MARINE MACROALGAL EXTRACTS AS SUSTAINABLE BIOCONTROL AGENTS: SUPPRESSION OF TOBACCO MOSAIC VIRUS IN TOMATO THROUGH INDUCED RESISTANCE MECHANISMS

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Abstract. This study aimed to isolate and identify the most prevalent and economically significant viruses causing severe yield losses in tomato crops, and to evaluate the potential of *Turbinaria ornate* and *Sargassum vulgare* extracts as biotic inducers of systemic acquired resistance (SAR) against tobacco mosaic virus (TMV). Viral isolates were confirmed through biological and serological assays, and the two marine macroalgal extracts were applied individually at 50% (v/v) to tomato plants. Foliar treatments administered every 15 days under greenhouse conditions were assessed for their effectiveness in inducing resistance. Both extracts effectively activated SAR, suppressed TMV infection, and triggered the expression of the pathogenesis-related (PR-1a) gene, confirmed through sequencing and comparison with GenBank records. The results showed that these bioelicitors significantly enhanced resistance in tomato plants by activating defence pathways. In conclusion, marine macroalgal extracts are promising, cost-effective, and environmentally sustainable agents for integrated crop protection.
Keywords: *TMV, tomato, macroalgae, Turbinaria, Sargassum, resistance*

Introduction

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated members of the Solanaceae family, which also includes economically important crops such as tobacco, potato, and chilli pepper (Ilyas and Ahmad, 2023). As an indeterminate vine producing edible berries, the tomato is valued worldwide as both a staple food and a cash crop. However, its cultivation is highly vulnerable to environmental stressors. In temperate regions, tomatoes are typically grown as annuals because their tissues are susceptible to freezing; frost exposure damages cellular structures, impairs physiological processes, and ultimately reduces yield (Kwak et al., 2021; González and Pålsson, 2023). Controlled environment agriculture, especially greenhouse systems, has partially alleviated these challenges by moderating thermal stress and extending productive cycles (Zhu et al., 2022). Recent advances in molecular breeding and crop management have also improved

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chilling tolerance, reflecting broader efforts to stabilise production amid increasingly unpredictable climatic conditions (Kwak et al., 2021; González and Pålsson, 2023).

Plant viral diseases remain among the most serious biotic constraints to tomato production, with Tobacco mosaic virus (TMV) is one of the most extensively studied plant viruses. Since its discovery in the late 19th century, TMV has played a pivotal role in shaping modern plant virology. Beijerinck's demonstration in 1898 that TMV was neither fungal nor bacterial but a novel infectious agent requiring host cells for replication established the foundation of virology as a discipline (Hull, 2014; Zhu and Scholthof, 2020). TMV has since become a model system for elucidating viral structure, replication, systemic movement, and host–virus interactions, while also contributing to biotechnology and nanoscience applications through its well-characterized virion architecture (Domingo-Calap and Sanjuán, 2016; Zhu and Scholthof, 2020; Amin and Kühne, 2022).

TMV and related viruses, such as Tomato mosaic virus (ToMV), were historically devastating to the tobacco and tomato industries during the early 20th century. The deployment of resistant cultivars and strict seed sanitation measures has substantially reduced their prevalence. Nonetheless, TMV remains a persistent threat due to its broad host range and remarkable environmental stability. The rod-shaped virions (18 × 300 nm) contain a single-stranded RNA genome encapsidated by a protein coat, enabling long-term survival in infected debris and soil for up to two years under favorable conditions. TMV infects more than 200 plant species, particularly within Solanaceae (tomato, pepper, potato, eggplant) and Cucurbitaceae (squash, cucumber, pumpkin), as well as ornamentals such as begonia, verbena, geranium, and petunia. In most cases, plant mortality due to infection is low, although substantial yield and quality losses may occur. In tomato, infections cause mosaic-patterned mottling of leaves, chlorosis, stunting, and leaf malformations. Fruits may ripen unevenly, display rugose deformation, or develop necrotic ringspots, rendering them unmarketable (Hull, 2014). Current estimates indicate that while only ~1% of tobacco and ~20% of tomato crops are affected annually, the associated economic losses remain considerable.

The search for sustainable and environmentally safe alternatives to synthetic pesticides has increased interest in natural bioresources with antiviral potential. Marine macroalgae, especially brown species like *Turbinaria ornata* and *Sargassum vulgare*, are becoming promising candidates due to their rich content of bioactive metabolites, including minerals, amino acids, vitamins, and phytohormones such as auxins, cytokinins, and gibberellins (Senn, 1987; Stirk and Van Staden, 1997a,b). These compounds promote plant growth and enhance pathogen resistance, offering new options for safer biopesticides and organic crop protection strategies (Zhao et al., 2017; Shereen et al., 2018; Najeeb et al., 2025). Macroalgae produce a wide range of antiviral agents—including polysaccharides, polyphenols, alkaloids, flavonoids, and proteins—that have shown inhibitory effects against various plant viruses (Hamed et al., 2018). Previous research indicates that algal polysaccharides can prevent viral adsorption by competing with viral binding sites (Duarte et al., 2004). These compounds may interact with host cells, thereby blocking viral entry and limiting subsequent replication (Feldman et al., 1999). Additional studies have identified bioactive compounds such as betaines and dictyols from *Dictyota ciliolata* with cytotoxic and antiviral properties (Manzo et al., 2009; El Gamal, 2010), as well as kappa/beta-carrageenans from *Tichocarpus crinitus* that inhibit TMV infection in *Nicotiana tabacum* leaves (Nagorskaia et al., 2008a). Red algae also contain vitamin C, amino acids, peptides, omega-3 fatty acids, and proteins with notable antiviral effects (Dawczynski et al., 2007; MacArtain et al., 2007; Matanjun

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et al., 2009). Likewise, lectins derived from the green alga Ulva pertusa have demonstrated activity against TMV (Wang et al., 2004; Liu et al., 2005). Screening efforts further support the strong antiviral potential of several algal extracts, with some species showing inhibition rates exceeding 80% (Pardee et al., 2004).

Taken together, these findings suggest that marine macroalgae represent a valuable but underutilized resource in the development of biologically based antiviral agents for crop protection. However, systematic evaluations of their efficacy against TMV in tomato remain limited. Therefore, this study was conducted to assess the antiviral activities of *Turbinaria ornata* and *Sargassum vulgare* extracts against TMV infection in tomato plants. We hypothesize that bioactive compounds in these algae can significantly suppress viral infection and mitigate disease symptoms, thereby enhancing tomato growth and yield while offering a sustainable alternative to conventional chemical control measures.

Materials and methods

Disease incidence and frequency of virus(es)

Tomato (*Solanum lycopersicum*) crops in Riyadh, Saudi Arabia, with particular attention to their presence in both cultivated fields and local markets. Tomato represents one of the most important vegetable crops for Saudi agriculture and food security, yet its production is increasingly threatened by viral diseases. Documenting TMV infections in commercial farms and produce sold in local markets highlights not only the risk to growers but also the potential impact on market quality and consumer supply chains.

Field incidence was estimated at the plot level based on inspection/diagnosis of total plants, while symptomatic plants were preferentially used for laboratory isolation to ensure adequate virus titre for downstream assays.

Tomato is globally recognised as a crop of high economic and nutritional value, but it is highly susceptible to a wide range of pathogens, particularly viruses. TMV remains among the most significant viral agents, capable of causing yield reduction and fruit quality deterioration across diverse production systems. Effective detection and characterisation of TMV are therefore critical for disease management and for safeguarding tomato productivity (Hull, 2014; López-Moya and García, 2021).

To assess TMV incidence, symptomatic plants were surveyed in ten tomato farms (including smallholder/home-garden plots) in Riyadh, Saudi Arabia, during [Month–Month, Year]. Young, fully expanded leaves showing typical viral symptoms were collected using sterilised tools disinfected with 70% ethanol between samples to prevent cross-contamination. Samples were placed in labelled polyethene bags, maintained on ice during transport, and either processed immediately or stored briefly at 4 °C to ensure their suitability for serological (DAS-ELISA) and molecular (RT-PCR) diagnostics. Although symptomatic plants were preferentially sampled for isolation/propagation to maximise virus titre, asymptomatic infections can occur and may be detected by diagnostic screening when included.

A total of twenty-five samples displaying mosaic, mottling, crinkling, leaf deformation, and chlorotic patterns were collected and tested. Disease incidence and disease severity were calculated using standard plant disease assessment procedures (Campbell and Madden, 1990; Madden et al., 2007).

Disease incidence (%) = Number of infected plants per location / Total number of plants per location × 100

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Disease severity (DS%) = Σ (disease grade × number of plants in each grade) / (Total number of plants × highest disease grade) × 100

Serological detection of TMV

Tomato samples were examined serologically using the double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). The assay was conducted with a Tecan Infinite M200 PRO microplate reader using antisera specific to *Tobacco mosaic virus* (TMV) supplied by LOEWE Company (BIOREBA GmbH), and followed standard protocols for plant virus detection (Hull, 2014; López-Moya and García, 2021).

Virus isolation, propagation, and identification

Mechanical inoculation was employed to isolate and propagate TMV. Infectious sap was extracted from TMV-infected tomato leaves in 0.1 M phosphate buffer (pH 7.0) containing 1% sodium sulphite (1:2 w/v). Healthy test plants were dusted with 400-mesh carborundum and gently rubbed with the prepared inoculum, while buffer-treated plants served as negative controls. Inoculated plants were maintained in a controlled greenhouse at 25 ± 2 °C (day)/20 ± 2 °C (night), under a 16-h photoperiod (200–250 μmol m^{-2} s^{-1} PPFD) and 55–65% relative humidity. Environmental conditions were continuously monitored and regulated to ensure uniformity across replicates. Viral infection was confirmed serologically using TMV-specific antisera (Adams et al., 2022).

Host-range and test plants. To assess the biological host range of the TMV isolate, thirteen plant species representing four botanical families were selected. The panel included economically important solanaceous hosts and cultivars, standard indicator hosts for local lesion assays (e.g., Chenopodium spp.), and additional representative species to provide taxonomic diversity and facilitate symptom comparison. Such a panel is commonly used for biological characterisation of tobamoviruses and supports reproducible symptom-based assessment (Lee et al., 2011; Hull, 2014). The tested species and their reactions are presented in *Table 1*.

