Research Article

Biotic Stress Response of Local Host on Cassava Mosaic Virus and its Control with selected Essential Oils: An Experimental Approach

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ABSTRACT

Aim: The present investigation was done to study the antiviral property of essential oils collected from *Cymbopogan flexuosus, Ocimum tenuiflorum* and *Piper nigrum*, on local lesion host *Gomphrena globosa*, which was mechanically infected with Cassava Mosaic Virus (CMV). **Materials and Methods:** Isolation of essential oil from the aerial parts of *Cymbopogan flexuosus, Ocimum tenuiflorum* and *Piper nigrum* was done using GC/MS analysis. The pathogenicity assay was done with the viral inoculum prepared from *Mannihot esculenta* leaves and was applied to local host, *Gomphrena globosa* at varying concentrations (100-500 ppm). Further, different biochemical assays like estimation of photosynthetic pigments, flavonoid, proline, MDA and aldehyde were conducted to find out biotic response of local lesion host. **Results:** It was observed that Concentrated Viral inoculum (CMV-C) was effective in infecting the local lesion host. The local lesion host plant showed many morphological changes like presence of local necrotic lesions, yellowing of leaf and early defoliation of leaves. The yield of the essential oils was 1.4% from *C. flexuosus*, 0.8% from *O. tenuiflorum* and 0.6% from *P. nigrum* dried samples. The most effective oil reducing local lesion number was the oil isolated from *C. flexuosus* (97.2%), followed by *O. tenuiflorum* (94.4%) and *P. nigrum* (91.6 %). Probit analysis data revealed that LC_{50} value was minimum (133.116 ppm) in *C. flexuosus* oil where as it was maximum (172 ppm) in *P. nigrum* oil. From the result it was observed that inhibitory effect of essential oil was dose dependent. Dominant component (>60%) present in *O. tenuiflorum* and *P. nigrum* oil was sesquiterpenes. From the three essential oil tested, *C. flexuosus* oil showed high inhibition activity. **Conclusion:** Thus, it could be concluded that essential oil from the tested plants can be used to control CMV infection in controlled condition

Keywords: *Cassava mosaic virus, Pathogenicity, Essential oils, Biotic stress*.

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INTRODUCTION

Plant viruses are a threat to crops in agriculture and horticulture round the World. However, very less chemical agents viz., fungicides and bactericides are effective against plant viruses. Cassava (*Manihot esculenta Crantz*) of the family Euphorbiaceae is one of the most consumed food crop for its starch containing tubers, fed over 500 million people worldwide.[1] The major difficulty in the production of Cassava in developing countries[2] is the Cassava Mosaic Disease (CMD) caused by viruses in the genus *Begomovirus* (f*amily Geminiviridae*). These are often transmitted from one plant to another by *Bemisia tabaci* Gennadius. The begomoviruses infecting cassava consist of genomes with single stranded DNA components (bipartite), represented as DNA A and B. DNA A codes for functions replication and encapsidation of virus and DNA B encodes the movement of viruses.[3] The very first visible symptom of CMD is the appearance of leaf mosaic. The leaves infected with CMV has pale green or yellow areas, commonly small and distorted. The size of tubers and stem are reduced in overall size and number,

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thus affecting yield. Even though different strains of the virus are recognized,[4] they are not important for its control in field.

Till date, no effective chemical methods to inhibit the replication as well as the multiplication of viruses in host plants is unavailable. In such a scenario, natural substances could be developed as agents that can alter the replication of viruses. Studies have proved that metabolites like steroids, quassinoids and alkaloids have shown to exhibit anti-TMV activity.[5] It has also been identified that, essential oils could control viral infection in plants.[6] There exists only limited knowledge in the antiviral effect of essential oil and hence this has to be explored to reduce the spread of viral infections. Essential oils are secondary metabolites of plants, stored in the flowers, leaves, fruits, roots, rhizomes and bark.[7] These are erratic blends of terpenoids, mainly sesquiterpenes and monoterpenes, however diterpenes might also be included. Many researches have demonstrated that essential oils are essential for the growth and development of plants.[8] Apart from this, they also show resistance against diseases and insects.[9] Research has demonstrated that essential oils and their constituents possess antiviral, antimycotic, antioxidative, antiparasitic and insecticidal characteristics.[10] Research findings has shown that, essential oil isolated from *Teucrium* species have reduced the number of lesions in *Chenopodium* affected with Cucumber Mosaic Virus.[11,12]

Hence, an attempt to isolate an active essential oil from selected plants for the control of Cassava Mosaic Virus (CMV) is much needed. Thus, the aim of this investigation was to study the effect of essential oils isolated from *Cymbopogon flexuosus* (Nees ex Steud.) Wats., *Ocimum tenuiflorum* L., *Piper nigrum* L., on cassava mosaic virus infected local lesion host plant and thereafter determine the compound present in the essential oil, responsible for the inhibition of virus multiplication.

