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Phytochemicals from *Moringa oleifera* Exhibit Antiviral Activity against Bean Yellow Mosaic Virus in *Vicia faba* L.

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FABA BEAN (*Vicia faba* L.) is a globally important legume and the most widely cultivated pulse crop in Egypt due to its high nutritional value. However, its productivity is severely constrained by Bean yellow mosaic virus (BYMV), a widespread and economically significant pathogen. This study aimed to isolate and characterize BYMV in Egypt and to evaluate the antiviral efficacy of *Moringa oleifera* leaf extract as a natural biocontrol agent. BYMV was biologically purified using *Chenopodium amaranticolor* and confirmed through DAS-ELISA and RT-PCR, which yielded an 832 bp amplicon. Sequencing of the Egyptian isolate (GenBank Accession No. OR242736.1) revealed 89-100% identity with global BYMV strains. Aqueous *M. oleifera* extract was applied via stem injection and foliar spray at concentrations of 25, 50, and 75 mg/mL, either 24 hours before or after virus inoculation. The post-infection foliar spray at 50 mg/mL (VM) resulted in the highest suppression of viral accumulation (88%) and significantly enhanced host defense responses, including total phenolic content (13.04 mg/g), peroxidase (12.14 U/mg/min), PAL (489.29 µg/mg), and catalase activity (32.94 U/mg/min). GC-MS profiling identified 27 phytochemicals dominated by terpenoids (34%), fatty acid derivatives (29%), sterols (17%), and hydrocarbons (15%). Major constituents-such as neophytadiene, linoleoyl chloride, and cholestanol derivatives-possess known antiviral and antioxidant properties. Neophytadiene, in particular, may contribute to viral inhibition by disrupting membrane integrity and replication processes. Overall, the findings highlight *M. oleifera* extract as a promising, eco-friendly antiviral agent that suppresses BYMV through a combination of direct virucidal activity and the activation of host defense pathways.

Keywords: BYMV, *Moringa oleifera*, DAS-ELISA, RT-PCR, GC-MS.

Introduction

Faba bean (*Vicia faba* L.), belonging to the family Fabaceae, is considered one of the most important food legumes worldwide. In Egypt, it ranks as the leading legume crop in terms of cultivated area, total production, and consumption. Both its green and dry seeds are widely consumed by humans and animals, as they are rich in protein (24.60–30.68%) and carbohydrates (51.11–58.15%), and serve as significant sources of vitamins, essential amino acids, and mineral salts (**Mahdi et al., 2021**; **Abdelaal**, **2023**).

In addition to its nutritional value, faba bean contributes to soil fertility by improving soil structure and fixing atmospheric nitrogen, leaving behind approximately 20–30 nitrogen units per feddan after harvest, benefiting subsequent crops. The major Egyptian governorates known for faba bean cultivation include Dakahlia, Sharqia, Kafr El-Sheikh, Assiut, and Beheira (**Sofy et al., 2014; Elsharkawy et al., 2021**). According to the **FAO (2018)**, Egypt cultivated approximately 113,810 feddans of faba bean, with an average yield of 9.2 ardebs/feddan over the previous five years.

Bean yellow mosaic virus (BYMV), a member of the genus *Potyvirus*, family *Potyviridae*, is a globally distributed virus with a wide host range that includes both monocot and dicot plants. BYMV is considered one of the most economically important plant viruses, causing severe disease symptoms and yield losses in various crops. Infections during early stages of plant growth can result in systemic necrosis and eventual plant death **Jones *et al.*, 2003**).

In Egypt, bean yellow mosaic virus (BYMV) is among the most prevalent and destructive viruses affecting

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leguminous crops. Faba bean has been identified as the most susceptible host, with infections leading to substantial reductions in seed yield (**Elsharkawy et al., 2021**). BYMV is a filamentous virus approximately 750 nm in length, possessing a single-stranded, positive-sense RNA genome. Although seed transmission occurs at a low rate (**McKirdy et al., 2000**), the virus is primarily spread in a non-persistent manner by several species of aphids (**Younes et al., 2021**).

Infected faba bean plants typically exhibit a range of symptoms, including mosaic patterns, mottling, yellowing, leaf flexing, rolling, and patchy green areas. According to visual assessments, the incidence of BYMV infection among symptomatic faba bean plants in four Egyptian governorates ranged from 14.4% to 46.4% (**Attia et al., 2024**).

Numerous studies have confirmed the significant role of plant extracts in managing various plant viral diseases. For instance, carnation leaves have been identified as a potent source of viral inhibitors and have also been reported to induce systemic resistance against viral infections (**Ostermann et al., 1987**). Compounds with antiviral properties have been detected in several angiosperm families, including Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Solanaceae, and Verbenaceae (**Cheesin et al., 1995**). **Awad et al. (2017)** demonstrated that fresh leaf extracts of *Dianthus caryophyllus* exhibited both antiviral activity and the ability to induce systemic resistance against BYMV in faba bean plants. Similarly, **Elsharkawy et al. (2021)** evaluated various plant extracts for their ability to control BYMV and found that cinnamon extract was the most effective in reducing disease severity, followed by ginger and fennel extracts. More recently, foliar application of *Clitoria ternatea* flower extract has been shown to enhance the plant's defense mechanisms, significantly reducing both the incidence and severity of BYMV infection in faba bean (**Attia et al., 2024**).

Historically, the Moringa tree (*Moringa oleifera*) has been recognized as a "miracle tree" and has long been used in traditional medicine to treat a wide range of ailments (**Jung, 2014**). As an antiviral agent, *M. oleifera* leaf extract has demonstrated notable effectiveness against herpes simplex viruses, as well as antiviral activity against the H1N1 strain of influenza A and SARS-CoV-2 (**Xiong et al., 2021; El-Meidany et al., 2023**).