For induction assays, one-month-old seedlings were foliar-sprayed with *Turbinaria ornata* or *Sargassum vulgare* extracts (100–300 mg/L) either 24 h before or 48 h after inoculation. Symptomatic plants were sampled at 7- and 25-days post-treatment, and infection was confirmed by DAS-ELISA, RT-PCR, and transmission electron microscopy (TEM) (López-Moya and García, 2021; Adams et al., 2022).

Marine macroalgal material and extract preparation

Marine brown macroalgae (*T. ornata* and *S. vulgare*) were collected from the Red Sea coast (Amlaj region – Jeddah, Saudi Arabia) in 2022. Identification and authentication were performed in collaboration with King Abdulaziz University (Jeddah) and verified by Dr. Sarah Al-Rashed (King Saud University, Riyadh).

Phytochemical analysis confirmed that *T. ornata* contained 18.44 μg gallic acid/mg extract of total phenolics, 10.33 μg rutin/mg of total flavonoids, and high levels of glutamic acid (9.91%) and aspartic acid (8.68%). It also contained 264.72 μg/g glucose and 63.32 μg/g fructose. *S. vulgare* showed higher phenolic content (25.49 μg gallic acid/mg), with notable amino acids such as glutamic acid (11.74%), alanine (7.26%), and glycine (5.00%), alongside 58.45 μg/g disaccharides and 142.84 μg/g glucose. These compounds are known for their antioxidant, antiviral, and elicitor activities.

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Stock aqueous extracts were prepared by dissolving 1 g of dried algal material in 1 L of distilled water heated to 65 °C. Filtrates were passed through sterilized muslin cloth and stored at 4 °C until use.

Phytochemical characterization of macroalgal extracts. Dried algal biomass was macerated overnight at room temperature in boiled distilled water, filtered, and concentrated under reduced pressure using a rotary evaporator to obtain crude aqueous extracts. Total phenolic content (TPC) was determined by a microplate Folin–Ciocalteu assay (Attard, 2013): 10 µL of extract (10 mg/mL) or gallic acid standard was mixed with diluted Folin reagent and 1 M Na_2CO_3, incubated for 20 min (25°C, dark), and read at 630 nm. Total flavonoid content (TFC) was measured by the aluminium chloride microplate method (Kiranmai et al., 2011): 15 µL of extract (20 mg/mL) was mixed with methanol, 1.25% $AlCl_3$ and 0.125 M sodium acetate, incubated for 5 min, and read at 420 nm; results were expressed as µg rutin/mg extract. Proximate composition (lipids, carbohydrates, proteins) and GC–MS profiling were performed following established protocols and instrument conditions described below.

Experimental design and treatments

Tomato seedlings (*Solanum lycopersicum* L.) at one month of age were transplanted into clay pots (25 cm diameter), with five seedlings allocated per pot. A total of 53 pots were used. Upon reaching the four-leaf stage, plants were treated via foliar spraying to runoff (~30 mL per plant) using aqueous algal extracts. The aqueous extracts of *Turbinaria ornata* and *Sargassum vulgare* were applied at five graded concentrations (100, 150, 200, 250, and 300 mg/L) to enable a rigorous dose–response assessment and to identify both the minimum effective concentration and the optimal concentration window for each extract. This low-to-moderate concentration range is consistent with foliar application rates commonly used for seaweed-derived biostimulants/elicitors in previous studies and is expected to elicit measurable defense-related and physiological responses without confounding effects associated with overly concentrated preparations (du Jardin, 2015; Battacharyya et al., 2015; Ali et al., 2016; Shukla et al., 2019). Moreover, the use of multiple concentrations is necessary to distinguish concentration-dependent antiviral/induced resistance effects and to avoid drawing conclusions based on a single arbitrary dose (Vera et al., 2011; Abdelkhalek et al., 2020). Treatments were arranged in a completely randomized design (CRD). Each treatment included 4–5 replicate pots (total = 53 pots), and the pot was considered the experimental unit. Experimental groups included plants sprayed with *Turbinaria ornata* extract (100–300 mg/L) either 24 h prior to Tobacco mosaic virus (TMV) inoculation or 48 h post-inoculation, and plants treated with *Sargassum vulgare* extract (100–300 mg/L) under the same pre- and post-inoculation intervals. Two control groups were included: a healthy, non-inoculated control (H), and an infected, untreated control inoculated with TMV only (V). Seven days following foliar treatment, plant samples were harvested for resistance assays, while a subset of plants was mechanically inoculated with TMV using 10−1 diluted viral sap. Healthy control plants were mock-inoculated using phosphate extraction buffer. Final samples for molecular and biochemical analyses were collected 25 days post-inoculation. Throughout the experimental period, plants were monitored daily for symptom development. Chenopodium amaranticolor was employed as an indicator host for both qualitative and quantitative virological assays. The percentage of infection was determined relative to the infected control, and the reduction of infection (RI%) was calculated as:

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$$Reduction of Infection (RI = \frac{Control - treated x 100}{Control} \quad \text{(Eq.1)}$$

Disease severity was assessed weekly up to 25-days post-inoculation.

Microscopy studies

Light microscopy

Leaf sections (15–17 µm thick) from the midrib region of the 4th apical leaf were fixed in FAA, stained with safranin O and fast green (Ruzin, 1999), and examined using an SEIWA OPTICAL light microscope (10×). Epidermal hair counts and histological observations were recorded and analyzed with Image Manager 50 software (Hull, 2014; Kharadi et al., 2021).

Transmission Electron Microscopy (TEM)

Representative tissues were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated, and embedded in epoxy resin. Ultrathin sections (~70 nm) were stained with uranyl acetate and lead citrate, then observed under a JEOL JEM-1400 TEM (80 kV) equipped with a Gatan Orius SC1000 CCD camera.

Biochemical and physiological analyses

- *Salicylic Acid (SA):* Free and total SA were extracted and quantified by HPLC following Raskin et al. (1989) with modifications (Salem, 2004).
- *Enzyme Assays:* Peroxidase (POD) activity was measured spectrophotometrically using guaiacol and H_2O_2 (Hammerschmidt et al., 1982). Polyphenol oxidase (PPO) activity was assayed by monitoring quinone formation from catechol at 420 nm (Coseteng and Lee, 1978).
- *Photosynthetic Pigments:* Chlorophyll a, b, and carotenoids were determined according to Wettstein (1957) using 85% acetone extracts.
- *RNA Content:* Total RNA was quantified using the orcinol reaction (Lee et al., 2011).

Molecular detection of TMV

Tobacco mosaic virus (TMV) infection in tomato plants was assessed using a combination of serological and molecular techniques. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was first performed on pooled leaf samples, of which 55% tested positive for TMV. To validate these findings, quantitative PCR (qPCR) was conducted on representative samples. In the infected controls, amplification curves from samples A and B appeared at cycles 30–32, with high fluorescence intensities indicative of elevated viral RNA accumulation. In contrast, tomato plants treated with *Turbinaria ornata* and *Sargassum vulgare* extracts displayed delayed and attenuated amplification signals, suggesting markedly lower viral loads. The reduction in qPCR signal was consistent with the DAS-ELISA results, reinforcing the reliability of the detection. Together, these complementary methods confirmed that seaweed extracts mitigated TMV infection by reducing viral replication and accumulation, likely through induced resistance mechanisms involving secondary metabolites and defense-related enzymes. Recent studies have emphasized the utility of combining serological and molecular diagnostics to provide robust and sensitive detection of plant viral pathogens in resistance-induction experiments (Pecman et al., 2017).

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Statistical analysis

Data from each treatment panel (*T. ornata*–TMV, *S. vulgare*–TMV) were analyzed separately. Results were expressed as mean ± SE (n = replicate pots per treatment; 4–5 biological replicates; total = 53 pots). Analyses were performed using IBM SPSS Statistics and R. One-way ANOVA was applied, with Shapiro–Wilk and Levene tests used to verify normality and homogeneity (Brown and Forsythe, 1974; Ghasemi and Zahediasl, 2012). Nonparametric Kruskal–Wallis tests were applied when assumptions were violated (Blanca et al., 2017). Post-hoc comparisons used Fisher's LSD ($\alpha = 0.05$), with groupings reported on figures (Gomez and Gomez, 1984). Forest plots presented effect sizes (absolute and percent differences) with 95% confidence intervals (Cumming, 2014), while heatmaps visualized per-variable z-scores (Atia et al., 2021; Omar et al., 2021).

Results

Tomato leaf samples displaying characteristic viral symptoms, including mosaic, mottling, blistering, crinkling, vein yellowing, and malformation, were tested using DAS-ELISA with TMV-specific antisera. The serological assay confirmed the presence of TMV in the collected samples, with disease incidence values presented in *Figures 1 and 2*. Infected plants exhibited severe symptoms on tobacco indicator hosts, validating the pathogenicity of the isolate. Based on these findings, the present study was designed to evaluate the ability of marine macroalgal extracts to induce systemic acquired resistance in tomato plants against TMV infection.

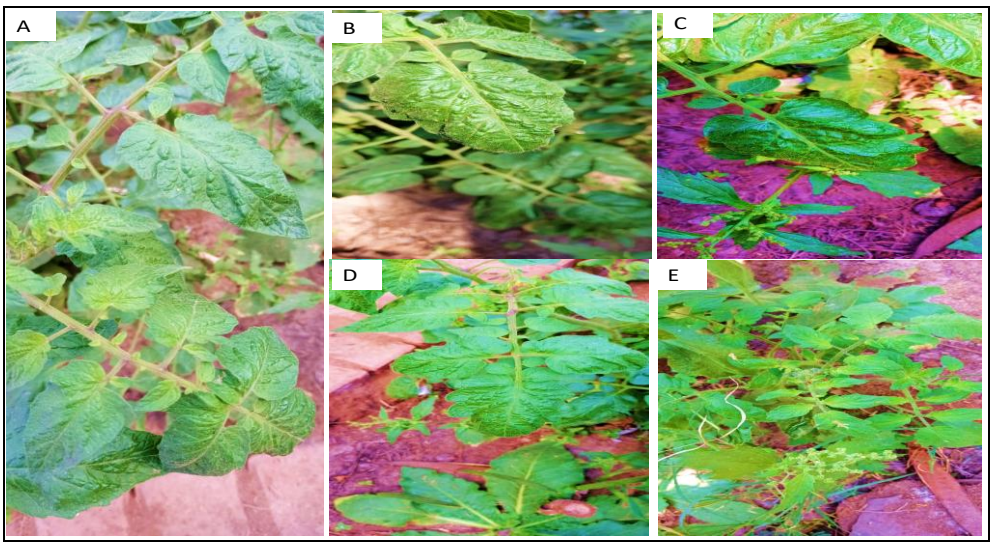

Figure 1. *Tomato leaves showing different types of natural infection symptoms: - (A) Healthy control. (B) tomato leaves showing blisters. (C) mottling. (D) mosaic and crinkle. (E) yellowing, malformation and erecting*

Serological screening of field-collected leaves. DAS-ELISA readings at 405 nm revealed marked variability in TMV antigen accumulation across 25 tomato samples relative to the negative control *Figure 2*. Using a positivity threshold defined as [2× the negative control (Aneg) / meanneg + 3SD], the majority of samples were classified as

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positive. In contrast, a small subset fell near the cut-off and were considered equivocal. These borderline samples were earmarked for confirmatory RT-PCR. The overall ELISA pattern is consistent with field symptomatology (mosaic/blistering) and with downstream molecular evidence, supporting a high TMV incidence in the surveyed sites.