MATERIALS AND METHODS

Collection of plant materials

Cassava Mosaic Virus (CMV) infected on leaves of *Manihot esculenta* Crantz (Cassava) were used as systemic host and was confirmed by consulting with Dr. C. Mohan, Principal Scientist, Central Tuber Crop Research Institute, Sreekaryam, Thiruvananthapuram. Healthy plants of *Gomphrena globosa* L., grown at a height of 15 cm was used as local lesion host and was maintained in green house, Department of Botany, University of Kerala. For essential oil analysis, aerial parts of *Cymbopogon flexuosus*, *Oscimum tenuiflorum* and *Piper nigrum* were collected from the Department of Botany, University of Kerala.

Isolation of essential oil and its GC-MS analysis

About 50 g leaves of *C. flexuosus*, *O.tenuiflorum* and *P.nigrum* were taken for the present study.The samples were shade dried for 48 hr and used for extraction. Samples were transferred for 3-4 hr in a Clevenger apparatus. The obtained oil was dried and stored under refrigeration (4ºC). The percentage yield of the oil was also noted. GC analysis was performed by using Trace GC Ultra Thermo scientific. The individual peaks were located by comparing their mass spectral databases from NIST02 (Gaithersburg, MD, USA) and literature, as well as by comparing their retention indices to those from the library. Using the normalisation method, the component percentages were computed as mean values from the GC and GC-MS peak areas.

Preparation of viral inoculum for pathogenicity assay

About 5 g of Cassava leaf with visible CMV symptom was grounded in 50 mL of distilled water using pestle and mortar. Through sterile muslin cloth, the leaf debris and sap were squeezed apart. To infect *Gomphrena globosa*, the filtrate was produced in concentrated and diluted solutions (to a 25% concentration). A small number of plants were also maintained as a control and notes were made.

Preparation and application of essential oil solution to local lesion host

Essential oils were dissolved in Tween 20 at a ratio of 1:10. A series of concentrations ranging from 100 to 500 ppm was prepared for treatment. Test solutions were prepared by adding essential oil (100 ppm to 500 ppm) to virus inoculum. Standardised dose of virus inoculum was added to each sample of essential oil (50 mL) to produce required amount of test solutions. Test solution was sprayed to *G. globosa* leaves to find out the activity of essential oil on CMV. Three control plants maintained for comparative study were normal plant without infection (control), essential oil sprayed plants (control EO) and virus inoculated plants (control CMV). All treatments were repeated thrice and observations were taken at regular intervals. By comparing the amount of viral lesions on the treated and control groups using the formula, localised lesions were tallied and the inhibition percentage was computed.

Biochemical parameters for the study

Biochemical analysis like estimation of photosynthetic pigments (chlorophyll and carotenoids), flavonoid, proline, MDA and aldehyde were done on control as well

as treated plants of *G. globosa* based on standard protocols.[13-15] The test groups were divided as control (C), viral inoculum treated plant (V), plant treated with Essential Oil (EO), plant treated with 100 ppm essential oil and viral inoculum (V+EO-100) and plant treated with 500 ppm essential oil and viral inoculum (V+EO-500).

Statistical Analysis

IBM SPSS Statistics was used to statistically estimate the significance of the difference between the treatment and control mean values (Version 22.0).

RESULTS

Collection of plant materials

The different plant materials for the present study were collected as shown in Figures 1 A to F. The local lesion host plant showed many morphological changes like presence of local necrotic lesions, yellowing of leaf and early defoliation of leaves.