The present study aims to isolate and identify Bean yellow mosaic virus (BYMV) and to evaluate the antiviral potential of aqueous *Moringa oleifera* leaf extract in controlling BYMV infection in faba bean plants.

Materials and Methods:

Source of Virus Isolate

Faba bean plants exhibiting characteristic viral symptoms, such as mosaic and mottling on the leaves, were collected from open field sites in the Menoufia Governorate, Egypt.

Virus Propagation

Symptomatic leaf tissues were homogenized in a phosphate buffer solution (pH 7.2), and the resulting sap was mechanically inoculated onto healthy Faba bean plants (cultivar Misr 1) pre-dusted with carborundum powder (600 mesh). The inoculated plants were maintained under greenhouse conditions and monitored regularly for symptom development.

Biological Purification of the Virus Isolate

Upon symptom manifestation, leaf samples from infected Faba bean plants were used to inoculate *Chenopodium amaranticolor* via mechanical transmission. The single local lesion technique was applied and repeated three successive times according to the protocol described by **Kuhn (1964)**, to biologically purify the virus. The purified isolate was then propagated in Faba bean plants under controlled greenhouse conditions and subsequently used as an inoculum for further experimentation.

Serological Detection

Detection of Bean yellow mosaic virus (BYMV) was performed using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as outlined by **Clark and Adams (1977)**. Specific polyclonal antibodies provided in a commercial ELISA kit (LOEWE®, Germany) were used at a dilution of 1:200 for both IgG and IgG conjugate. Absorbance was measured at 405 nm using a microplate reader (CLINDIAG Systems Co. Ltd.). Samples were considered positive for BYMV if their optical density (OD) readings were at least twice that of the mean OD value of healthy (negative control) samples.

RNA Extraction and RT-PCR Amplification of BYMV Coat Protein Gene

Total RNA was extracted from BYMV-infected faba bean leaves using the RNeasy Plant Mini Kit (QIAGEN, Germany; Cat. No. 74106) following the manufacturer's protocol. One-step RT-PCR was conducted to amplify the full-length coat protein (CP) gene using the Verso™ 1-Step RT-PCR Ready Mix Kit (Thermo Scientific, USA) and gene-specific primers BYMV-pnsF (5′-TCAGATCAAGAGCAACTCAATGCA-3′) and BYMV-pnsR (5′-GACGGATACTCTAAATACGAACA-3′), as reported by **Sharma et al. (2015)**. The 25 µL

reaction mix contained 12.5 µL of 2X RT-PCR Ready Mix, 1.5 µL of each primer (10 µM), 0.5 µL of Verso Enzyme Mix, 1.25 µL of RT Enhancer, and 3 µL of RNA template. Thermal cycling was performed with an initial cDNA synthesis at 50 °C for 15 min, denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 15 s, 48 °C for 40 s, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. Amplified products were resolved on a 1.5% agarose gel in 1X TAE buffer, stained with EZview nucleic acid stain (Cat# A4205, BIOMATIK, Canada), and visualized under UV light alongside a 100 bp DNA ladder. The target 832 bp band was excised and purified using the QIAquick Gel Extraction Kit (QIAGEN, Germany), then sequenced using the Sanger method. Multiple sequence alignment with 15 reference BYMV CP sequences from GenBank and phylogenetic analysis were performed using CLC Genomics Workbench version 25.0, applying the Maximum Likelihood method with 1000 bootstrap replicates.

GC-MS Analysis

Fresh *Moringa oleifera* leaves were shade–dried at room temperature, ground to a fine powder, and extracted by maceration in HPLC-grade methanol (1:10 w/v) for 24 h at room temperature with occasional shaking. The extract was filtered through Whatman No. 1 paper and then passed through a 0.22 µm PTFE syringe filter before GC–MS analysis. One microliter of the filtered extract was injected in splitless mode into a Thermo Scientific™ Trace™ GC Ultra gas chromatograph coupled to a TSQ™ 8000 Evo triple quadrupole mass spectrometer. Separation was achieved on a TG-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injector temperature was held at 280 °C, and helium served as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was as follows: initial temperature of 50 °C held for 3 min, ramped to 200 °C at 5 °C/min, then to 280 °C at 10 °C/min, and finally held at 280 °C for 10 min (total run time ≈ 45 min). The mass spectrometer operated in electron-impact (EI) mode at 70 eV, scanning from *m/z* 50 to 750. Detector voltage and transfer line temperatures were set according to the manufacturer's recommendations to ensure optimal sensitivity and reproducibility. Chromatograms were processed using the instrument software to integrate peak areas and heights. Compound identities were assigned by comparison of mass spectra against the Wiley Registry 8th Edition and the NIST/EPA/NIH mass spectral library, considering match factors >700 and retention times. Relative abundances were calculated as each compound's peak area divided by the total ion current, expressed as a percentage.

Preparation of Aqueous Extract of Moringa oleifera

Moringa oleifera seeds were obtained from the Horticultural Research Institute and cultivated in sterilized pots (40 cm diameter) filled with a 1:1:1 mixture of silt, sand, and peat moss. One month after germination, fresh leaves were harvested, thoroughly washed under running tap water, and air-dried at room temperature for 48 hours. The dried leaves were then ground into a fine powder using an electric grinder, following the method described by **El Awady et al. (2023)**. For extraction, 15 g of leaf powder was mixed with 150 mL of distilled water in a sterile flask and agitated on a rotary shaker at room temperature for 48 hours to release water-soluble phytochemicals. The mixture was filtered through Whatman No. 1 filter paper, and the resulting crude extract (100 mg/mL) was stored at 4 °C for further use. Working solutions were freshly prepared by diluting the crude extract with distilled water to final concentrations of 25, 50, and 75 mg/mL for application in biological assays.

