Integrated Approaches to Combat Cucurbit Mosaic Disease in *Cucumis sativus*: Insights from Assam

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ABSTRACT

Aims: This study was conducted to evaluate the efficacy of various integrated management strategies for management of cucurbit mosaic disease on cucumbers (*Cucumis sativus*). The aim was to study the effects of various treatments on disease incidence and vector population and promote sustainable farming through eco-friendly management approaches in an integrated manner.

Study design: Randomised Block Design with six treatments and one control with three replications

Place and Duration of Study: BiswanathChariali, Assam, India (2021-2022).

Methodology: Local cucumber variety "Ganga" was used to evaluate the treatments, including insect-proof seedling raising, yellow sticky traps, straw mulch, foliar sprays with neem formulation (Azadirachtin 0.03%), mineral oil, biopesticides (*Beauveria bassiana* and *Bacillus thuringiensis*), and a chemical insecticide (Imidacloprid). Disease incidence were recorded at intervals of 15 days from 30 to 75 days after transplanting. Statistical tests; *viz.,* t-tests, ANOVA, correlation analysis were performed to analyse the treatment effects.Correlation analysis was performed between disease incidence and yield.

Results: Treatment with neem formulation (Azadirachtin 0.03%) at 5 ml/L (T2) significantly reduced disease incidence (38.89%) and increased yield (32.83 q/ha), comparable to the insecticide treatment (Imidacloprid, T6), which had the lowest disease incidence (22.22%) and highest yield (38.55 q/ha).The disease incidence and aphid vector population were mild in treatment T2 and T6, while the control (T0) showed severe disease and high vector density. Correlation analysis revealed a significant negative relationship between disease incidence and yield (r = -0.969).

Conclusion: Neem formulation has shown high efficacy in managing cucurbit mosaic disease with less hazardous impact on the environment and can be used as an alternative to chemical pesticides. Integrated management strategies incorporating neem-based botanicals provide eco-friendly alternatives for disease management. Integrated management strategies were chosen over traditional methods because they combine biological, cultural, and chemical controls, reducing pesticide reliance, minimizing environmental impact, and promoting long-term sustainability.

Keywords: Cucurbit mosaic disease, integrated management, neem formulation, biopesticides, yield improvement.

1. INTRODUCTION

Cucumber mosaic virus (CMV), a common member of the genus Cucumovirus in the family Bromoviridae, infects a wide range of plants, including vegetables, pulses, ornamentals, medicinal plants, and weeds. Its broad host range including economically important crops indicate the importance of effective management strategies for the disease to minimize yield losses (Joshi *et al.* 2023). Cucurbit mosaic disease is one of the most devastating viral diseases severely infects cucurbits across the globe. Several viruses have been repoted accountable for this disease *i.e.* Cucumber Mosaic Virus (CMV), Watermelon Mosaic Virus (WMV), Zucchini Yellow Mosaic Virus (ZYMV), Pumpkin Yellow Vein Mosaic Virus (PYVMV), and Papaya Ringspot Virus-Watermelon Strain (PRSV-W) (Biswas and Ghosh 2018; Kumar *et al.* 2008). These viruses are commonly transmitted through aphid vectors such as *Aphis gossypii* and *Myzuspersicae* in a non persistent manner (Panno*et al.* 2021). Aphid feeding accelerates virus acquisition and transmission, affecting cucurbit crops and related weeds that act as virus reservoirs (Gilligan, 2007; Roy *et al*., 2023). A prolonged feeding period is not necessary for the aphid to acquire or spread the virus because it is frequently spread in a non-persistent way (McKirdy and Jones 1994). The green peach aphid (*Myzuspersicae*), cotton aphid (*Aphis gossypii*), and cowpea aphid (*Aphis craccivora*) are the primary aphid species involved. They are all known to colonize cucurbit crops and weeds that can act as virus reservoirs (Shi *et al.* 2016).