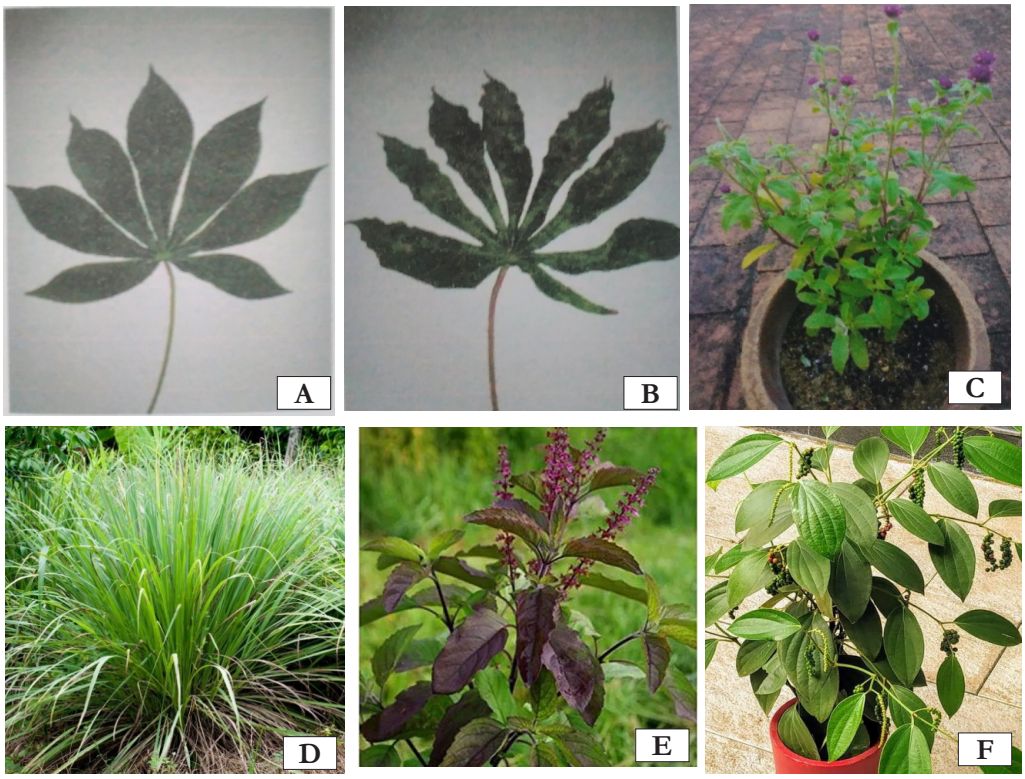

Figure 1 A:
Uninfected leaf; B: Leaf infected with CMV; C: *Gomphrena globosa*; D: *Cymbopogon flexuosus*; E: *Oscimum tenuiflorum*; F: *Piper nigrum*.

Table 1: Yield and characteristics of essential oil of plants studied

Plant	Experimental conditions					
	Percentage yield		Colour		Odour	
	Fresh	Stored (48 hr)	Fresh	Stored (48 hr)	Fresh	Stored (48 hr)
Cymbopogan flexuosus	0.8	1.4	Dark yellow-amber	Dark yellow-amber	Lemony sweet	Lemony sweet
Ocimum tenuiflorum	0.6	0.8	Pale yellow	Pale yellow	Balsamic	Balsamic
Piper nigrum	0.4	0.6	Light amber-yellow green	Light amber-yellow green	Spicy	Spicy

Chemical characterisation of essential oil *C. flexuosus*, revealed that endo-1,5,6,7-tetramethyl bicyclo (3,2,0) hept-6-en-3-ol has maximum abundance with a retention time 17.07 similarly in *O. tenuiflorum* and *Piper nigrum* 3-ethyl-6-methoxyphenol with retention time 20.48 and Bicyclo[5,3,0] decan, 2-methylene-5-1-methylvinyl-8-methyl with retention time 25.33 respectively showed maximum abundance. The percentage of components present in the oil and their retention time is given in Table 2a, b, c.

Table 2: GC-MS analysis of essential oil.
Table 2a: Identified major components of *C. flexuosus* oil

Sl. No.	Retention time	Components	Percentage
1	16.24	Phenyl thio aectic acid 2,7-dime thylol-7en-5yn-4yl ester	3.07

2	17.07	Endo-1,5,6,7-tetramethyl bicyclo (3,2,0) hept-6-en-3-ol	34.37
3	18.04	Myrtenol	11.25
4	18.96	1-methyl verbenol	23.40
5	19.55	Ethanol, 2-(3,3-dimethyl bicyclo (2,2,1) hept-2-yildene	10.37
6	19.73	2-pinene-10-ol	1.77
7	24.44	Isoledene	7.70
8	26.29	Caryophyllene oxide	4.14
9	26.91	Cubenol	0.59