Evaluation of the Antiviral Activity of Moringa oleifera Extract Against BYMV in Faba Bean Plants

A greenhouse experiment was conducted under insect-proof conditions to evaluate the antiviral efficacy of *Moringa oleifera* leaf extract against Bean yellow mosaic virus (BYMV) in faba bean (*Vicia faba* L., cv. Misr 1). Seeds were sown in 30 cm diameter pots, three plants per pot, with five replicate pots per treatment group. Plants were maintained under natural daylight conditions with standard irrigation and fertilization practices. Two weeks after planting, plants at the fourth leaf stage were inoculated with BYMV using sap extracted from infected faba bean leaves. The leaves were lightly dusted with 600 mesh carborundum powder to facilitate mechanical inoculation. Viral inoculation was performed either 24 hours before or 24 hours after *Moringa oleifera* extract application, depending on the treatment group. Aqueous *Moringa oleifera* leaf extract was prepared at three concentrations (25, 50, and 75 mg/mL) by dilution in distilled water. Two application methods were used: foliar spray and stem injection. For foliar spray, the extract was applied evenly onto the leaves using a handheld sprayer until runoff. For injection, 1 mL of extract was injected per plant at

multiple points along the stem using sterile syringes. In both methods, treatments were applied either 24 hours before (MV) or after (VM) BYMV inoculation. Control groups included a positive control (BYMV-infected, untreated) and a negative control (uninoculated, untreated). Thirty days post-treatment, newly developed leaves were collected for virus quantification using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) according to **Clark and Adams (1977)**. Absorbance at 405 nm was measured using a microplate reader (CLINDIAG Systems Co. Ltd). Samples were considered BYMV-positive if OD values were at least twice that of the negative control. The percentage of virus reduction was calculated as described by **Abdelkader et al., (2025)** using the formula:

$$\text{Reduction } \% = \left(\frac{\text{OD}_{\text{positive control}} - \text{OD}_{\text{treatment}}}{\text{OD}_{\text{positive control}}} \right) \times 100$$

Where:

- $\text{OD}_{\text{positive c}}{}_{\text{ontrol}}$ = Average optical density of virus-infected untreated plants
- $\text{OD}_{\text{treatment}}$ = Average optical density of *Moringa*-treated plants

Total Phenolic Content

Total phenolic content (TP) was measured in faba bean leaves subjected to various treatments, including foliar application of *Moringa oleifera* extract at a 50 mg/mL concentration applied either 24 hours before (MV) or after (VM) BYMV inoculation, as well as Moringa-only treatment (M), virus-infected untreated plants (V), and healthy controls (N). Leaf samples (0.2 g of air-dried tissue) were extracted with 10 mL of 50% aqueous methanol and incubated at 40 °C for 1 hour, followed by centrifugation at 14,000 × g for 15 minutes. The supernatants were collected and stored at 4 °C. TP was quantified using the Folin–Ciocalteu colorimetric assay (**Lim et al., 2014**). Briefly, 0.125 mL of the extract was mixed with 0.5 mL deionized water and 0.125 mL Folin–Ciocalteu reagent. After 1 minute, 1.25 mL of 7% Na_2CO_3 was added, and the mixture was incubated in the dark at room temperature for 90 minutes. Absorbance was measured at 760 nm using a UV-Visible Spectronic 601 spectrophotometer.

Peroxidase (POD) Activity

Peroxidase activity was measured spectrophotometrically using a UV-Vis Spectronic 601 at 420 nm. The assay employed pyrogallol as the substrate following the method described by **Falade et al. (2019)**. The enzymatic reaction involved the oxidation of pyrogallol in the presence of hydrogen peroxide, and the increase in absorbance at 420 nm was recorded to determine peroxidase activity.

Phenylalanine Ammonia-Lyase (PAL) Activity

PAL activity was determined using a UV-Vis Spectronic 601 spectrophotometer at 290 nm, based on the protocol described by **Şirin et al. (2016)**. The assay measures the formation of trans-cinnamic acid from L-phenylalanine, with the absorbance change at 290 nm reflecting PAL enzyme activity.

Catalase (CAT) Activity

Catalase activity was assayed spectrophotometrically at 240 nm using hydrogen peroxide as the substrate, following the method of **Beers Jr. and Sizer (1952)**. The breakdown of hydrogen peroxide by catalase results in a decrease in absorbance at 240 nm, which was monitored to quantify catalase activity.

Statistical Analysis

Statistical analysis was performed using SPSS version 23. To evaluate the antiviral effects of *Moringa oleifera* extract and its impact on viral concentration in Faba bean plants, data were subjected to Univariate Analysis of Variance (UNIANOVA). Post-hoc comparisons between treatment groups were conducted using the Bonferroni test. Statistical significance was considered at $p < 0.05$. All experiments were performed in triplicate, and the mean values were used for comparative analysis.

Results and Discussion:

Virus Isolation and Detection Methods

Faba bean (*Vicia faba* L.), a key crop in the Fabaceae family, is increasingly threatened by viral diseases, with *Bean yellow mosaic virus* (BYMV) recognized as one of the most prevalent and destructive pathogens in Egypt. Previous studies have shown faba bean to be highly susceptible to BYMV, leading to significant yield losses. **Elsharkawy et al. (2021)** reported substantial seed yield reduction due to BYMV, while recent field surveys by **Attia et al. (2024)** recorded infection rates ranging from 14.4% to 46.4% across multiple Egyptian governorates. In the present study, BYMV was isolated from naturally infected faba

bean plants exhibiting typical mosaic and mottling symptoms. For biological purification, sap extracted from symptomatic leaves was mechanically inoculated onto *Chenopodium amaranticolor*, a local lesion host. Inoculated *C. amaranticolor* plants developed characteristic necrotic local lesions, from which single lesions were excised and used for serial reinoculations to ensure purification. The purified isolate was subsequently propagated in healthy faba bean plants, which developed clear mosaic symptoms similar to those observed in the field, confirming successful isolation and pathogenicity of the virus. Serological confirmation was conducted using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). All mechanically inoculated plants tested positive for BYMV, while no reaction was observed in the negative controls, confirming the presence of the virus and the specificity of the detection method. These findings support the use of DAS-ELISA as a reliable diagnostic tool for BYMV and reaffirm the widespread prevalence and impact of the virus on faba bean production in Egypt. The integration of classical biological purification using *C. amaranticolor* and serological verification with DAS-ELISA underscores the importance of combining traditional and modern diagnostic approaches to accurately identify and confirm plant virus infections. The results align with previous reports and provide a solid foundation for subsequent antiviral evaluation of treatment strategies.