Common symptoms of Cucurbit mosaic disease has been reported as mosaic patterns of dark and light green to yellow, leaf distortion, yellow streaking/spots, and vein yellowing (Loebenstein and Lecoq 2012). Severely infected plants exhibit epinasty, reduced leaf size, and petiole/leaf surface bending (Zitter and Murphy 2009). Fruit symptoms include lumps, bumps, rings, and, if infected at pre-pollination stage; then the fruits show green/yellow blotches or stripes. Severe cases result in little to no fruit production, with deformities, discoloration, reduced size, yield, and potential fruit death (Anon 2021).

The cucurbit mosaic disease significantly reduces both the quality and quality of cucurbit yield. It has been reported that the yield loss due to cucumber mosaic virus (CMV) can be as high as 100 per cent (Khan e*t al.*2015). India contributed to about 5.6 per cent of the world's total vegetable production in the year 2019 (Brar *et al.* 2021). Major cucurbit growing states of India are Orissa, Assam, Rajasthan and Punjab (Anon 2020). Among the North-Eastern states of India, Assam is one of the major states growing cucurbit vegetables (Anon 2020).

There are reports of significant incidence of cucurbit mosaic disease in various cucurbit growing areas of Assam. In Assam, cucurbit mosaic disease in pumpkin crop was documented by Gogoi*et al.* (2023) with disease incidence of 35.71 from Jorhat district and 52.38 per cent from Golaghat district. Also,90.91 per cent incidence of cucurbit mosaic disease in Sonitpur district, 66.67 per cent in Biswanath district, 14.29 per cent in Jorhat district, 11.00 per cent in Sivasagar district and 25.00 per cent in Dibrugarh district on various cucurbit crops through molecular assay was reported by Dey *et al.* (2023). This necessitates studying the efficacy of various possible interventions to manage the disease in field condition with an integrated approach combining some eco- friendly strategies. Therefore, research was undertaken to address the expanding incidence of cucurbit mosaic disease in the farmers' fields of cucurbit growing areas of Assam. A comprehensive field experiment was conducted to examine eco-friendly and sustainable management practices such as nursery seedling raising in insect-proof condition, use of botanicals such as neem extracts, and biocontrol agents (*Beauveria bassiana* and *Metarhiziumanisopliae*), use of yellow sticky traps etc.

The findings from this study provided insights for the management of cucurbit mosaic disease through an integrated and eco- friendly approach. The integrated approach showed some sustainable management strategies which significantly reduced the yield losses due to cucurbit mosaic disease. The study helped in understanding the virus-vector-host dynamics with respect to various strategies which have less hazardous impacts on the ecosystem for sustainable disease management minimizing yield loss and severity of the disease.

2. MATERIAL AND METHODS

A field experiment comprised of seven treatments (including the control) with 3 replications was conducted to formulate an integrated management strategy for cucurbit mosaic disease in the experimental field of Biswanath College of Agriculture, BiswanathChariali using a local cucumber variety of Assam named "Ganga" having crop duration of 90-120 days and a potential yield of 50-60 q/ha.

Nursery treatment (TA)= Cucumber seeds were germinated and seedlings were grown in polythene bags within an insect-proof net house equipped with a 40-mesh net for protection. Seedlings were transplanted at 2-3 leaf stage in the main field.

The field experiment was comprised of the following treatment combinations:

1. T_0=Control
2. T_1= TA + Yellow sticky trap + Straw mulch
3. T_2= T_1 + Spraying of neem formulation (Azadirachtin 0.03%) @ 5 ml/L at 30, 45, 60 and 75 days after transplanting
4. T_3=T_1+ Application of mineral oil @ 5ml/L at 30,45, 60 and 75 days after transplanting
5. T_4= T_1 + Foliar spraying with Bio-sona (Bio formulation with *Beauveria bassiana*) @ 20 ml/L at 30,45, 60 and 75 days after planting
6. T_5 = T_1 + Spraying of Bio-Bt (Bio formulation with *Bacillus thuringiensis*) @ 20 ml/L at 30, 45, 60 and 75 days after transplanting

7. $T_6 = T_1$ + Foliar spraying with Imidacloprid @0.2ml/L at 30, 45, 60 and 75 days after planting

In the experiment, a commercial neem formulation, “Nimbecidine” with 0.03 per centAzadiractin was used. The bio pesticides *viz.;* Biosona and Bio- Bt standardized by Department of Plant Pathology, AAU, Jorhat were used for spraying. The commercial Imidacloprid insecticide “Dzire” containing 70 per cent imidacloprid was used as chemical check.