Table 2b: Identified major components of *O. tenuiflorum* oil

Sl. No.	Retention time	Components	Percentage
1	20.48	3-ethyl-6-methoxyphenol	60.39
2	21.29	Guain-(5),11-diene	21.72
3	22.17	Caryophyllene	16.6
4	24.51	Isoledene	1.19

Table 2c: Identified major components of *P. nigrum* oil

Sl. No.	Retention time	Components	Percentage
1	20.67	(-)Cyclosalivni	3.26
2	22.04	Humulen-(v1)	6.52
3	23.62	(+)Epibicycloses-quiphellandrene	3.26
4	25.33	Bicycle [5,3,0] decane, 2-methylene-5-(1-methylninyl)-8-methyl	63.04
5	28.42	Longilinene	23.91

Pathogenicity assay

The pathogenicity of the virus inoculum on the local lesion host showed that the concentrated samples (CMV-C) were more effective than diluted sample (CMV-D) and hence for further studies, CMV-C was used. Local necrotic lesions appeared in G. globosa plants were studied and it is shown in the Figure 2 A, B.

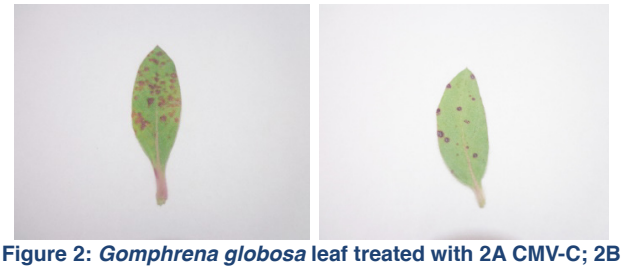

Figure 2: *Gomphrena globosa* leaf treated with 2A CMV-C; 2B CMV-D.

Morphological observation in the local lesion host

Prominent morphological changes were observed in the local lesion host after inoculation. Green leaves changes to yellow, early defoliation on the leaves and presence of local necrotic lesion were studied and it is shown in Figure 3A, B.

Figure 3 A: Control plant; B: Virus affected plant.

Table 3: Effect of essential oil on CMV infectivity

Sl. No.	Dose of essential oil in ppm	Inhibition percentage of different essential oil treatments		
		EOC	EOO	EOP
1	0	0	0	0
2	100	38.8	33.3	27.7
3	200	66.6	58.3	58.3
4	300	77.7	75.0	72.2
5	400	83.3	80.5	77.7
6	500	91.6	88.8	88.8

EOC: Essential Oil Cymbopogon; **EOO:** Essential Oil Oscimum; **EOP:** Essential Oil Piper.

Anti-viral effect of essential oil

Anti-viral effect of three essential oils obtained from *C. flexuosus*, *O. tenuiflorum* and *P. nigrum* is detailed in Table 3 and the probit analysis data is given in Table 4.

Table 4: Probit analysis data of essential oils stored in different durations.

Sl. No.	Samples	LC_{50} (ppm)	LC_{90} (ppm)
1	EOC-2D	96.72 (130.819-52.807)	358.668 (564.04-278.385)
2	EOC-30D	133.116 (170.047-86.669)	501.591 (855.516-378.535)
3	EOO-2D	102.245 (139.345-54.067)	426.361 (743.878-320.967)
4	EOO-30D	156.929 (194.968-111.146)	569.380 (984.152-426.224)
5	EOP-2D	149.693 (185.144-106.927)	503.971 (805.840-388.099)
6	EOP-30D	172.000 (209.821-128.265)	582.027 (970.209-440.536)

Biochemical assay

In biochemical assay the presence of substances like flavonoid, proline, chlorophyll a, chlorophyll b, Total Chlorophyll, carotenoid, MDA and aldehyde were detected in both control and treated plants. It was observed that the amount of flavonoid, proline, carotenoid, MDA and aldehyde increased considerably in the plants treated with viral inoculum and decreased in plants treated with oil and the viral inoculum at different concentrations. The oils were treated in two stages viz., storing for 2 day and 30 days after. The results obtained for *Cymbopogon flexuosus*, *Ocimum tenuiflorum*, *Piper nigrum* oil treated after storing for 2 days are documented in Tables 5, 6 and 7 respectively. The results obtained for *Cymbopogon flexuosus*, *Ocimum tenuiflorum*, *Piper nigrum* oil treated after storing for 30 days are documented in Tables 8, 9 and 10 respectively.