RT-PCR and Sequence Analysis of BYMV Coat Protein Gene

The presence of BYMV in faba bean was confirmed through serological and molecular assays. Symptomatic leaf samples tested positive by double antibody sandwich-ELISA (DAS-ELISA; data not shown) and yielded a distinct 832 bp amplicon in RT-PCR targeting the coat protein (CP) gene, whereas no amplification was observed in healthy control plants (**Figure 1A**). This PCR product size is consistent with the expected CP gene fragment and with previous reports of BYMV detection in legumes (**Sharma *et al.*, 2015**). The successful amplification in all symptomatic samples, coupled with the absence of products in negatives, definitively confirms BYMV as the causal agent in the diseased faba bean plants. Sequencing of the 832 bp RT-PCR product (GenBank accession OR242736.1, denoted here as BYMV-EG) verified its identity as Bean yellow mosaic virus. Comparative analysis with fifteen reference BYMV isolates from GenBank revealed a high degree of sequence homology, with nucleotide identities ranging from ~89% to ~99.5%. Notably, BYMV-EG shared the highest identity (99.5%) with two Japanese isolates (S-22N and E-24N; accessions AB029435.1 and AB029438.1) and showed very high similarity to several other Asian and European strains – including a German isolate (99.3%, DSMZ_PV-1213) and a Chinese isolate (99.0%, Saffron2). Other Japanese (e.g., isolates 35-1 and B-33) and Chinese (e.g., Hangzhou) BYMV strains also exhibited ≥98% identity to the Egyptian isolate. Even geographically distant isolates retained substantial similarity: for instance, the Egyptian BYMV shared about 92% identity with an Argentine isolate (Arg) and U.S. isolate (Pullman, USA), 94% with an Iranian isolate (BYSun), and approximately 88.9% with an Iraqi isolate (BYMV-Iraq5). These results indicate that the CP gene of BYMV-EG is highly conserved relative to global BYMV populations. The exceptionally close genetic relatedness to East Asian strains (especially those from Japan and China) suggests a possible common lineage or origin, whereas the somewhat lower identities with isolates from other continents reflect regional genomic divergence among BYMV strains worldwide. Phylogenetic analysis of the CP gene sequences further supports these relationships. In the neighbor-joining tree (**Figure 1B**), the Egyptian isolate clustered tightly with BYMV isolates from Asia and Europe in a distinct clade, separate from those of more distant geographic origin. The bootstrap values for these groupings were high (>50%), reinforcing the reliability of the clustering pattern. Notably, BYMV-EG grouped most closely with the Japanese and Chinese isolates, mirroring the nucleotide identity results. This close phylogenetic placement confirms that the Egyptian strain is evolutionarily allied with strains circulating in East Asia and parts of Europe. Similar clustering of BYMV by regional origin has been documented previously, where isolates from distant regions like North America or the Middle East form distinct sub-lineages (**Sharma *et al.*, 2015**). Our findings underscore that despite being isolated in North Africa, the BYMV-EG strain shares a recent common ancestor with Asian/European strains, while still exhibiting the genetic signatures of geographic separation evident in more distantly related isolates (e.g., from the Americas or Iraq).

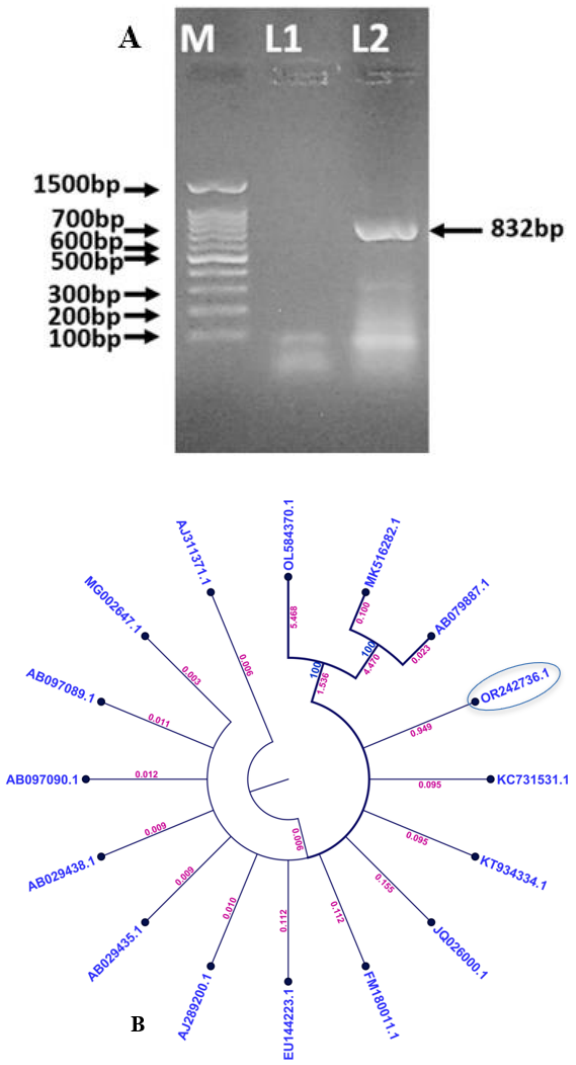

Fig. 1. (A): RT-PCR amplification of BYMV-CP gene (OR242736.1). Lane M: DNA ladder; Lane L1 - negative control (healthy Faba bean); Lane L2 - BYMV-infected Faba bean sample showing a specific band at 832 bp. (B): Phylogenetic tree based on nucleotide sequence alignment of the coat protein gene of the newly identified BYMV isolate from Egypt (highlighted by its GenBank accession number) and reference BYMV isolates retrieved from GenBank. The tree was constructed using the Maximum Likelihood method with bootstrap values (1,000 replicates) shown at nodes. Branch lengths represent genetic distances.