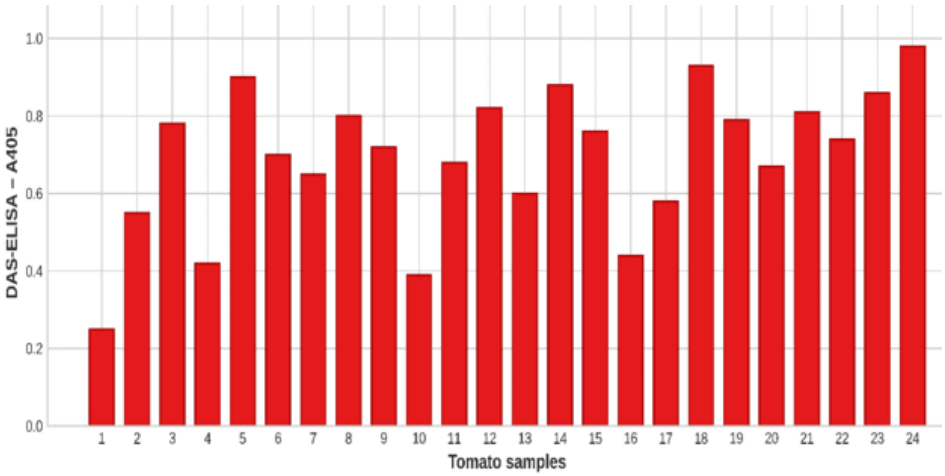

Figure 2. *Incidence of Tobacco Mosaic Virus associated with symptomatic tomato samples*

Confirmation of Tobacco mosaic virus (TMV)

The host range of the TMV isolate was assessed by mechanical inoculation of thirteen plant species representing four botanical families (*Table 1*). Based on symptom expression, responses were categorized into three groups. The first group included plants that developed local lesions: *Chenopodium amaranticolor*, *C. quinoa*, and *C. murale* exhibited chlorotic local lesions. In contrast, *Datura metel* developed necrotic lesions on inoculated leaves approximately 10-days post-inoculation. The second group comprised species that exhibited systemic infection. *Nicotiana glutinosa* developed severe mosaic symptoms accompanied by leaf malformation, whereas *N. tabacum* cv. Samsun displayed pronounced mosaic patterns and blistering. Tomato plants showed vein clearing, mosaic, vein necrosis, and blistering, while cucumber plants developed severe systemic mosaic symptoms. The third group consisted of species that remained symptomless, including *Vigna unguiculata* and *Vicia faba*, which did not display visible symptoms under the tested conditions (*Figure 3*).

Evaluation of marine macroalgae as antiviral agents against TMV

Antiviral activity assessment

Extracts from two brown marine macroalgae, *Turbinaria ornata* and *Sargassum vulgare*, were evaluated for their antiviral potential against *Tobacco mosaic virus* (TMV) in tomato plants. Treatments were applied both prior to and following inoculation to examine their protective and curative effects. Antiviral activity was assessed through multiple approaches, including histopathological observations, biochemical analyses [antiviral protein expression, peroxidase (POD) and polyphenol oxidase (PPO) activities], and phytochemical profiling [salicylic acid (SA), chlorophyll content, phenolic compounds, and total phenols]. The biological impact of algal extracts on viral infectivity was further validated through microscopy and symptomatology.

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Table 1. *The reactions of plant host species and cultivars inoculated with TMV isolate*

Families	Host plant	Symptoms	DAS-ELISA
Chenopodiaceae	quinoa Wild.	CLL	0.865
	C. amaranticolor	CLL	0.766
	C. murale	CL	0.875
Cucurbitaceae	Cucumis sativus L.	SM	0.566
	Cucurbita pepo L.	SM	0.655
Leguminosae	Phaseolus vulgaris L.	M	0.484
	Vigna unguiculata L.	NS	0.288
	Vicia faba L.	NS	0.265
Solanaceae	Datura metel L.	NLL	0.657
	esculentum	SM, Mf , S, yell	0.657
	Capsicum spp	M, chl.	0.878
	tabacum L., cvs. Samsun	SM, Mf	0.899
	N. glutinosa	SM	0.798

M mosaic and leaf distortion, stunting, and necrosis. CLL = Chlorotic local lesion, CHL = Chlorosis, NLL = Necrotic local lesion, M = Mosaic, S= Stunting, SM = Severe mosaic, Mf = Malformation, NS = No symptoms

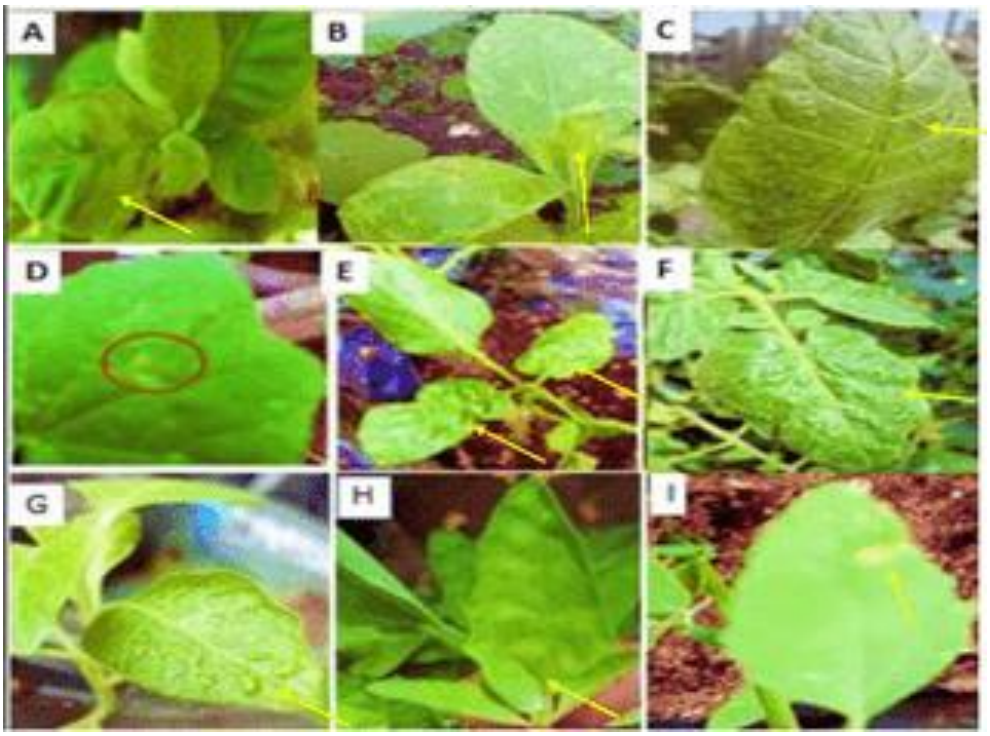

Figure 3. *Plant leaves inoculated with TMV isolate showing.:(A&B) Nicotiana glutinosa appeared severe mosaic and malformation. (C) N. tabaccum cv. Samsun showing severe mosaic and blisters. (D) cucumber plants showing chlorotic spotsc. (E) pepper plants showing mosaic and chlorosis. (F) tomato plants showing vein clearing, mosaic, vein necrosis, blisters. (G), Datora metal, necrotic local lessions and mosaic. (H) Chenopodium amaranticolor, systemic chlorotic spots. (I) Chenopodium quinioa Necrotic local lession*

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Effects on TMV infectivity

Application of *T. ornata* and *S. vulgare* extracts significantly suppressed TMV replication compared to untreated infected controls, which developed severe mosaic symptoms and stunting (*Figures 4 and 5*). Notably, plants treated with higher concentrations (250 and 300 mg/L) exhibited complete suppression of symptom development when applied 48 h post-inoculation, suggesting strong antiviral protection.

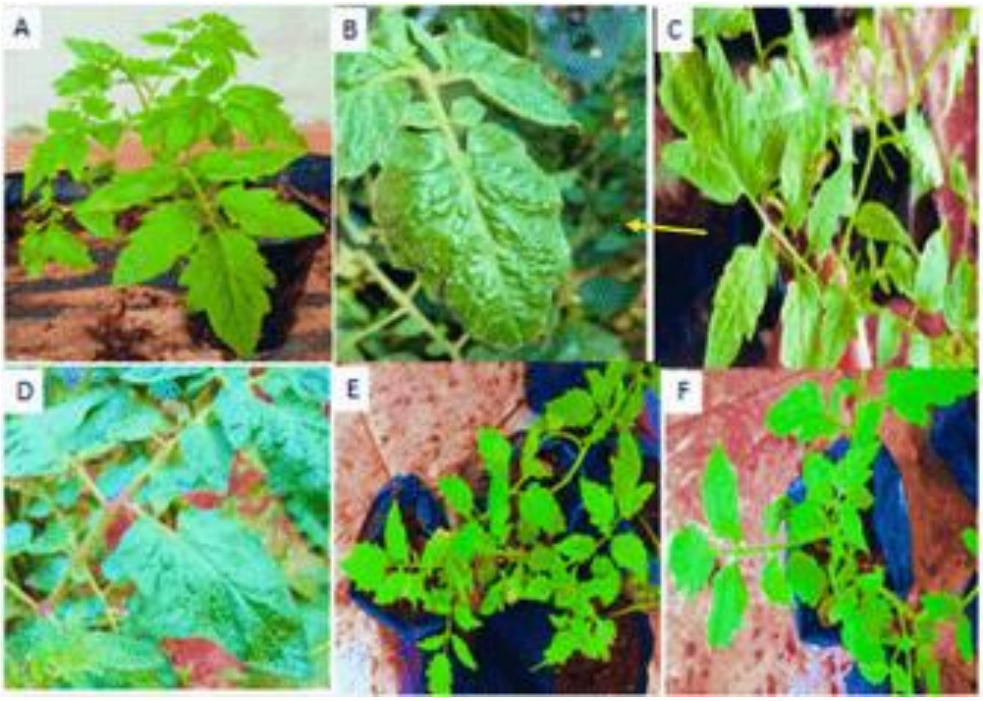

Figure 4. *Antiviral activity of different concentrations of Turbinaria (Turbinaria ornata) marine macroalgae on TMV infectivity in tomato plants. (A) Healthy control. (B) Tomato leaf treated with 100 mg / L of T. omata shown blister symptoms. (C) Tomato leaf treated with 150 mg / L of T. omata shown mild TMV symptoms. (D) Tomato leaf treated with 200 mg / L of T. omata shown very mild TMV symptoms. (E) Tomato leaf treated with 250 mg / L of T. omata showed no symptoms. (F) Tomato leaf treated with 300 mg / L of T. omata shown no symptoms*

At lower concentrations (100–200 mg/L), tomato plants treated with *T. ornata* showed partial protection with milder symptom expression relative to the infected control, whereas *S. vulgare* produced comparatively modest effects at the same doses (*Figures 4 and 5*).