Table 5: Effect of *C. flexuosus* oil on Cassava Mosaic virus.
Table 5 a: Oil stored for 2 days

Sl. No	Parameter	Experimental conditions				
		C	V	EO-500	V+EO-100	V+EO-500
1	Flavonoid (g/mL)	0.48± 0.007^{e}	2.50± 0.007^{a}	1.50± 0.007^{b}	1.31± 0.012^{c}	1.21± 0.010^{d}
2	Proline (mg/mL)	3.27± 0.005^{d}	7.49± 0.009^{a}	4.38± 0.009^{b}	3.46± 0.007^{c}	3.27± 0.008^{d}
3	Chlorophylla (mg/g)	1.01± 0.005^{a}	0.24± 0.005^{e}	0.65± 0.007^{b}	0.42± 0.005^{c}	0.64± 0.007^{b}
4	Chlorophyllb (mg/g)	0.96± 0.007^{a}	0.07± 0.005^{e}	0.37± 0.005^{b}	0.32± 0.007^{c}	0.36± 0.005^{b}
5	Total chlorophyll (mg/g)	2.06± 0.598^{a}	0.53± 0.007^{b}	1.01± 0.005^{b}	0.75± 0.005^{b}	0.83± 0.007b
6	Carotenoid (mg/g)	0.24± 0.007^{e}	1.06± 0.007^{a}	0.58± 0.005^{b}	0.51± 0.005^{c}	0.27± 0.007^{d}
7	MDA(n mol)	0.01± 0.0005^{e}	0.08 ± 0.0007^{a}	0.06 ± 0.0007^{b}	0.03± 0.0006^{c}	0.01 ± 0.0005^{d}
8	Aldehyde(n mol)	0.15± 0.0005^{e}	0.55 ± 0.0005^{a}	0.27 ± 0.0008^{b}	0.19± 0.0005^{c}	0.15 ± 0.0007^{d}

Table 5b: Oil stored for 30 days.

Sl. No	Parameter	Experimental conditions				
		C	V	EO-500	V+EO-100	V+EO-500
1	Flavonoid (g/mL)	0.48± 0.007^{e}	2.50± 0.007^{a}	1.26± 0.007^{d}	1.75± 0.007^{b}	1.65± 0.007^{c}
2	Proline(mg/mL)	3.27± 0.005^{d}	7.49± 0.009^{a}	3.46± 0.008^{d}	5.85± 0.007^{b}	4.75± 0.008^{c}
3	Chlorophylla (mg/g)	1.01± 0.005^{a}	0.24± 0.005^{e}	0.87± 0.006^{b}	0.38± 0.007^{d}	0.63± 0.010^{c}
4	Chlorophyllb (mg/g)	0.96± 0.007^{a}	0.07± 0.005^{e}	0.48± 0.005^{b}	0.25± 0.005^{d}	0.31± 0.005^{c}
5	Total chlorophyll (mg/g)	2.06± 0.598^{a}	0.53± 0.007^{c}	1.15± 0.006^{b}	0.47± 0.007^{e}	0.50± 0.007^{d}
6	Carotenoid (mg/g)	0.24± 0.007^{e}	1.06± 0.007^{a}	0.28± 0.007^{d}	0.75± 0.008^{b}	0.60± 0.005^{c}
7	MDA(n mol)	0.01± 0.0005^{e}	0.08 ± 0.0007^{a}	0.03 ± 0.0007^{d}	0.07± 0.0007^{b}	0.07± 0.0007^{c}
8	Aldehyde (n mol)	0.15± 0.0005^{e}	0.55± 0.0005^{a}	0.19± 0.0007^{d}	0.35± 0.0010^{b}	0.29± 0.0007^{c}

Table 6: Effect of *O. tenuiflorum* oil on Cassava Mosaic virus.
Table 6 a: Oil stored for 2 days.