GC/MS analysis of Moringa oleifera leaf extract

Phytochemical Composition of *Moringa oleifera* Leaf Extract and Its Antiviral Implications

Gas chromatography–mass spectrometry (GC-MS) analysis of the aqueous leaf extract of *Moringa oleifera* revealed a complex phytochemical profile comprising 27 distinct compounds, with the run spanning 0-45.27 minutes (**Figure 2**). The total ion chromatogram (TIC) identified five major constituents-each exceeding 5% relative abundance-including neophytadiene (RT 24.83 min; 11.04%), linoleoyl chloride (RT 30.50 min; 9.73%), 5α-cholestan-3α-ol, 2-methylene- (RT 43.77 min; 9.30%), (Z)-8,11,14-eicosatrienoic acid methyl ester (RT 30.41 min; 6.20%), and cis-8,11,14-eicosatrienoic acid (RT 41.56 min; 5.86%). Two additional mid-abundance peaks-hexadecanoic acid, 2,3-dihydroxypropyl ester (RT 27.28 min; 5.38%) and dotriacontane (RT 36.52 min; 5.22%)-also contributed notably to the chromatographic profile. These compounds collectively represented four major chemical classes: terpenoids (~34% of total ion current), fatty acid derivatives (~29%), sterols (~17%), and long-chain aliphatic hydrocarbons (~15%). The abundance of terpenoids and unsaturated fatty acid derivatives is particularly significant in light of their well-documented antiviral properties in both plant and animal systems. Neophytadiene, a diterpenoid hydrocarbon and the most abundant constituent in the extract, is a strong candidate for the observed antiviral activity. Previous studies have demonstrated its capacity to disrupt viral envelopes and interfere with viral replication, primarily through interactions with host cell membranes and viral structural proteins (**Gabbianelli et al., 2023**). The ability of neophytadiene to inhibit viral protein–receptor interactions may be especially relevant for plant viruses like BYMV, which depend on efficient cell-to-cell and systemic movement facilitated by membrane-bound viral replication complexes. Fatty acid methyl esters, such as (Z)-8,11,14-eicosatrienoic acid methyl ester and linoleoyl chloride, are also implicated in antiviral mechanisms. These amphipathic molecules may integrate into lipid bilayers, disrupting viral entry, uncoating, and intracellular transport-critical steps in the infection cycle of positive-sense RNA viruses such as BYMV. The co-occurrence of sterol derivatives, notably 5α-cholestan-3α-ol, 2-methylene-, further strengthens the extract's potential efficacy. Sterols have been shown to regulate membrane fluidity and are involved in the formation of lipid rafts-microdomains essential for virus replication and movement within host plants (**Wani et al., 2023**). In this context, the disruption of lipid raft function by phytosterols may impair BYMV's ability to establish systemic infection via plasmodesmata. The characteristic mass spectral fragmentation patterns, including major fragment ions (m/z 123, 207, 259), confirmed the structural identities of the compounds. The detection of methylated fatty acids such as eicosatrienoic acid methyl ester and linoleic acid derivatives aligns with findings from other medicinal plants where these metabolites contribute to antiviral defense by interfering with host lipid signaling and viral replication (**Ghildiyal et al., 2020; Ahmad et al., 2024**). From a mechanistic standpoint, the extract's antiviral activity is likely attributable to a

multifaceted interplay between terpenoids, sterols, and polyunsaturated fatty acid derivatives. These compound classes may act synergistically-targeting different stages of the viral life cycle such as entry, replication, and systemic movement-thus amplifying the overall inhibitory effect on BYMV. This synergism is a hallmark of many effective botanical antivirals and provides a compelling rationale for the superior performance observed in foliar application trials. The findings of this phytochemical analysis not only affirm the chemical richness of *Moringa oleifera* leaf extract but also provide mechanistic insight into its antiviral potential. The high concentration of neophytadiene (>11%) in particular underscores its potential as a lead antiviral compound for the development of plant-based biopesticides. Moreover, the presence of bioactive compounds in the aqueous extract supports its suitability for eco-friendly foliar formulations, compatible with organic farming practices. Taken together, these results highlight the promise of *Moringa oleifera*-derived products as a sustainable and biologically active solution for viral disease management in faba bean and other economically important crops. The integration of such natural antivirals into plant protection regimes could significantly reduce the reliance on synthetic chemicals, enhance crop resilience, and contribute to sustainable agricultural practices in virus-prone regions.

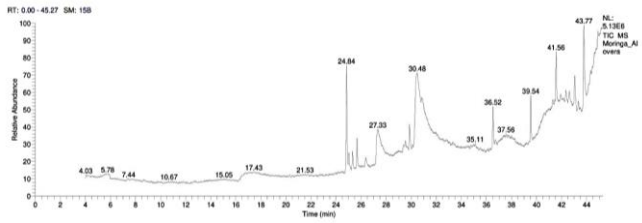

Fig. 2. Total-ion chromatogram of *Moringa oleifera* leaf extract acquired by GC-MS over a 0-45.27 min run. Arrows indicate the five major peaks: neophytadiene (RT 24.84 min), hexadecanoic acid, 2,3-dihydroxypropyl ester (RT 27.33 min), 9,12-octadecadienoyl chloride (RT 30.48 min), cis-8,11,14-eicosatrienoic acid methyl ester (RT 41.56 min), and cholestan-3-ol, 2-methylene- (RT 43.77 min).