Plants were examined on a routine basis for appearance of any symptoms of mosaic disease. The disease incidence was noted every 15 days between 30 and 75 days after planting. Disease incidence was calculated using the following formula:

Per cent incidence of cucurbit mosaic disease = (Number of infected plants/Total number of plants observed) x 100.

Based on per cent disease incidence, severity of the cucurbit mosaic disease was classified as mild (less than 50% disease incidence), moderate (50-75% disease incidence) and severe (more than 75% disease incidence) for different locations. (Dey *et al.* 2023).

For each plot, the vector population count was determined by randomly selecting five plants from each plot.Three leaves were chosen from each identified plants; one leaf each from the top, middle, and bottom portion of the plant. The average vector count was then calculated across three replications for each treatment.

Data on fruit yield were also recorded for each treatment and further correlated with disease incidence

3. RESULTS AND DISCUSSION

Characteristic symptoms of cucurbit mosaic disease were observed in the experimental plot (Fig 1). Early symptoms included light and dark green mosaic patterns on the leaves and upward curling of the leaf edges. There was reduction in size of the leaves of infected plants, resulting in small, crinkled, and abnormal leaves. The infected plants developed chlorosis, deformed leaves, and unmarketable fruits as the disease progressed. Older leaves on infected plants showed vein banding and yellow spots. Vein clearing and leaf yellowing were also prominent in severely infected plants.

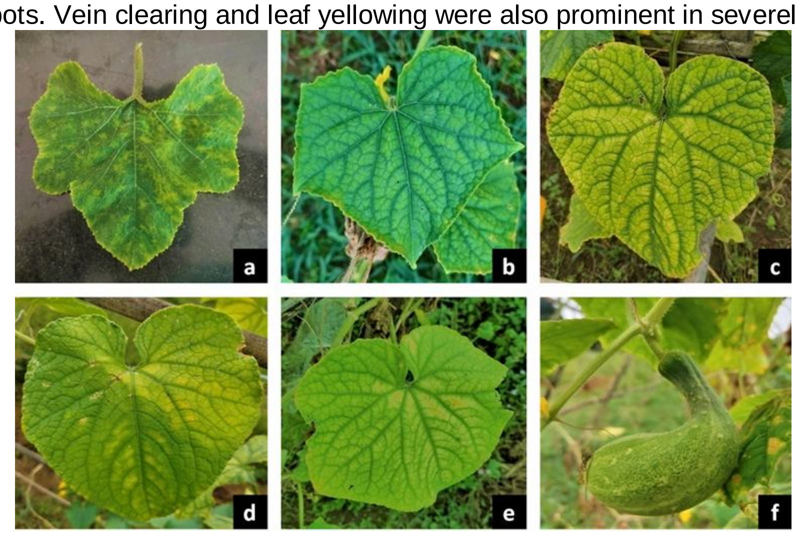

Fig 1.Differentsymptoms observed of cucumber mosaic disease in the experimental research plot. a= mosaic pattern on leaf. b= upward curling of leaf margins. c=vein banding. d= leaf chlorosis. e= deformed leaf. f= deformed fruit

There was comparatively low disease incidence (38.89%) in the treatment no.3 ($T_2 = T_1$ + Foliar spraying with Azadirachtin 0.03% at 30, 45, 60 and 75 days after planting) than the other treatments and this was effective in reducing disease incidence similarly as that of the insecticide check; *i.e.,* treatment no.7 ($T_6 = T_1$ + Foliar spraying with Imidacloprid @0.2 ml/L at 30, 45, 60 and 75 days after planting) which showed the lowest disease incidence of 22.22 per centthroughout the cropping period. In case of treatments; the treatment no.3 ($T_2 = T_1$ + Spraying of neem formulation (Azadirachtin 0.03%@ 5 ml/L at 30, 45, 60 and 75 days after transplanting) was followed by the treatment no.5 ($T_4 = T_1$ + Foliar spraying with Bio-sona (*Beauveria bassiana*at 30, 45, 60 and 75 days after planting) with 55.56 per centdisease incidence (Table I). Highest yield of 38.55 q/ha (8 no. of fruits per bed) was obtained from the treatment no.7 (T_6) (insecticide check). Amongst various treatments, higher yield was obtained from the treatment no.3 (T_2) (32.83 q/ha)**.** Table II shows effects of different treatments on cucumber yield (75 days after planting).