Sl. No	Parameter	Experimental conditions				
		C	V	EO-500	V+EO-100	V+EO-500
1	Flavonoid (g/mL)	0.48± 0.007^{e}	2.50± 0.007^{a}	1.50± 0.007^{b}	1.33± 0.008^{c}	1.26± 0.007^{d}
2	Proline (mg/mL)	3.27± 0.005^{d}	7.49± 0.009^{a}	4.17± 0.008^{b}	3.77± 0.007^{c}	3.27± 0.009^{d}
3	Chlorophylla (mg/g)	1.01± 0.005^{a}	0.24± 0.005^{e}	0.79± 0.005^{b}	0.36± 0.005^{d}	0.53± 0.005^{e}
4	Chlorophyllb (mg/g)	0.96± 0.007^{a}	0.07± 0.005^{e}	0.42± 0.005^{b}	0.19± 0.005^{d}	0.26± 0.005^{c}
5	Total chlorophyll (mg/g)	2.06± 0.598^{a}	0.53± 0.007^{b}	1.07± 0.005^{b}	0.63± 0.005^{b}	0.70± 0.005^{b}
6	Carotenoid (mg/g)	0.24± 0.007^{e}	1.06± 0.007^{a}	0.53± 0.007^{b}	0.46± 0.005^{c}	0.21± 0.005^{d}
7	MDA (n mol)	0.01± 0.0005^{e}	0.08± 0.0007^{a}	0.05± 0.0007^{b}	0.03± 0.0005^{c}	0.02± 0.0007^{d}
8	Aldehyde (n mol)	0.15± 0.0005^{e}	0.55 ± 0.0005^{a}	0.25 ± 0.0005^{b}	0.22± 0.0008^{c}	0.15 ± 0.0008^{d}

Table 7: Effect of *Piper nigrum* oil on Cassava Mosaic virus.
Table 7a: Oil stored for 2 days.

Sl. No	Parameter	Experimental conditions				
		C	V	EO-500	V+EO-100	V+EO-500
1	Flavonoid (g/mL)	0.48± 0.007^{e}	2.50± 0.007^{a}	1.15± 0.007^{d}	1.88± 0.007^{b}	1.67± 0.007^{c}
2	Proline (mg/mL)	3.27± 0.005^{d}	7.49± 0.009^{a}	3.27± 0.006^{d}	6.14± 0.009^{b}	4.85± 0.008^{c}
3	Chlorophylla (mg/g)	1.01± 0.005^{a}	0.24± 0.005^{e}	0.91± 0.005^{b}	0.35± 0.005^{d}	0.52± 0.005^{c}
4	Chlorophyllb (mg/g)	0.96± 0.007^{a}	0.07± 0.005^{e}	0.53± 0.005^{b}	0.17± 0.006^{d}	0.21± 0.005^{c}
5	Total chlorophyll (mg/g)	2.06± 0.598^{a}	0.53± 0.007^{c}	1.23± 0.006^{b}	0.42± 0.007^{e}	0.48± 0.007^{d}
6	Carotenoid (mg/g)	0.24± 0.007^{e}	1.06± 0.007^{a}	0.25± 0.005^{d}	0.89± 0.005^{b}	0.63± 0.007^{c}
7	MDA(n mol)	0.01± 0.0005^{e}	0.08± 0.0007^{a}	0.02± 0.0005^{d}	0.08± 0.0007^{b}	0.07± 0.0007^{c}
8	Aldehyde (n mol)	0.15± 0.0005^{e}	0.55 ± 0.0005^{a}	0.17 ± 0.0005^{d}	0.45± 0.0007^{b}	0.30 ± 0.0007^{c}

Table 7b: Oil stored for 30 days.

Sl. No	Parameter	Experimental conditions				
		C	V	EO-500	V+EO-100	V+EO-500
1	Flavonoid (g/mL)	0.48± 0.007^{e}	2.50± 0.007^{a}	1.09± 0.007^{d}	2.08± 0.008^{b}	1.70± 0.007^{c}
2	Proline (mg/mL)	3.27± 0.005^{d}	7.49± 0.009^{a}	3.25± 0.007^{e}	6.65± 0.007^{b}	5.31± 0.008^{c}
3	Chlorophylla (mg/g)	1.01± 0.005^{a}	0.24± 0.005^{e}	0.96± 0.008^{b}	0.28± 0.016^{d}	0.36± 0.005^{c}
4	Chlorophyllb (mg/g)	0.96± 0.007^{a}	0.07± 0.005^{e}	0.73± 0.005^{b}	0.13± 0.005^{d}	0.16± 0.005^{c}
5	Total chlorophyll (mg/g)	2.06± 0.598^{a}	0.53± 0.007^{c}	1.33± 0.007^{b}	0.37± 0.007^{e}	0.42± 0.008^{d}
6	Carotenoid (mg/g)	0.24± 0.007^{e}	1.06± 0.007^{a}	0.12± 0.007^{d}	0.77± 0.005^{b}	0.69± 0.005^{c}
7	MDA(n mol)	0.01± 0.0005^{e}	0.08± 0.0007^{a}	0.02± 0.0007^{d}	0.08± 0.0007^{b}	0.07± 0.0007^{c}
8	Aldehyde (n mol)	0.15± 0.0005^{e}	0.55± 0.0005^{a}	0.17± 0.0007^{d}	0.45± 0.0007^{b}	0.34± 0.0007^{c}