Transmission electron microscopy further supported these findings: plants treated with macroalgal extracts exhibited defective and incomplete viral particles, whereas untreated infected controls contained well-developed TMV virions. These results highlight the capacity of marine macroalgal metabolites to disrupt viral assembly and reduce infectivity.

Effect of marine macroalgae on viral accumulation

Application of marine macroalgal extracts markedly reduced TMV accumulation in tomato plants, with efficacy depending on extract type and concentration (*Table 2*).

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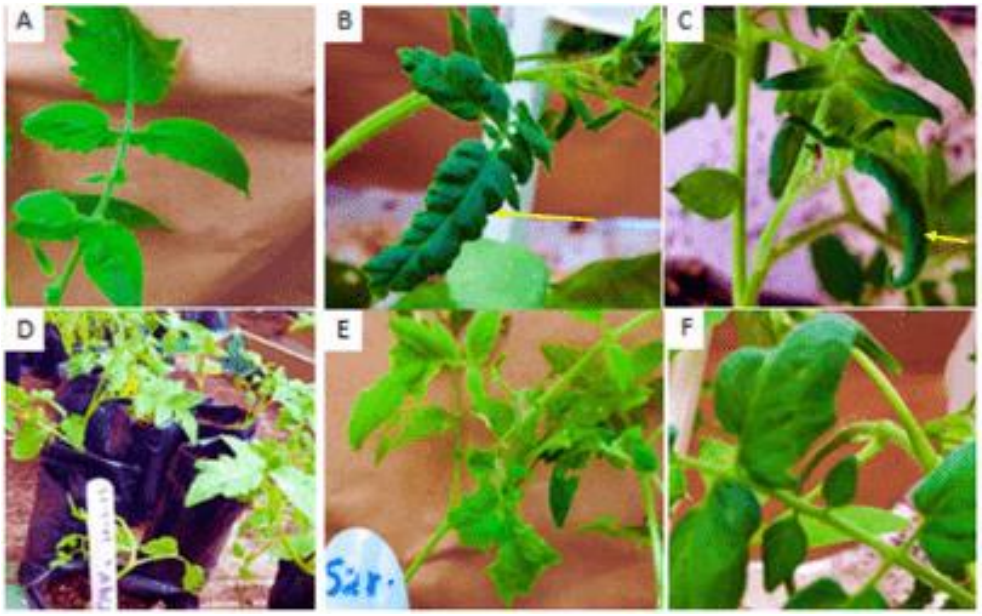

Figure 5. *Antiviral activity of different concentrations of Sargassum: (Sargassum vulgare marine macroalgae on TMV infectivity in tomato plants. (A) Healthy control. (B) Tomato leaf treated with 100 mg / L of S. vulgare shown blister symptoms. (C) Tomato leaf treated with 150 mg / L of S. vulgare shown mild TMV symptoms. (D) Tomato leaf treated with 200 mg / L of S. vulgare shown very mild TMV symptoms. (E) Tomato leaf treated with 250 mg / L of S. vulgare shown no symptoms. (F) Tomato leaf treated with 300 mg / L of S. vulgare shown no symptoms*

Table 2. *Effect of the marine macroalgae on virus concentration and accumulation in treated plants at tested concentrations compared to healthy and infected controls using ELISA reaction*

Concentration (mg/L)	Virus titre (optical density (OD) at 405 nm)					
	(*Turbinaria ornata*)			(*Sargassum vulgare*)		
	OD	+/ –	%	OD	+/ –	%
Positive untreated control	0.653^{e}	(+)	-	0.568^{d}	(+)	-
Negative healthy control	0.256^{a}	-	-	0.254^{a}	-	-
100	0.525^{b}	+	+	0.550^{a}	=	+
150	0.411^{c}	+	+	0.490^{b}	+	+
200	0.313^{d}	+	+	0.390^{c}	+	+
250	0.248^{e}	+	+	0.285^{d}	+	+
300	0.238^{e}	-	-	0.270^{d}	-	-
p≤0.05	0.04	-	-	0.032	-	-

(%): Efficiency index in reducing virus concentration over the positive control in samples reacting positively (+) with tested TMV antibodies. The proportion of reduction in each negative sample was considered with a value of 100%. Values with the same letters in each experiment were not significantly different. Values are means of three repeats ±s.e. (p≤0.05)

For *Turbinaria ornata*, all tested concentrations except 100 mg/L significantly decreased viral content when applied 48 h post-inoculation. The strongest inhibitory effects were observed at 250 and 300 mg/L, where optical density (OD) values reached 0.248 and 0.238, respectively, and were statistically indistinguishable from the healthy control, indicating a negative ELISA reaction. Treatments with 150 and 200 mg/L also

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produced significant reductions in viral accumulation (0.411 and 0.313 OD, respectively) compared with the untreated infected control.

In the case of *Sargassum vulgare*, reductions in TMV accumulation were observed across all tested concentrations except 100 mg/L. The most effective treatments were 250 and 300 mg/L, yielding OD values of 0.285 and 0.270, respectively, both comparable to the negative control. Moderate reductions were recorded at 150 and 200 mg/L (0.490 and 0.390 OD, respectively), whereas the lowest concentration (100 mg/L) showed no significant difference from the untreated control (0.550 OD).

These findings demonstrate that higher concentrations of both *T. ornata* and *S. vulgare* are capable of lowering viral accumulation to near-undetectable levels, highlighting their potential as effective biocontrol agents against TMV.

Histopathological changes

Histopathological alterations were evident in tomato leaf tissues following treatment with marine macroalgal extracts, providing clear evidence of their antiviral activity. Seven days after spraying, distinct tissue modifications were observed in treated plants compared with untreated controls (*Figure 6*).

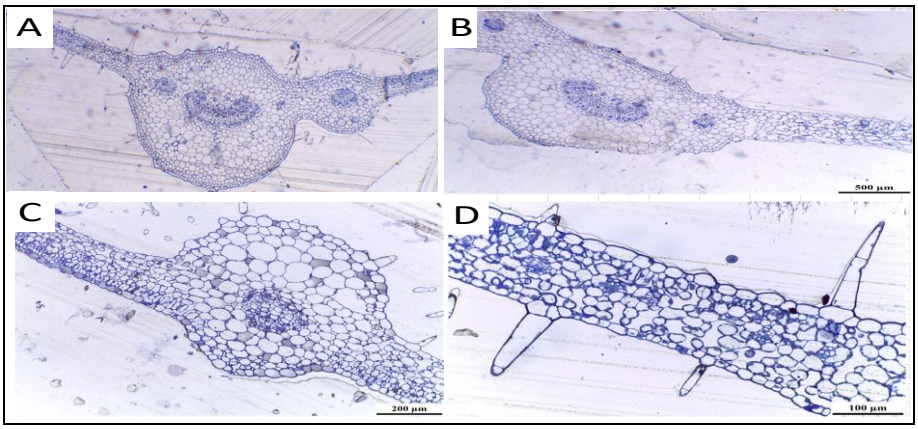

Figure 6. *Anatomical variations in tomato leaves infected with TMV and treated with marine macroalgae]. (A) Healthy control. (B) Infected control (C) tomato leaf treated with (Turbinaria ornata). (D) tomato leaf treated with Sargassum vulgare*

Notable changes included increased lignin deposition in epidermal cells, a higher number of epidermal hairs, thickening of the leaf blade, and an increase in both xylem arms and phloem layers. Additional alterations comprised tissue shrinkage, intense staining, and lignin precipitation in the substomatal cavities. Mesophyll cells exhibited folding and layering of the cell walls, along with remnants of palisade cell wall structures.

Collectively, these histological modifications suggest that *T. ornata* and *S. vulgare* extracts enhance structural defenses in tomato leaves, potentially contributing to reduced TMV replication and symptom expression.

Transmission Electron Microscopy

Ultrastructural examination of infected tomato leaves using TEM confirmed the presence of characteristic TMV particles (*Figure 7*). The virions appeared as rigid, rod-shaped structures, measuring approximately 18 × 300 nm, which corresponds to the

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typical morphology previously described for TMV. In some fields of view, partially aggregated rods were observed, forming parallel arrays, a common feature of tobacco viruses.