Table I Effect of different treatments on cucurbit mosaic disease incidence

Treatment No.	Treatments	Disease incidence through visual observation at different intervals after planting (%)			
		30 DAP	45 DAP	60 DAP	75 DAP

1	T_0	12.57 (19.20)	45.36 (42.29)	83.60656 (12.88)	88.89 (13.34)
2	T_1	7.10 (13.28)	34.43 (35.93)	72.6776 (12.00)	83.33 (12.88)
3	T_2	1.64 (7.36)	1.64 (7.36)	23.49727 (6.54)	38.89 (8.75)
4	T_3	12.57 (19.20)	23.50 (28.73)	56.28415 (10.50)	66.67 (11.48)
5	T_4	1.64 (7.36)	7.10 (13.28)	34.42623 (8.13)	55.56 (10.50)
6	T_5	1.64 (7.36)	18.03 (25.13)	50.81967 (9.88)	61.11 (11.02)
7	T_6	1.64 (7.36)	1.64 (7.36)	18.03279 (5.74)	22.22 (6.54)
SEd		5.781349	3.625833	5.12	7.44
CD(P=0.05)		NS	7.90	1.28	1.45
CV		61.11	19.42	7.65	7.64

*There was no disease development up to 30 days after planting.
Data are sum of three replications. Data within parentheses are angular transformed values

Table II Effect of different treatments on yield

Treatment No.	Treatments	No. of cucumber per plant	Weight of cucumbers (g) per plant	Yield (q/ha)
1	T_0	1	170	3.09
2	T_1	3	182	9.93
3	T_2	7	258	32.83
4	T_3	4	190	13.82
5	T_4	6	247	26.95
6	T_5	5	220	20.00
7	T_6	8	265	38.55
SEd				0.60
CD (P=0.05)				1.317
CV				15.236

Severe symptoms and highest vector population were observed in treatment no.1 (Control, T_0) and treatment no. 2 (T_1= TA + Yellow sticky trap + Straw mulch). Mild symptoms with lowest vector population were observed in treatment no.3 (T_2= T_1 + Spraying of neem formulation (Azadirachtin 0.03%) @ 5 ml/L at 30, 45, 60 and 75 days after transplanting) along with treatment no.7 (T_6 = T_1 + Foliar spraying with Imidacloprid @0.2ml/L at 30, 45, 60 and 75 days after planting.)

Table III Severity of cucurbit mosaic symptoms and vector population count in different treatment combinations

Treatment No.	Treatment	Symptoms observed in the field	Vectors
1	T_0	+++	***
2	T_1	+++	***
3	T_2	+	*
4	T_3	++	**
5	T_4	++	**
6	T_5	++	**
7	T_6	+	*

(+) = Mild (less than 50%), (++) = Moderate (50-75%), (+++) = Severe (75% and more)
(*) = Low (Less than 50%), (**) = Medium (50-75%), (***) = High (75% and more)

The correlation analysis showed that the yield of cucumber was negatively correlated with cucurbit mosaic disease incidence with coefficients of correlation for disease incidence and yield was -0.969 (**Significance at 1%probability level). Hence, it was evident that with increase in cucurbit mosaic disease incidence there was reduction in yield of cucumber (Fig 2).