DISCUSSION

The observed biotic stress response of the local cassava host to CMV corroborates previous studies, confirming the detrimental impact of the virus on plant health. Symptoms such as mosaic patterns, leaf distortion and stunted growth were evident, indicating a compromised physiological state. Our findings align with the general understanding that CMV induces alterations in gene expression and physiological processes in the host. The upregulation of certain stress-responsive genes and the activation of defense mechanisms were consistent with the plant's attempt to counteract the viral invasion.

Essential oils are chief elements in controlling viral infections. In the present study, anti-viral activity of essential oils from *Cymbopogan flexuosus*, *Ocimum tenuiflorum* and *Piper nigrum*, on local lesion host *Gomphrena globosa*, mechanically infected with Cassava Mosaic Virus (CMV), was studied and compared. Results obtained from the standardization protocol revealed that *G. globosa* plants are suitable for local lesion assay of CMV. Present study also revealed that mechanical inoculation of concentrated incula offers better result. Little and no attention has been devoted to the problem of improving the usefulness of local-lesion assays, even though some viruses lack a known local lesion host. Consequently local-lesion assay method is capable of providing data on a large number of infection sites with use of few test plants. Holm, *et al* (1997)[16] first reported the local lesion assay for Tobacco Mosaic Virus (TMV). Currently there are very few reports about local lesion assays for CMV. *G. globosa* is one of the known local lesion host of many mosaic viruses[17] Therefore, for the present study this host was tested for Cassava mosaic virus and confirmed that *G. globosa* prevents systemic infection of virus and promote localized growth around the infected area. This is the first report of Cassava Mosaic Virus local lesion assay. Morphological changes observed during infection in *G. globosa* plant suggests that the infection is related to CMV. Studies suggested that mechanical transmission of Sri Lankan cassava mosaic virus (SLCMV) on *Nicotiana benthamiana* exhibited severe downward leaf curl, chlorosis and stunted growth.[18] Indian Cassava Mosaic Virus (ICMV) infected plants showed downward curling of upper leaves. Symptom expression can range from the mild symptoms to severe symptoms (foliar distortion, stunting and defoliation) depending on the varietal response, age of the plant at infection and virus strain.[19] In the present study symptoms such as necrotic lesions, foliar distortion, defoliation, chlorosis and downward leaf curl were noticed in the local lesion host mechanically infected with CMV. The antiviral effect of the *Thymus serpyllum* and *Lavandula officinalis* essential oils, on the treated *Nicotiana tabacum* plants with Potato virus Y infection.[20] Research works suggest that the essential oils of three genera of *Lamiaceae*, *Satureja*, *Teucrium* and *Micromeria* reduced the number of local lesions on both Tobacco Mosaic Virus and Cucumber mosaic virus infected plants of local hosts *Chenopodium amaranticolor* Coste and Reyn. and *Chenopodium quinoa* Willd.[21] There are no reports about the anti-phytoviral activity of essential oil isolated from *Cymbopogan flexuosus*, *Ocimum tenuiflorum* and *Piper nigrum* against CMV. In the present study essential oils isolated from aerial parts of *C. flexuosus*, *O. tenuiflorum* and *P. nigrum* was analysed by GC/MS and 18 different compounds were identified. Experiments conducted with fresh samples was also shown that *C. flexuosus* yield more oil compared to others. The main constituents of the investigated essential oils are monoterpene and sesquiterpene hydrocarbons. A comparison of the mean number of lesions on the oil treated *G. globosa* plants with the corresponding control showed that essential oils isolated from the investigated species significantly reduced CMV infections. The most effective oil reducing local lesion number was the oil isolated from *C. flexuosus* (97.2%), followed by *O. tenuiflorum* (94.4%) and *P. nigrum* (91.6 %). Probit analysis data revealed that LC_{50} value was minimum in *C. flexuosus* oil (EOC-2D) where as it was maximum in EOP-30D. From the result it was observed that inhibitory effect of essential oil was dose dependent. The common feature of all the investigated oils is the presence of sesquiterpenes in relatively high percentages compared to monoterpenes. Dominant component (>60%) present