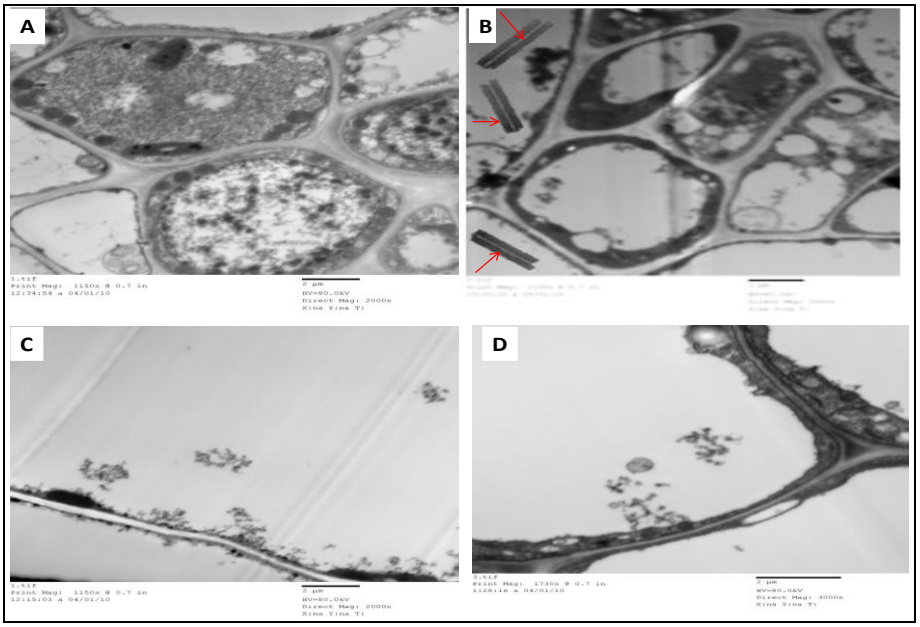

Figure 7. *Transmission electron micrograph of Tobacco mosaic virus (TMV) particle unit obtained from marini macroalgae -treated plants (b, c, d) and untreated control (a). Healthy plant, (b). inoculated plant, red arrows indicate the viral particles in the cells. (c) treated tomato plants with Turbinaria ornata. (d) tomato leaf treated with Sargassum: Sargassum vulgare treated plants. Photos were captured under direct magnification of 100 000 x with scale of 100 nm, HV=80.0 kV*

Leaf-dip preparations of symptomatic tissues consistently showed high densities of these particles scattered across the background, reflecting the systemic spread of the virus within host cells. In contrast, tissues from healthy control plants lacked such structures, confirming the specificity of the observations.

In addition to whole virions, incomplete or fragmented rod-shaped particles were occasionally detected, particularly in plants treated with algal extracts, suggesting an interference effect on viral assembly. Within infected cells, cytoplasmic inclusions resembling crystalline arrays of TMV particles were visible, often aligned along the cytoplasm and occasionally adjacent to organelles, consistent with massive viral replication sites.

Together, these ultrastructural features provided direct visual evidence of TMV infection in tomato plants and revealed differences between untreated and treated samples, as reflected in the density and integrity of viral particles.

Peroxidase activity in tomato plants sprayed with marine macroalgae

Enzyme activities (pre- and post-inoculation)

The activities of peroxidase (POD), polyphenol oxidase (PPO), and total phenols were quantified in tomato plants treated with marine macroalgal extracts, both before and after inoculation with TMV.

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For *Turbinaria ornata*, preventive treatments (pre-inoculation) elicited marked increases in defense enzyme activities. At baseline doses, POD, PPO, and total phenols reached 2.028, 1.521, and 129.02, respectively, with further increases at 300 mg/L (2.224, 2.508, and 167.42, respectively). In contrast, post-inoculation treatments produced comparatively lower responses: 250 mg/L yielded 1.946, 1.429, and 92.445, while 300 mg/L resulted in 2.328, 1.532, and 119.113 for POD, PPO, and phenols, respectively. These results indicate that pre-inoculation applications were more effective in enhancing biochemical defenses than post-inoculation treatments (*Figure 8*).

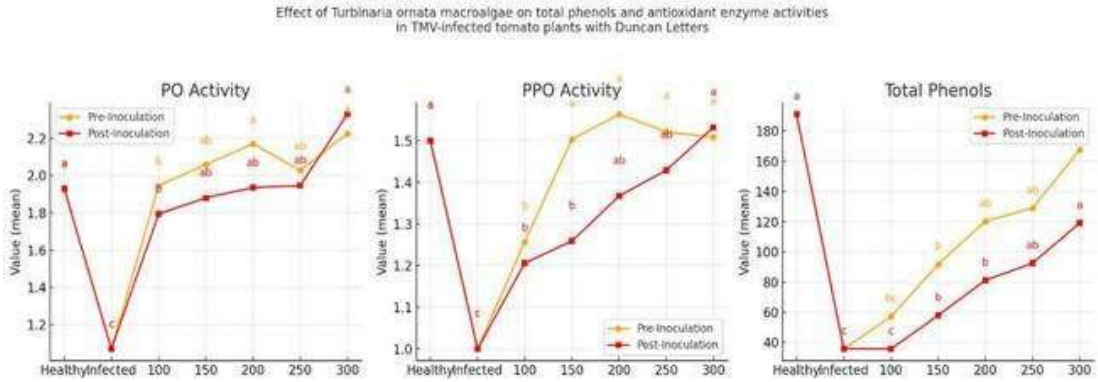

Figure 8. *Effect of Turbinaria ornata macroalgae on PO, PPO, and total phenol contents in TMV-infected tomato plant. Bars represent mean ± SE (n = replicate pots per treatment). Different letters indicate significant differences at p ≤ 0.05 (Fisher's LSD)*

For *Sargassum vulgare*, similar trends were observed. At 250 mg/L, POD, PPO, and total phenols recorded 2.258, 1.693, and 125.93, respectively, compared with 2.373, 1.742, and 158.504 under pre-inoculation conditions. At 300 mg/L, the respective values were 2.258, 1.709, and 133.75 (pre-inoculation) and 2.386, 1.759, and 166.147 (post-inoculation). In addition, *S. vulgare* treatment at 250 mg/L increased POD and PPO activities to 8.4056 and 11.552 U/g FW, significantly higher than the healthy control (6.914 U/g FW) (*Figure 9*).

Figure 8 illustrates the effect of *Turbinaria ornata* extracts on peroxidase (PO), polyphenol oxidase (PPO), and total phenol accumulation in tomato plants subjected to TMV infection. A clear trend emerges whereby both pre- and post-inoculation treatments enhance enzymatic activities and phenolic content compared with untreated controls. Notably, pre-inoculation generally induces stronger responses, indicating a priming effect that equips plants with heightened defensive capacity prior to viral challenge. The Duncan letters further confirm.

Figure 9 illustrates z-scored tomato responses under *Sargassum vulgare* treatments during TMV infection for the same defense-related indicators (POD, PPO, and total phenols). The separation of pre- and post-inoculation columns allows evaluation of whether pre-inoculation applications confer a "priming effect" relative to post-inoculation treatments. The alternating warm and cool bands across concentrations highlight heterogeneity in defense responses as a function of both dosage and timing. In particular, darker red cells mark doses associated with relatively elevated defense indicators, suggesting potential dose–response relationships.

As heatmaps provide descriptive visual patterns, the interpretation of "hotspots" should be cross-validated against inferential analyses, including post-hoc comparisons

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(e.g., LSD groupings) and uncertainty around means. This ensures that the observed color patterns reflect statistically meaningful differences rather than random variability or scaling artifacts.

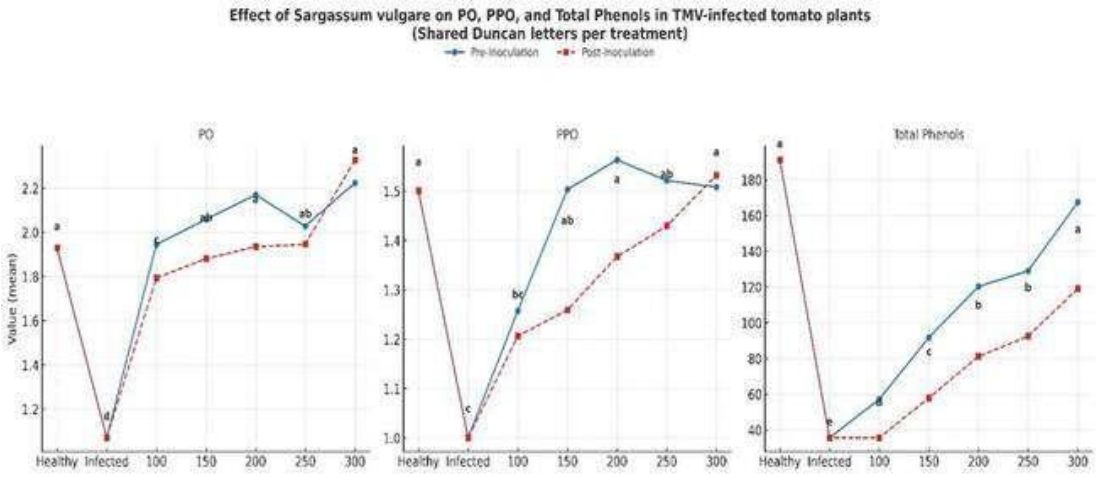

Figure 9. *Effect of Sargassum vulgare treatments on PO, PPO, and total phenols in TMV-infected tomato plants (Pre- and Post-inoculation), Bars represent mean ± SE (n = replicate pots per treatment). Different letters indicate significant differences at p ≤ 0.05 (Fisher's LSD)*

Leaf mineral, chlorophyll and proline contents

The results presented in *Figure 10* demonstrate that foliar application of marine macroalgae significantly enhanced nutrient concentrations in tomato leaf tissues compared with both healthy and infected controls.

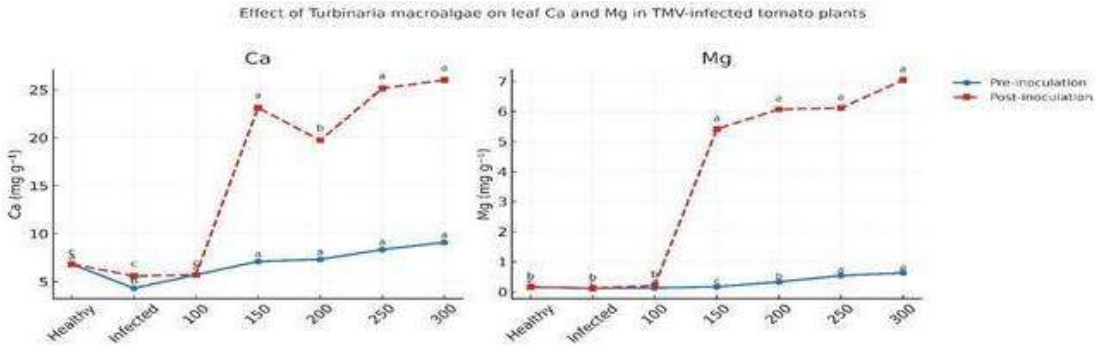

Figure 10. *Histogram showing the Effect of Turbinaria marine macroalgae on nutrient minerals contents in treated plants compared to healthy and infected untreated controls. Bars represent mean ± SE (n = replicate pots per treatment). Different letters indicate significant differences at p ≤ 0.05 (Fisher's LSD)*

Turbinaria ornata

Treatment with *T. ornata* extracts increased calcium (Ca) and magnesium (Mg) contents in tomato leaves. At 250 mg/L (pre-inoculation), Ca and Mg concentrations reached 8.35 and 0.54, respectively, compared with 6.84 and 0.16 in healthy controls and 4.32 and 0.12 in infected controls. At 300 mg/L, pre-inoculation treatments further

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elevated Ca and Mg to 9.10 and 0.63, respectively, whereas post-inoculation treatments resulted in comparatively lower values of 7.04 and 0.26.