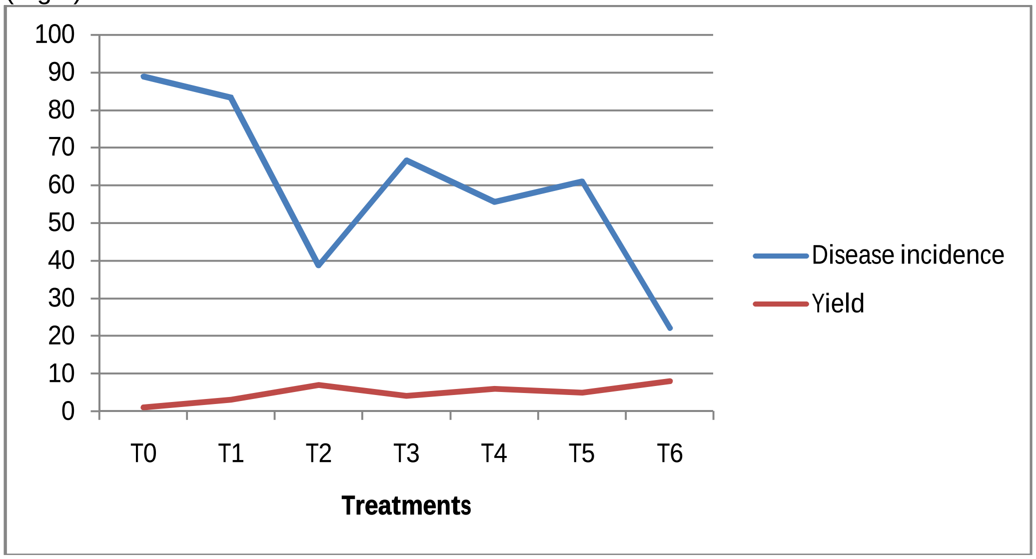

Fig 2. Correlation of disease incidence with yield

Discussion: The integrated management module showed some effective strategies for management of cucurbit mosaic disease. The neem formulation, could give effective results with low disease incidence with low vector population and it is having minimum environmental residual effect, thus; making it a safer option than chemical pesticides. This combined with early detection and routine field inspections are therefore crucial for effective disease management. The use of botanicals like neem formulations as well as entomopathogenic biopesticides, showed good results in management of the cucurbit mosaic disease; hence could be suggested as effective strategies for the same.

The complex interactions between the virus, vector, and host plants frequently make it difficult to design efficient management strategies. Identification of the causal agent and application of different control measures under integrated approach has been one of the important components of viral disease management. Under field conditions, monitoring and trapping of insect vectors can help to reduce the spread of the disease. The use of a systemic insecticide may also aid in aphid population reduction (Jam *et al.* 2014; Daunde*et al.* 2020). However, chemical insecticides are not considered as a long-term strategy for controlling CMV, as these can harm beneficial insects in addition to the targeted pest (Wang and Uchida 2014).

Insecticide use on a regular basis can also result in the development of insecticide-resistant vectors. Furthermore, insecticides are expensive and contribute to environmental imbalance. Neem based formulations have been shown to be highly effective in controlling aphid populations in cucurbits (Sharma *et al.* 2017). Biopesticides can also be used as an alternative management strategy of chemical treatments.

4. CONCLUSION

Among all the treatments, T_6 (foliar spraying with imidacloprid) was the most effective treatment which showed the highest yield (38.55 q/ha) and the lowest disease incidence (22.22%). Promising results were also demonstrated by T_2 (spraying of neem formulation), which was an environmentally safer alternative than the chemical pesticide with a high yield (32.83 q/ha) and low disease incidence (38.89%). The study highlighted the potential of neem-based formulations and biopesticides as sustainable approaches for management cucurbit mosaic disease. Combining with regular monitoring, these integrated approaches can be cited as environment friendly and sustainable strategies for the long run reducing the requirement for chemical pesticides.

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COMPETING INTERESTS

The authors declared that no conflicts of interest.

AUTHORS' CONTRIBUTIONS

'Author 1' designed the manuscript, performed the statistical analysis and wrote the first draft of the manuscript. 'Author 2' and 'Author 3' guided for the statistical analyses of the study. 'Author 4' guided to conduct the research as major advisor and guided during writing of the manuscript. All authors read and approved the final manuscript.

CONSENT (WHEREEVER APPLICABLE)

Consent from all the authors were taken before submitting this manuscript.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

This manuscript is ethically approved by all the authors.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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