in *O. tenuiflorum* and *P. nigrum* oil was sesquiterpenes. Sesquiterpene-rich essential oils have been shown in studies to be powerful inhibitors of Cucumber Mosaic Virus infection and to be natural compounds that may help manage plant viral infections.[11] The current study also demonstrated how effective these essential oils with high sesquiterpene content are at preventing CMV infection. Other literature data dealing with antiviral activity of essential oils do not compare composition of oils and their antiviral effectiveness. Research in recent years has primarily focused on comprehending how plants react to specific biotic or abiotic challenges, even though the response to many stresses would inevitably result in a far more complex situation.[22] Viral pathogen can affect chloroplast number, size morphology and content as well as the size and number of chloroplast inclusions (plastoglobuli, starch grains etc). It was summarised that chloroplast aberrations and metabolism appear as a common feature of pathogen infection.[23] In marrow inoculated with the Cucumber Mosaic Virus (CMV) starch accumulating cells display increased photosynthetic capacity relative to uninfected cells or cells in which virus replication is actively occurring.[24] It was reported that the total Chlorophyll (Chl) and carotenoids (Car) concentrations in yellow mosaic virus infected leaves were significantly reduced by 64% and 62%, respectively.[25] It was reported that elevated amounts of MDA and H2O2 in *Cucurbita pepo* leaves indicating lipid peroxidation and oxidative stress is in response to viral infection.[26] Studies demonstrated that Tobacco mosaic virus induced oxidative stress in a necrotic host plant is signalled by an elevated level of Monodehydroascrobate (MDA) radicals detected by electron paramagnetic resonance spectroscopy.[27] The essential oils tested, demonstrated a significant reduction in CMV symptoms and viral load. This suggests a promising potential for these essential oils in mitigating the impact of CMV on cassava crops. The observed antiviral effects may be attributed to various bioactive compounds present in the essential oils. Terpenes, phenols and other constituents have been reported to possess antiviral properties and their interference with the CMV life cycle was evident in our results. Hence the present investigation provides sufficient information about the three studied essential oils and their action against CMV infection. It is essential to acknowledge the limitations of our study, including variations in environmental conditions, potential interactions with other pathogens and the need for further optimization of essential oil concentrations and application methods. Future research should explore additional essential oils with diverse chemical profiles to expand the repertoire of antiviral agents. Long-term studies are necessary to assess the sustainability of essential oil-based strategies and potential development of resistance in the CMV population.

CONCLUSION

The present study deals with the efficacy of essential oils isolated from *Cymbopogan flexuosus*, *Ocimum tenuiflorum* and *Piper nigrum* in determining the antiviral property on Gomphrena globosa infected with Cassava Mosaic Virus. This is the first report on testing the efficiency on the selected plant species. The local lesion host plant showed many morphological changes like presence of local necrotic lesions, yellowing of leaf and early defoliation of leaves. GC/MS analysis revealed 18 different compounds and the yield of the essential oils from plants under investigation were analysed. Experiments showed that *C. flexuosus* yield more oil compared to others. The main constituents of the investigated essential oils are monoterpene and sesquiterpene hydrocarbons. From the three essential oil tested *C. flexuosus* oil has been shown high inhibition activity than other two oils. Finally, it could be concluded that essential oil from the tested plants can be used to control CMV infection in controlled condition. The multifaceted implications of this research extend to sustainable agriculture, with the potential to revolutionize CMV management practices. Continued investigation into the mechanisms of action and optimization of essential oil applications will contribute to the development of effective, eco-friendly strategies for managing biotic stress in cassava crops.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The present study provides an insight into the ability of essential oils in drastically controlling the infection of viruses on plants. This can thereby help in developing new plant varieties with minimal infection ultimately resulting in high yield.

ABBREVIATIONS

CMV: Cassava Mosaic Virus; **EOC:** Essential Oil *Cymbopogon*; **EOO:** Essential Oil *Oscimum*; **EOP:** Essential Oil *Piper*.

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