Sargassum vulgare

Similarly, *S. vulgare* extracts substantially increased Ca and Mg accumulation. At 250 mg/L (pre-inoculation), Ca and Mg concentrations rose to 26.79 and 9.21, respectively, compared with 6.84 and 0.16 in healthy controls, and 5.72 and 0.12 in infected controls. At 300 mg/L, pre-inoculation values further increased to 26.95 and 9.85, whereas post-inoculation treatments recorded lower values of 18.03 and 2.02, respectively.

These findings highlight the ability of marine macroalgal extracts particularly at higher concentrations applied pre-inoculation—to improve the nutritional status of tomato plants. Enhanced mineral accumulation may contribute to improved metabolic function and increased resistance against TMV (*Figure 11*).

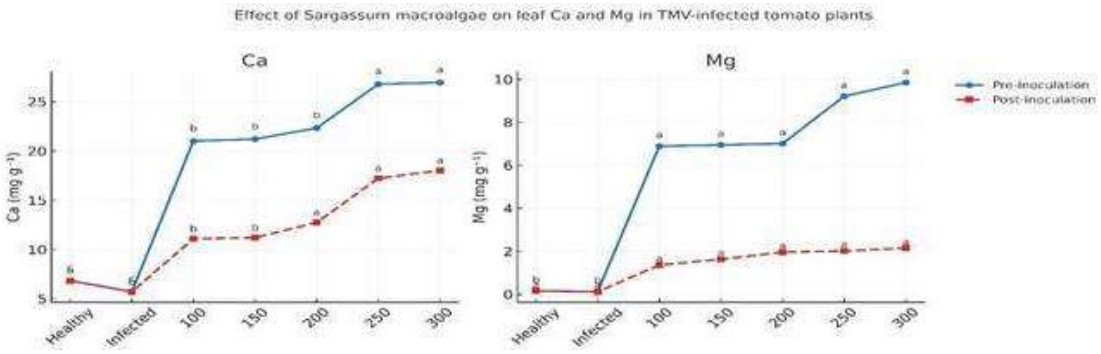

Figure 11. *Histogram showing the Effect of Sargassum marine macroalgae on nutrient minerals contents in treated plants compared to healthy and infected untreated controls.Bars represent mean ± SE (n = replicate pots per treatment). Different letters indicate significant differences at p ≤ 0.05 (Fisher's LSD)*

Photosynthetic pigments content

Inoculation with TMV markedly reduced chlorophyll and proline contents in tomato plants compared with non-infected controls. Infected plants recorded Chl a, Chl b, total chlorophyll, and proline contents of 3.8, 1.2, 5.04, and 0.76 mg/g FW, respectively, whereas healthy plants showed 8.0, 2.5, 10.6, and 1.82 mg/g FW.

Turbinaria ornata

Treatment with *T. ornata* extracts substantially improved chlorophyll and proline levels in infected tomato plants. At 250 mg/L (pre-inoculation), Chl a, Chl b, total chlorophyll, and proline reached 10.8, 3.4, 14.2, and 5.32 mg/g FW, compared with 10.09, 3.4, 13.4, and 4.96 mg/g FW post-inoculation. At 300 mg/L, pre-inoculation values were 10.4, 3.8, 11.2, and 5.32 mg/g FW, while post-inoculation values were slightly higher at 10.8, 3.8, 14.2, and 6.40 mg/g FW (*Figure 12*).

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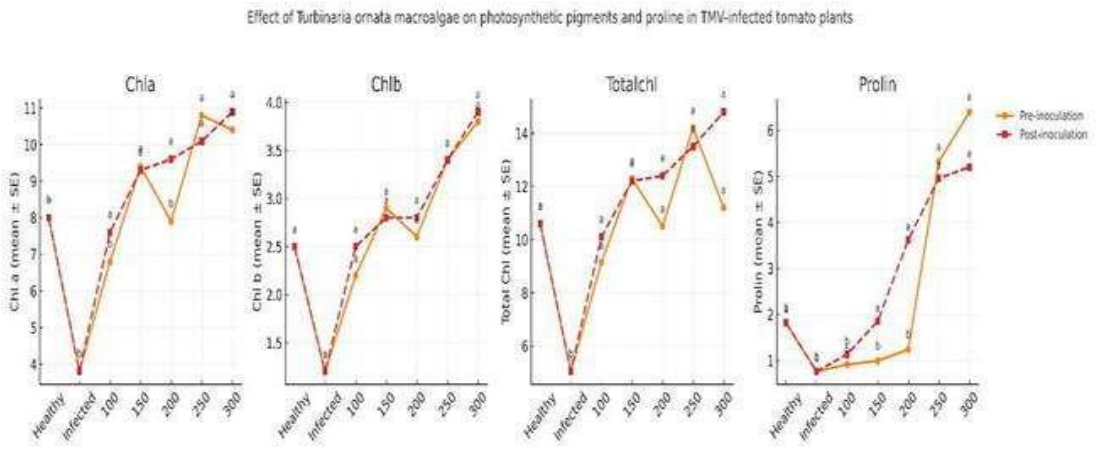

Figure 12. *Photosynthetic pigments and proline in tomato under Turbinaria ornata treatments during TMV infection (pre-and post-inoculation .Bars represent mean ± SE (n = replicate pots per treatment). Different letters indicate significant differences at p ≤ 0.05 (Fisher's LSD)*

Sargassum vulgare

A similar pattern was observed in *S. vulgare*-treated plants. At 250 mg/L (pre-inoculation), Chl a, Chl b, total chlorophyll, and proline contents were 11.5, 4.6, 16.2, and 4.17 mg/g FW, respectively, compared with 11.8, 3.8, 15.6, and 4.93 mg/g FW post-inoculation. At 300 mg/L, values further increased to 11.6, 4.9, 16.6, and 5.25 mg/g FW (pre-inoculation) and 14.1, 4.3, 18.4, and 5.73 mg/g FW (post-inoculation) (*Figure 13*).

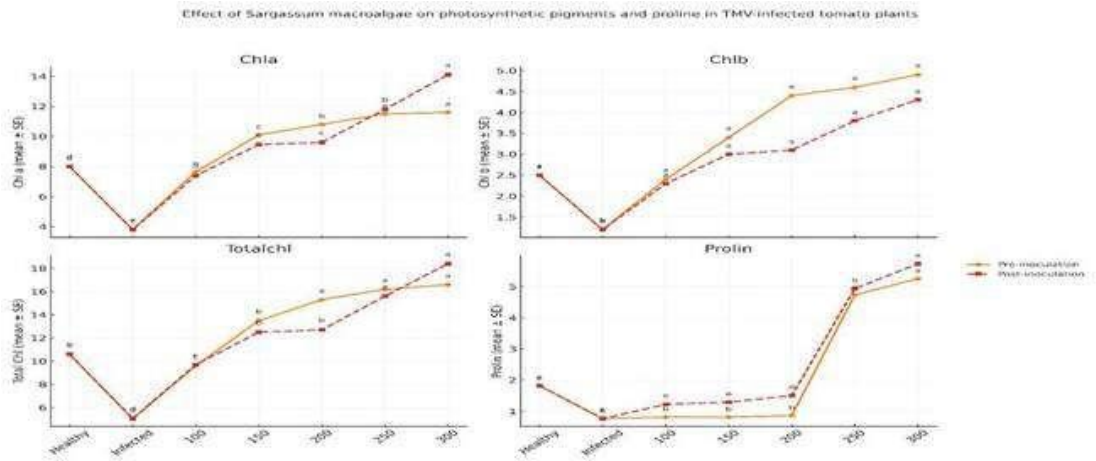

Figure 13. *Photosynthetic pigments and proline in tomato under Sargassum treatments during TMV infection (pre-and post-inoculation). Bars represent mean ± SE (n = replicate pots per treatment). Different letters indicate significant differences at p ≤ 0.05 (Fisher's LSD)*

Collectively, these results show that both *T. ornata* and *S. vulgare* enhanced chlorophyll and proline contents in TMV-infected tomato plants, with greater effects at higher concentrations. This increase in photosynthetic pigments and osmoprotectants provides additional evidence that marine macroalgal extracts contribute to induced resistance against TMV (*Figure 8*).

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Figure 12 presents a z-scored heatmap illustrating the relative responses of tomato plants to *Turbinaria ornata* treatments during TMV infection, focusing on photosynthetic pigments (Chl a, Chl b, total chlorophyll, carotenoids) and proline content. Standardization to z-scores within each variable enables visualization of relative changes across treatments. Warmer colors represent values above the mean for each variable, whereas cooler colors indicate values below the mean.

The treatments are arranged in the sequence Healthy → Infected → ascending doses, with columns distinguishing pre-inoculation (Pre) from post-inoculation (Post) applications. This organization facilitates direct assessment of dose–response patterns and the relative effectiveness of priming (preventive) versus curative treatments.

The statistical analysis was performed using ANOVA followed by Duncan's multiple range test to determine significant differences among treatments (*Figure 13*). Treatment means were compared at $p \leq 0.05$, and results were annotated with shared letters to indicate homogeneity groups. To enhance interpretation, results were visualized through multi-panel line graphs, showing pre- and post-inoculation responses across increasing macroalgal concentrations. Additionally, heatmaps were employed to illustrate standardized (z-scored) shifts in pigment and proline levels, thereby providing a comparative overview of treatment effects and enabling the identification of coordinated trends in tomato responses to macroalgal supplementation during TMV infection. This organization also allows qualitative comparison with the *Turbinaria* profile (*Figure 12*), highlighting similarities and differences in host responses to the two macroalgal treatments.

