato, lady finger, brinjal. Mediterranean fruit fly, oriental fruit fly and melon fly are attracted to methyl eugenol. Many species of insects belonging to orders *Melanostoma*, *Platycheirus, Ferdinanda, Meliscaena* and *Hadromyia* are also attracted to methyl eugenol. Also blera and melangyna (Symphora), Orchesia (Coleoptera), Empis

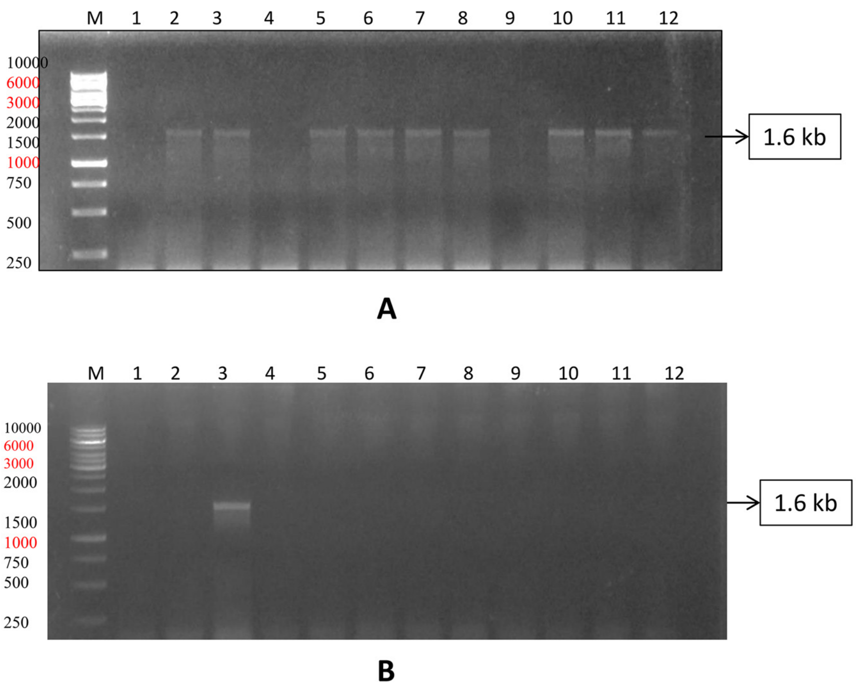

Fig. 4. Gel images showing PCR based detection of begomoviruses causing okra yellow vein mosaic disease through degenerate primers. Amplicons of 1.6 kb size on 1% agarose gel were used to identify the infection of Begomovirus of different samples from (A) field without multiplex trap, (B) field with multiplex trap.

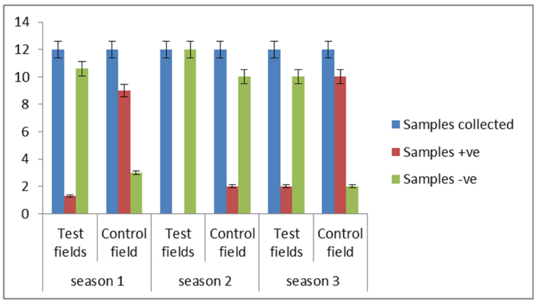

Fig. 5. Graph depicting the reduction in the disease incidence in all the test fields (with trap) and control fields (without trap) w.r.t. average mean value of samples found positive and negative for begomovirus in all the three different seasons. The respective standard errors mean are shown as bars ($p<0.05$).

(Diptera), and many other species of chrysopids are also attracted to methyl eugenol. Apart from this, this trap can also attract Solanaceous pests like Tomato fruit worm, Aphid, Beet armyworm, tobacco hornworm. Besides them, methyl eugenol is also a potent attractant for whitefly which are the insect vector of begomoviruses (Tan and Nishida 2012, Leblanc *et al.* 2015). To check the efficacy of the designed trap with respect to whitefly transmitting begomoviuses, four different field trials were setup and observed symptoms of okra yellow vein mosaic disease in all the fields in three different seasons (approx 12 plants from each field). Random sampling from12 plants exhibiting infection symptoms were collected from all the test fields and control fields i.e., field with trap and field without trap and checked for viral infection. Infected leaves showed typical symptoms of vein clearing, chlorosis, yellowing of veins and veinlets, mosaic like pattern and stunted plant growth in the field without multiplex trap. The plants in the field with multiplex trap showed healthy plants with more fruiting bodies without infection. The fields with traps showed sufficient decreased incidence of disease and presence of whiteflies (Fig. 2).