Effect of Moringa oleifera Extract on BYMV Concentration in Faba Bean Plants

The antiviral potential of *Moringa oleifera* leaf extract against Bean yellow mosaic virus (BYMV) was evaluated under controlled greenhouse conditions using two application methods-stem injection and foliar spray-at three concentrations (25, 50, and 75 mg/mL), administered either 24 hours before (MV) or after (VM) viral inoculation. Virus accumulation in treated and control plants was quantified 30 days post-infection using DAS-ELISA (OD_{405}), with the results summarized in **Figure 3** and **Supplementary Table 1**.

Injection Method:

Stem injection of M. oleifera extract led to a significant reduction in virus concentration compared to the untreated BYMV-infected control (OD = 1.082). Pre-inoculation treatments MV1 and MV2 (25 and 50 mg/mL) showed comparable OD values (0.619 and 0.621, respectively; $p > 0.05$), indicating moderate antiviral efficacy with no significant difference between the two concentrations. However, MV3 (75 mg/mL) yielded a significantly lower OD (0.519; $p < 0.05$), reflecting enhanced suppression of BYMV. Post-inoculation treatments VM1, VM2, and VM3 resulted in OD values of 0.533, 0.546, and 0.519, respectively-each significantly lower than the positive control ($p < 0.01$), with virus reduction reaching up to 52%. No significant differences were observed among the post-inoculation injections, suggesting that all concentrations were similarly effective in limiting systemic viral accumulation (**Figure 3**).

Foliar Spray Method:

Foliar application of Moringa extract showed superior antiviral performance compared to the injection method. All pre-inoculation treatments (MV1, MV2, MV3) significantly lowered BYMV concentration relative to the infected control (OD = 1.088), achieving OD values of 0.513, 0.528, and 0.441, respectively. Although these differences were not statistically significant among themselves ($p > 0.05$), MV3 exhibited the highest reduction, corresponding to a 60% suppression in virus titer. Post-inoculation foliar treatments demonstrated the most pronounced antiviral effects. VM1 and VM2 (25 and 50 mg/mL) recorded OD values of 0.134 and 0.147, respectively, both statistically indistinguishable from the healthy negative control (OD = 0.167; $p > 0.05$), indicating near-complete viral suppression (88% and 87% reduction, respectively). VM3 (75 mg/mL) also showed a significant effect (OD = 0.296), corresponding to a 72% reduction, though slightly less effective than VM1 and VM2 ($p < 0.05$). These findings confirm the efficacy of foliar spraying-particularly post-inoculation-over injection in reducing BYMV accumulation (**Figure 3**; **Supplementary Table 1**). Parallel to these experimental findings, *Moringa oleifera* has been widely recognized for its medicinal and antimicrobial potential. Its antiviral activity has been attributed to diverse bioactive compounds-including flavonoids, terpenoids, alkaloids, and polyphenols-that can interfere with

multiple stages of the viral life cycle such as entry, replication, and systemic spread (**Ghildiyal et al., 2020; El-Meidany et al., 2023**). Notably, studies in human virology have demonstrated that M. oleifera phytochemicals exhibit strong binding affinity to viral proteins and can modulate host immune responses. For instance, in silico models by **Siddiqui et al. (2022)** highlighted the binding potential of Vitexin and glucomoringin to the SARS-CoV-2 spike protein, while **Xiong et al. (2022)** identified Moringa A as an effective inhibitor of H1N1-induced inflammation. In the context of plant virology, this study is the first to document the antiviral efficacy of aqueous *M. oleifera* extract against BYMV in faba bean. The data clearly demonstrate that both application methods significantly suppressed viral accumulation compared to the positive control. Injection treatments produced moderate effects, with 43–52% reductions across concentrations. In contrast, foliar spraying-especially when applied after virus inoculation-resulted in significantly greater suppression, with reductions ranging from 72% to 88%. Importantly, the VM1 treatment (25 mg/mL foliar spray post-inoculation) achieved the highest level of virus suppression (88%) and yielded an OD value statistically equivalent to the healthy control.

Table 1. Effect of *Moringa oleifera* Extract via Injection and Foliar Spray on BYMV Concentration in Faba Bean Plants as Measured by DAS-ELISA.

Treatment	Injection ($OD_{405} \pm SE$)	+/–	Foliar Spray ($OD_{405} \pm SE$)	+/–
Positive Control	1.082 ± 0.002^{a}	(+)	1.088 ± 0.007^{a}	(+)
MV1 (25 mg/ml)	0.619 ± 0.002^{b}	(+)	0.513 ± 0.031^{b}	(+)
MV2 (50 mg/ml)	0.621 ± 0.003^{b}	(+)	0.528 ± 0.037^{b}	(+)
MV3 (75 mg/ml)	0.519 ± 0.009^{c}	(–)	0.441 ± 0.038^{b}	(+)
VM1 (25 mg/ml))	0.533 ± 0.006^{c}	(–)	0.134 ± 0.001^{d}	(–)
VM2 (50 mg/ml)	0.546 ± 0.005^{c}	(–)	0.147 ± 0.002^{d}	(–)
VM3 (75 mg/ml)	0.519 ± 0.001^{c}	(–)	0.296 ± 0.030^{c}	(–)
Negative Control	0.284 ± 0.006^{d}	(–)	0.167 ± 0.003^{d}	(–)

OD values represent the mean absorbance at 405 nm ± standard error (SE), measured by DAS-ELISA 30 days post-treatment. Treatments included three concentrations of *Moringa oleifera* extract (25, 50, and 75 mg/mL), applied either 24 hours before (MV) or 24 hours after (VM) BYMV inoculation. (+) indicates virus-positive samples; (–) indicates OD values below the virus detection threshold. Means followed by different superscript letters within each column are significantly different at $p < 0.05$ according to Bonferroni post hoc test.