The integrative heatmap and PCA biplot together provide a comprehensive view of how *Turbinaria ornata* and *Sargassum vulgare* treatments influence tomato responses during TMV infection (*Figures 14 and 15*). The heatmap highlights clear contrasts between untreated infected plants, which exhibit strong reductions across pigments, proline, and mineral nutrients, and treated plants, which show marked recovery trends. *Turbinaria* treatments are more strongly associated with enhanced chlorophyll content and proline accumulation, whereas *Sargassum* treatments distinctly promote calcium and magnesium enrichment, suggesting complementary physiological pathways. The PCA biplot supports these findings, showing infected controls clustering apart from healthy plants, while macroalgal treatments—particularly at 250–300 mg/L—shift responses closer to healthy profiles. Chlorophyll variables largely drive PC1 variance, while proline and mineral nutrients contribute more to PC2, reflecting their roles in treatment-specific differentiation. Together, these figures demonstrate that both macroalgae mitigate disease severity through distinct yet synergistic mechanisms, reinforcing their potential as eco-friendly biostimulants against viral stress.

Quantification of phytohormones and salicylic acid (SA)

The quantification of phytohormones and total salicylic acid (SA) in tomato plants treated with marine macroalgal extracts, both before and after TMV inoculation, is presented in *Figures 16–19*. The results closely paralleled disease incidence, severity, and viral concentration, confirming that biochemical defense responses were strongly activated in macroalgae-treated plants compared with untreated controls (H = healthy control, V = inoculated control).

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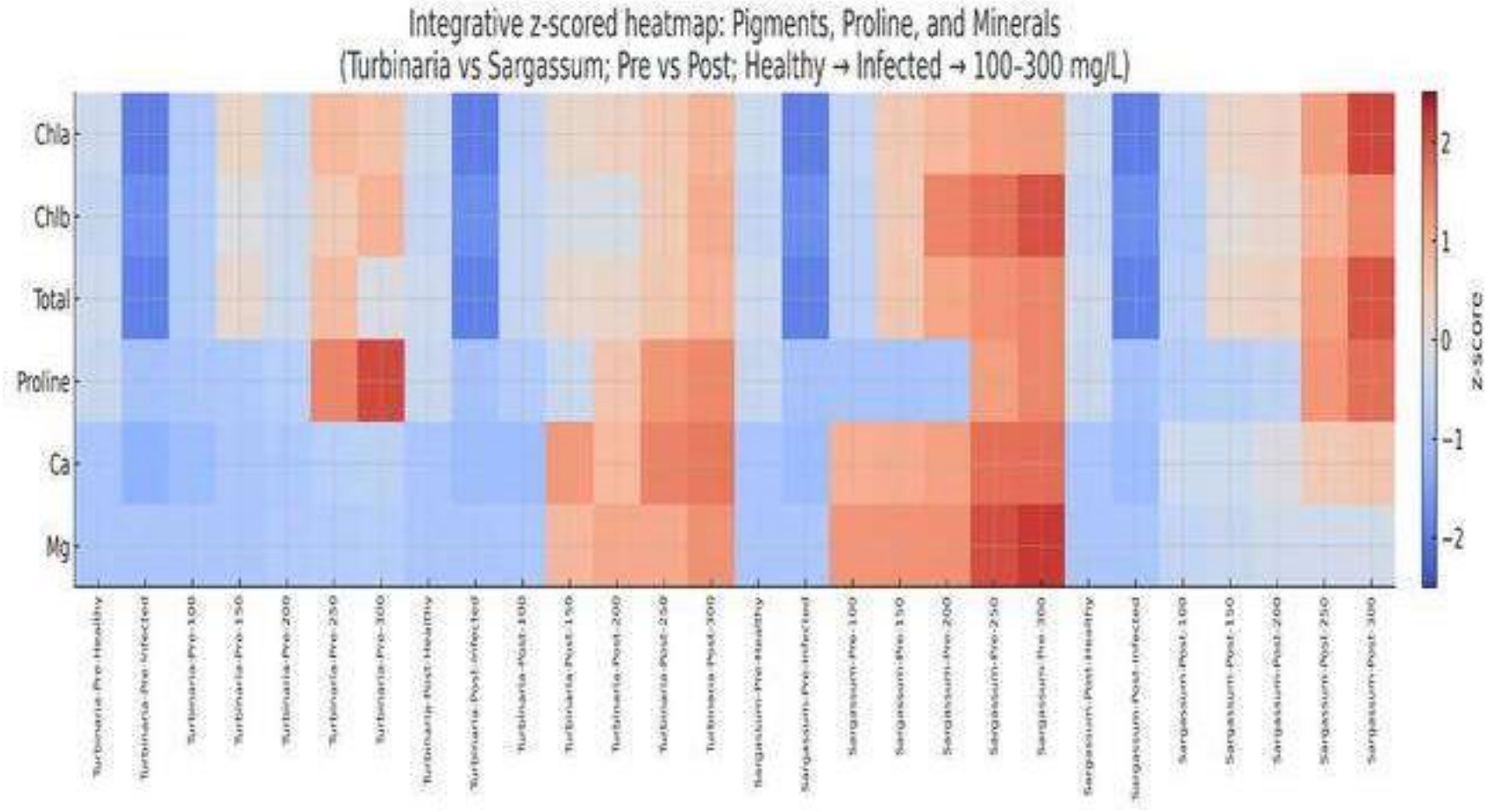

Figure 14. *Integrative z-scored heatmap illustrating the effects of Turbinaria ornata and Sargassum vulgare treatments on tomato plants during TMV infection. Data include photosynthetic pigments (Chl a, Chl b, total chlorophyll), proline, and mineral nutrients (Ca, Mg) under pre- and post-inoculation conditions across all treatments (Healthy → Infected → 100–300 mg/L)*

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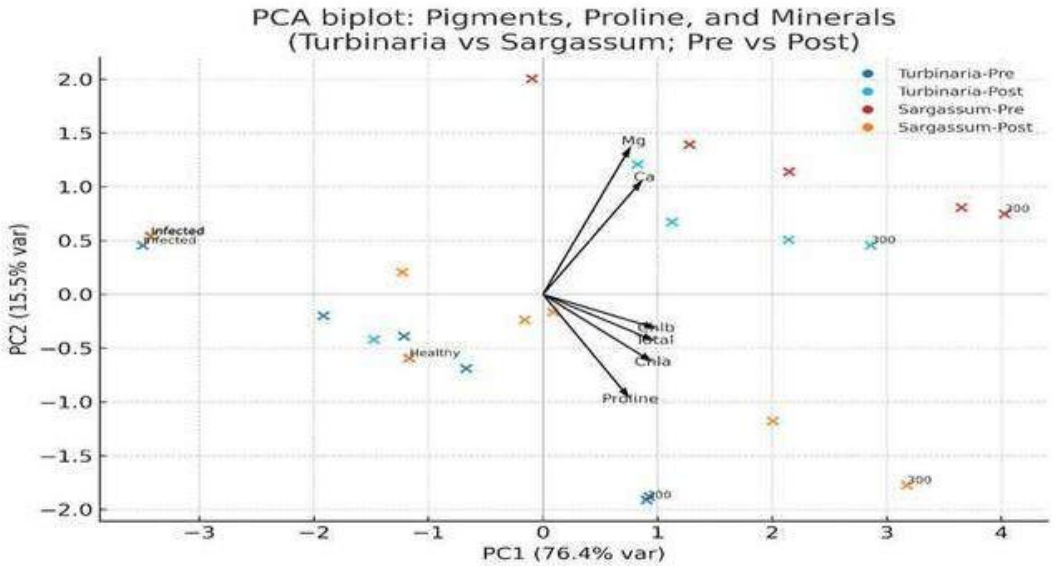

Figure 15. *Principal component analysis (PCA) biplot of tomato responses to Turbinaria ornata and Sargassum vulgare treatments during TMV infection. The analysis integrates photosynthetic pigments (Chl a, Chl b, total chlorophyll), proline, and mineral nutrients (Ca, Mg) under pre- and post-inoculation conditions. Treatments are clustered along PC1 and PC2 according to metabolic shifts, while loading vectors indicate the relative contribution of each variable to overall variance*

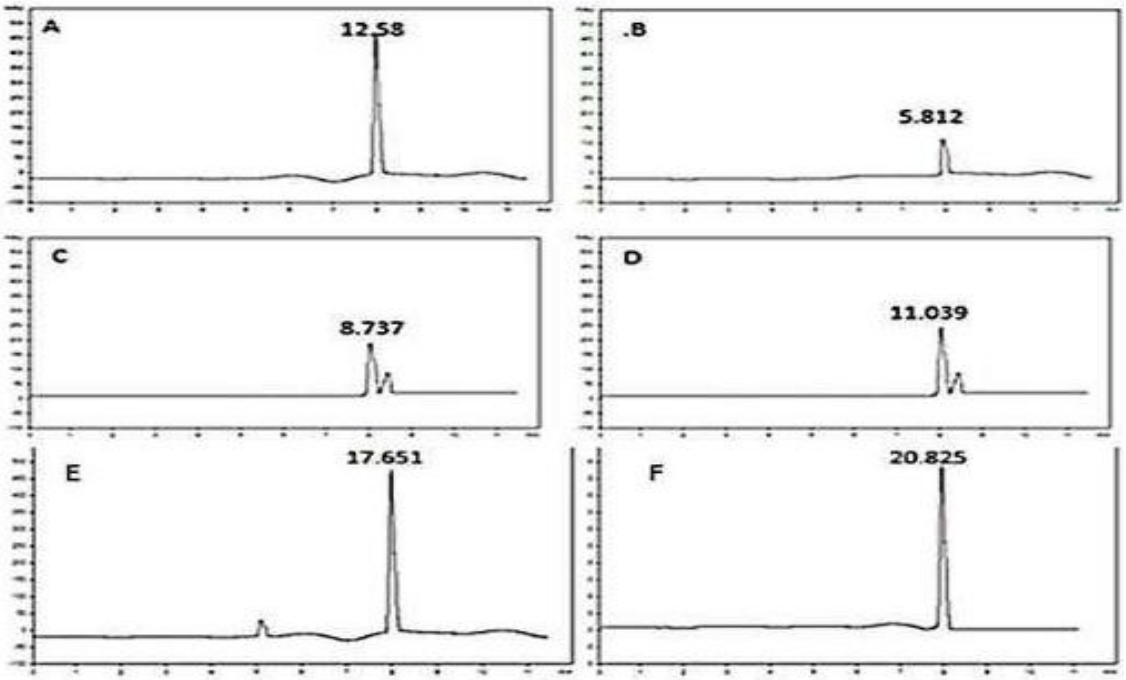

Figure 16. *HPLC quantification of free and endogenous SA in induced tomato plants treated with Turbinaria ornata: - A. inoculatedsample. B; Healthy sample. C; sample treated with T. ornata in 150 ml/L. D;sample treated with T. ornata in 200 ml/L. E; sample treated with T. ornata in 250 ml/L. E; sample treated with T. ornata in 300 ml/L*