Molecular diagnostics for the presence of incidence of okra yellow vein mosaic disease caused by begomoviruses

The incidences of the disease in the fields with and without traps were detected through PCR using virus specific primers. To check the decline in the disease incidence, DNA was isolated from randomly collected leaves from both the fields. Samples were checked for the presence of begomoviruses using begomovirus specific degenerate primers. It was observed that in the first season (March 2022 to May 2022), fields with trap (test fields) only1-2 out of 12 samples was found positive for begomovirus while the field without trap had 9 out of 12 samples positive for begomovirus (Fig. 4). In second season (August 2022 to November 2022), it was observed that no samples were found positive in any of the test fields while control field was also showed only 2 positive samples out of 12 randomly collected samples. This is due to the low survival rate of whiteflies in the low temperature during the winter season. In the third season (March 2023 to May 2023), it was also observed that the test fields on an average had only 2 samples found positive for begomovirus out of 12 samples while the field without trap had 10 out of 12 samples positive for begomovirus. Furthermore, the results showed the significant amount of reduction of begomoviruses in the test field as compared to the control field (Fig. 5). Thus, we conclude that the designed trap is potent enough to control the virus transmission ~85% as compared to control field by targeting the whitefly and used as efficient model to target not only whitefly but also other insects, aphids and pests.

DISCUSSION

In integrated pest management (IPM), pests are managed using a single, adaptable strategy that incorporates all practical, affordable, and ecologically responsible pest control techniques (Karlik and Tjosvold 2003). Since it is neither practical nor cost-effectively possible to completely eradicate all pests, IPM programs work to keep pest populations at levels that do not harm the economy.IPM practitioners must be able to identify the symptoms and outcomes of both biotic and abiotic natural controls and comprehend the significance of each. When human intervention is required to control pests, the least disruptive methods should be used. These methods should include host-plant resistance, biological control since they are the least likely to interfere with natural control and are also the most sustainable. Practises that are extremely disruptive or harmful to the environment should only be employed as a last option. In India, 54% of the okra plantations are lost to pests (Ghosal *et al.* 2012). One of the major reasons for this huge loss in production in okra fields is begomoviruse infection. Whitefly are not only the carrier and vector of begomoviruses (Family : Geminiviridae), but are also the vector of other plant viruses ex. torradoviruses, ipomoviruses, criniviruses, and some carlaviruses (Fiallo-Olivé *et al.* 2020). Major viral diseases in the serious losses in okra productivity are yellow vein mosaic disease (YVMD), okra leaf curl disease (OLCD), and okra enation leaf curl disease (OELCD) of begomoviruses family (Venkataravanappa *et al.* 2013).

White fly is the causal agent of Bean dwarf mosaic virus (BDMV) in beans, Tomato yellow leaf curl virus (TYLCD) in tomato, Cassava mosaic virus (CMD) and Cassava brown streak disease (CBSD) in cassava and Cotton leaf curl disease (CLCuD) in cotton (Lapidot *et al.* 2014). A number of colored traps were tested but yellow traps have been found to be most effective (Malik *et al*. 2012). To cater this problem of pest management, a multiplex trap is designed which is cost effective and eco-friendly. Methyl eugenol which used as a potent attractant in this trap has least impact to other pests and insects especially for bees (Vargas *et al.* 2010). Also yellow sticky traps at sides were efficient in trapping the whitefly and other bugs that are harmful to crops. When using chemical pesticides, especially ones with broad-spectrum activity, it is important to monitor pest populations frequently and regularly. Monitoring natural enemy populations is necessary to assess how they affect pests. When pesticides are required, those that are least harmful to natural -pollinator should be prioritized (Sponsler *et al.* 2019). The practise of integrated pest management is dynamic and ever-changing. Based on variations in pest populations and their natural controls, specific management techniques will alter from crop to crop, location to location and year to year (Kaur and Kaur 2020). As particular new techniques or technologies are created, they can also be included to the program as necessary. Effective pest controllers will have a thorough understanding of the pests, their natural adversaries and all available management measures.

CONCLUSION

After contemplating and analyzing the results it was clearly found that okra fields in which the traps were used performed better than the fields in which no traps were placed. Since the trap with Methyl Eugenol had 3x more whiteflies, therefore Methyl Eugenol is highly effective in attracting whiteflies. The multiplex trap was found to be highly efficient as it worked well both during day and night. Since this trap can catch insects apart from whiteflies and is non-toxic, eco-friendly and non-hazardous this trap is better than conventional traps which employ a single insect trapping mechanism and can be employed as a trap in fields of other commercial crops like tomato, beans, cotton, cassava. Which are usually infected by begomovirus harboring vectors.

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