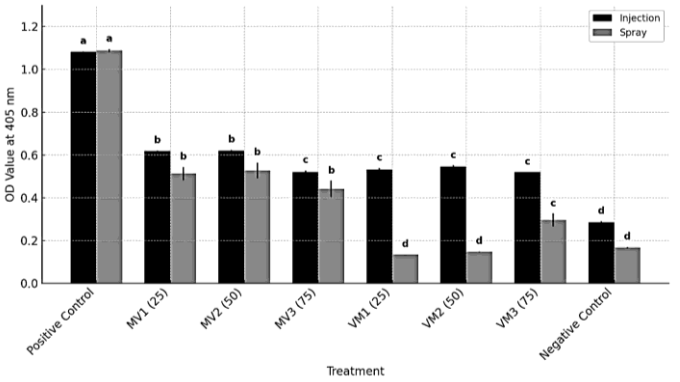

Fig. 3. Effect of *Moringa oleifera* extract (injected or sprayed) at different concentrations (25, 50, 75 mg/mL) on BYMV accumulation in faba bean plants, measured by DAS-ELISA at 405 nm, 30 days post-infection. Treatments were applied 24 h before (MV) or after (VM) inoculation. Different letters (a–d) indicate significant differences (Bonferroni test, $p < 0.05$).

Biochemical Responses to Moringa oleifera Treatment in BYMV-Infected Faba Bean Plants

Biochemical analyses were conducted to evaluate the physiological impact of *Moringa oleifera* foliar application (50 mg/mL) on *Vicia faba* plants under different treatment regimens following Bean yellow mosaic virus (BYMV) infection. Four key biochemical markers were examined: total phenolic content (TPC), peroxidase (POD), phenylalanine ammonia-lyase (PAL), and catalase (CAT) activities (**Fig. 4A-D**). Significant differences were observed across all treatments for each biochemical parameter ($p < 0.05$). The VM treatment (Moringa applied 24 hours post-infection) yielded the highest total phenolic content (13.04 mg/g), significantly surpassing all other groups ($p < 0.01$). The MV (pre-infection spray) and M (Moringa-only) treatments also induced elevated TPC levels (11.27 mg/g and 10.56 mg/g, respectively), both higher than the virus-infected untreated (V, 10.37 mg/g) and healthy controls (N, 9.82 mg/g) (**Fig. 4A**). Peroxidase activity showed a different trend: MV treatment exhibited the highest POD activity (14.74 U/mg/min), comparable to the healthy control (14.36 U/mg/min), whereas the M group recorded the lowest value (9.43 U/mg/min) (**Fig. 4B**). PAL activity peaked in the VM group (489.29 µg/mg), followed by MV, V, and N treatments, which exhibited similar intermediate values (471.42-475.32 µg/mg), while the M treatment showed the lowest PAL activity (462.63 µg/mg) (**Fig. 4C**). Catalase activity was markedly elevated in both VM and MV treatments (32.94 and 32.07 U/mg/min, respectively), significantly higher than in V (20.48 U/mg/min), N (15.76 U/mg/min), and especially the M group (5.03 U/mg/min) (**Fig. 4D**). Statistical comparisons were validated using Bonferroni post-hoc tests, with distinct letters indicating significant

differences ($p < 0.05$). The significant elevation of TPC in Moringa-treated plants, particularly in the VM group, highlights the extract's ability to enhance secondary metabolism and activate phenolic-mediated defense pathways. Phenolic compounds are well-established components of plant defense, acting as antioxidants, antimicrobial agents, and signaling molecules that contribute to systemic acquired resistance (SAR) (**Saini et al., 2024; Salman et al., 2024**). The highest TPC in VM-treated plants suggests that *M. oleifera* is effective not only as a prophylactic but also as a therapeutic agent, capable of stimulating the host immune response even after viral establishment. The modest increase in phenolics observed in BYMV-infected, untreated plants reflects a basal defense response, while Moringa-treated plants displayed an amplified and targeted defense reaction-consistent with elicitor-induced resistance (**Nürnberger & Kemmerling, 2009**). These findings align with earlier reports in other plant-virus systems. For instance, **Tariq et al. (2022)** reported a negative correlation between phenolic accumulation and Tomato spotted wilt virus (TSWV) replication in pepper. The current results suggest a similar mechanism in faba bean, wherein phenolics may restrict BYMV replication and movement either directly or through enhanced structural defense and signaling. Moreover, the phytochemical richness of *Moringa oleifera*, including flavonoids (e.g., quercetin, kaempferol), chlorogenic acid, and caffeic acid, has been previously linked to antiviral activity through the activation of key enzymes in the phenylpropanoid pathway (**Saini et al., 2024**). Peroxidase activity patterns provide additional insight into the defense priming capability of *M. oleifera*. The elevated POD levels in the MV treatment, similar to healthy plants, suggest that pre-infection Moringa treatment effectively primes the antioxidant machinery. The lower POD activity in the virus-only and Moringa-only groups supports the concept that *M. oleifera* does not act as a constitutive inducer of defense in unstressed plants but rather enhances enzyme activity in response to pathogen-associated molecular patterns (PAMPs). This context-dependent activation is a hallmark of induced systemic resistance (ISR), wherein pre-treated plants respond more rapidly and robustly to biotic stress (**Conrath et al., 2001**). These observations are corroborated by other studies where Moringa extract enhanced antioxidant enzyme activity under stress conditions (**Ibrahim & Namich, 2021**). **Abir-ul-Islam et al. (2022)** and **Mehmood et al. (2021)** reported similar increases in POD and other ROS-scavenging enzymes in maize and Nigella sativa, respectively, under salinity and drought stress. This supports the hypothesis that Moringa's effect is not merely nutritional but involves specific biochemical modulation of the plant's defense pathways. The modulation of PAL activity by Moringa extract further emphasizes its role as a defense elicitor. PAL is a key enzyme in the phenylpropanoid pathway, contributing to the biosynthesis of lignin, flavonoids, and phytoalexins-all critical in pathogen defense. The highest PAL activity was recorded in VM-treated plants, suggesting a therapeutic boost in secondary metabolism following viral infection. This mirrors findings in faba bean and sugar beet where Moringa seed extract elevated PAL activity under fungal and fungal-like pathogen stress (**Abdel-Monaim et al., 2017; Sehsah et al., 2022**). The observation that PAL levels were lowest in Moringa-only (unstressed) plants reinforce the notion that Moringa acts synergistically with pathogen cues to amplify defense, rather than initiating these pathways in isolation. Catalase activity also followed a consistent pattern with other antioxidant enzymes. The significant elevation in both VM and MV treatments-well above levels in infected or healthy controls-highlights Moringa's capacity to boost redox defense mechanisms during viral stress. Importantly, minimal CAT activity in the Moringa-only treatment confirms that enzyme induction is stress-dependent. This priming behavior is consistent with findings by **Azeem et al. (2023)**, who demonstrated that Moringa-derived compounds regulate ROS-detoxifying genes, likely via activation of transcription factors such as WRKY and NPR1. The mitigation of oxidative stress through CAT, especially during early viral infection when ROS levels spike, may help preserve cell integrity and limit virus movement (**Khan et al., 2025**). Taken together, these biochemical responses strongly support the dual role of *Moringa oleifera* extract in both direct antiviral action and host defense enhancement. Its application-particularly as a post-infection foliar spray-resulted in elevated phenolic content and enzymatic activities that are well known to correlate with enhanced resistance to viral infection. The synergy between primed antioxidant pathways and phenolic metabolism appears central to the observed reductions in BYMV accumulation. From a practical standpoint, foliar application of *Moringa oleifera* at 50 mg/mL appears to offer an environmentally safe and cost-effective strategy for managing BYMV in legume crops. Its ability to activate key physiological defenses without imposing metabolic burden on unstressed plants underscores its potential as a sustainable biostimulant within integrated pest management (IPM) frameworks. Future research should explore the transcriptomic and metabolomic profiles of Moringa-treated plants to identify specific regulatory networks and bioactive markers associated with viral suppression. Such insights will advance our understanding of how natural elicitors can be harnessed for eco-friendly crop protection.

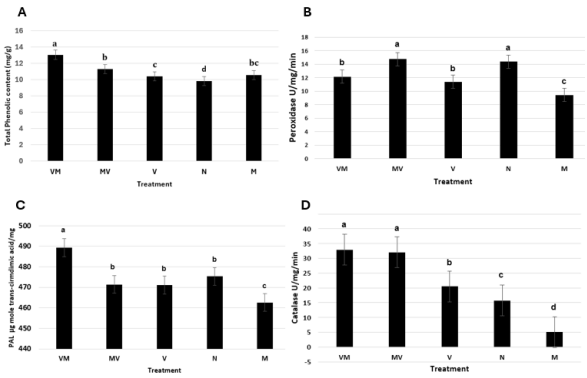

Fig. 4. Biochemical responses in *Vicia faba* leaves under different *Moringa oleifera* foliar spray treatments. Treatments include N (healthy control), MV (spray 24 h before virus inoculation), VM (spray 24 h after inoculation), M (Moringa only), and V (virus-infected untreated control). Data represent means ± SE of three replicates. Different letters indicate statistically significant differences (Bonferroni post-hoc test, $p < 0.05$). (A) Total phenolic content. (B) Peroxidase activity. (C) Phenylalanine ammonia-lyase (PAL) activity. (D) Catalase activity.

This study provides compelling evidence for the antiviral efficacy of aqueous *Moringa oleifera* leaf extract against Bean yellow mosaic virus (BYMV) in *Vicia faba* under controlled greenhouse conditions. Among the tested treatment regimes, foliar application at 50 mg/mL post-infection (VM) exhibited the most pronounced antiviral effect, achieving an 88% reduction in viral accumulation. This treatment also significantly elevated total phenolic content and enhanced the activity of key defense-related enzymes, including catalase (CAT), peroxidase (POD), and phenylalanine ammonia-lyase (PAL), indicating its role in priming host defense mechanisms. Phytochemical profiling via GC-MS analysis revealed a diverse array of bioactive constituents-such as phytol, hexadecanoic acid, and oleic acid-with known antiviral and antioxidant properties. These compounds likely contribute to the suppression of BYMV replication, and the stimulation of plant immune responses observed in this study. Collectively, the findings suggest that *M. oleifera* extract offers a promising, eco-friendly, and sustainable biocontrol strategy for managing viral infections in leguminous crops. Its dual function as both an antiviral agent and a defense elicitor presents a valuable alternative to synthetic chemical treatments. Nonetheless, further investigations—including multi-season field trials and molecular-level studies-are essential to fully elucidate its mechanisms of action and optimize its practical application within integrated viral disease management programs.

Consent for publication:
All authors declare their consent for publication.

Author contribution:
The manuscript was edited and revised by all authors.

Conflicts of Interest:
The author declares no conflict of interest.

Authors' contributions: All authors contributed equally to the development and execution of this study. Eman M. EL-Abagy, Sawsan M. Saied, Amira M. E. Aly, Radwa M. Shafie, Ahmed A. Kheder, and Hayam S. Abdelkader collaborated in the conceptualization, design, and implementation of the research. The team collectively performed the virus isolation, ELISA assays, enzymatic analyses, infection and treatment experiments, RT-PCR, sequencing, and data analysis. Manuscript preparation, writing, and revision were carried out jointly, with all authors actively involved in drafting, editing, and providing critical feedback. All authors have read and approved the final version of the manuscript.

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