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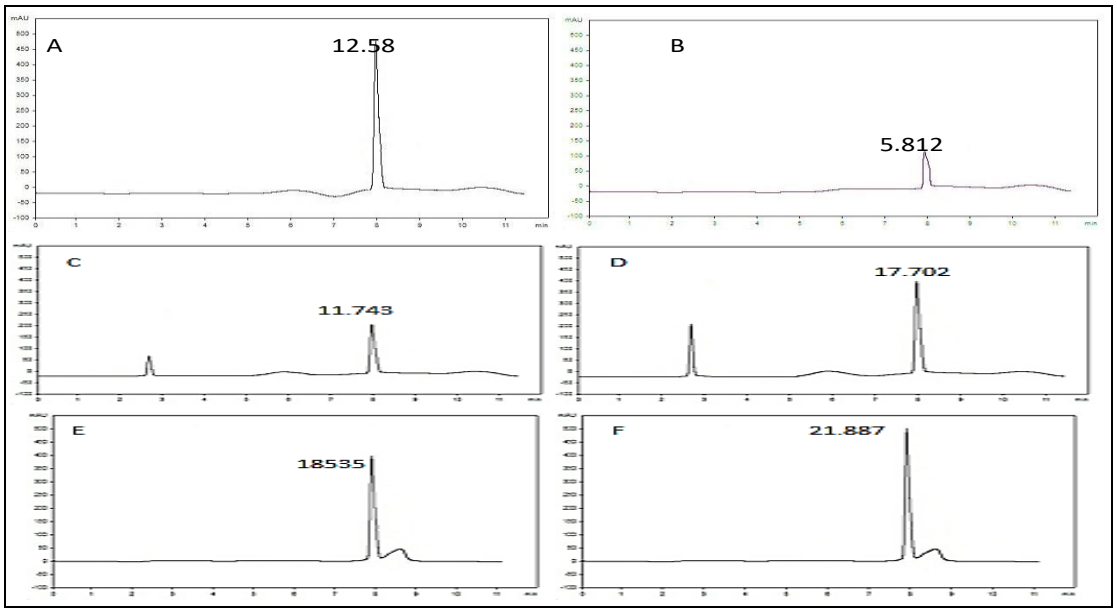

Figure 17. *HPLC quantification of free and endogenous SA in induced tomato plants treated with Sargassum: - A. inoculated sample. B; Healthy sample. C; sample treated with T. ornata in 150 ml/L. D;sample treated with T. ornata in 200 ml/L. E; sample treated with T. ornata in 250 ml/L. E; sample treated with T. ornata in 300 ml/L*

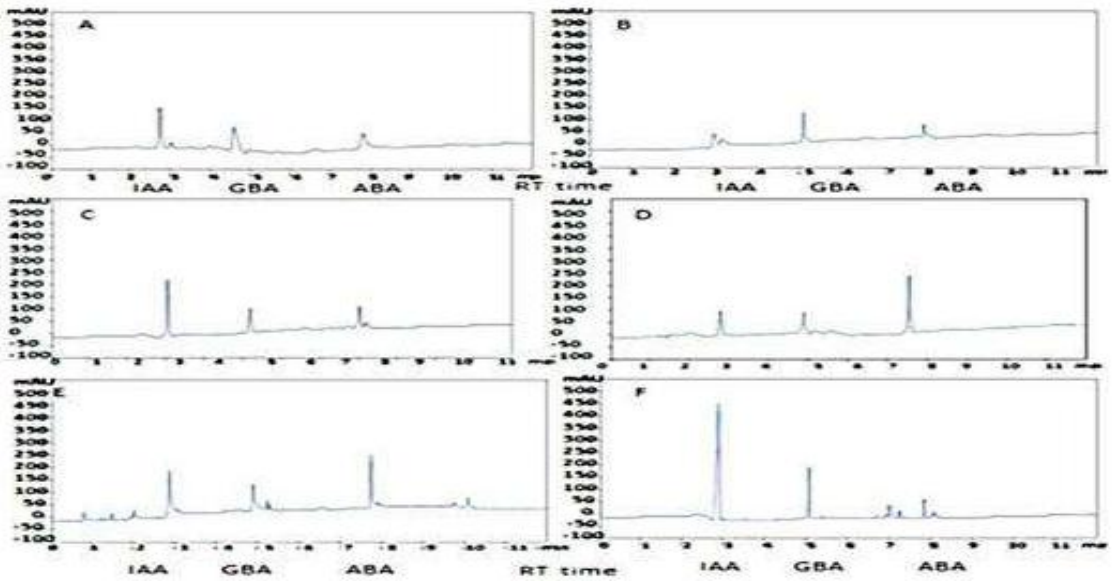

Figure 18. *HPLC quantification of free and phyto-hormons in induced tomato plants treated with Turbinaria ornata: - A. inoculated sample. B; Healthy sample. C; sample treated with T. ornata in 150 ml/L. D;sample treated with T. ornata in 200 ml/L. E; sample treated with T. ornata in 250 ml/L. E; sample treated with T. ornata in 300 ml/L*

Baseline SA levels in healthy, untreated plants remained relatively low, reflecting the absence of pathogen-induced signaling. By contrast, tomato plants treated with *Turbinaria ornata* and *Sargassum vulgare* displayed marked increases in SA, with *T. ornata* inducing the highest accumulation (9346.61 µg/g FW) followed by *S. vulgare*

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(8652.78 µg/g FW). HPLC analysis verified the accuracy of these measurements, as retention times corresponded precisely to authentic standards, and quantification based on peak areas provided reliable estimates of total SA accumulation.

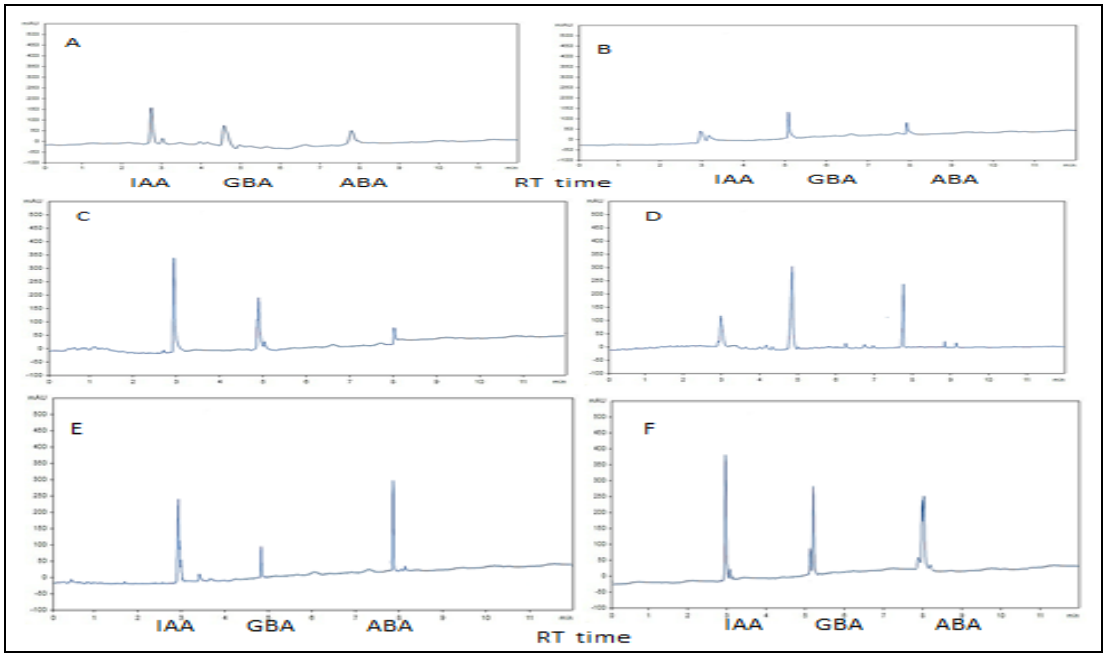

Figure 19. *HPLC quantification of free and endogenous phyto-hormons in induced tomato plants treated with Sargassum: - A. inoculated sample. B; Healthy sample. C; sample treated with T. ornata in 150 ml/L. D;sample treated with T. ornata in 200 ml/L. E; sample treated with T. ornata in 250 ml/L. E; sample treated with T. ornata in 300 ml/L*

Importantly, the elevated SA content was not an isolated response but was accompanied by enhanced activities of peroxidase (POD) and polyphenol oxidase (PPO), as well as higher phenolic compound accumulation. Together, these findings suggest a coordinated defense response in which macroalgal extracts act as elicitors, simultaneously stimulating multiple arms of the plant immune system. SA is a well-established regulator of systemic acquired resistance (SAR) and often functions synergistically with antioxidant enzymes and phenolic compounds to restrict viral replication and movement.

The observed lignification, increased trichome density, and thickened mesophyll tissues in treated plants further reinforce this interpretation, indicating that biochemical signaling was translated into anatomical strengthening of host barriers. Taken together, the convergence of elevated SA, enhanced POD/PPO activity, and higher phenolic accumulation provides compelling evidence that *T. ornata* and *S. vulgare* extracts induce a systemic and multi-layered defense response. This integrative mechanism likely explains the significant reduction in TMV accumulation and symptom severity in treated tomato plants, positioning marine macroalgae as promising eco-friendly bioelicitors for sustainable disease management.

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Detection of tobacco mosaic virus in tomato

The DAS-ELISA assay revealed that 55% of the pooled tomato leaf samples were positive for Tobacco mosaic virus (TMV), a result that was further confirmed by qPCR analysis (*Figure 20*). In the infected control, amplification curves for samples A and B emerged around cycles 30–32, reaching high fluorescence intensities that reflected elevated viral RNA accumulation. By contrast, plants treated with seaweed extracts from *Turbinaria ornata* and *Sargassum vulgare* exhibited delayed and attenuated amplification signals, indicating a significant reduction in viral load. This decline in qPCR signal corroborates the serological DAS-ELISA findings, collectively suggesting that the seaweed treatments triggered induced resistance responses, likely mediated through enhanced production of secondary metabolites and defense-related enzymes. Thus, the integration of serological and molecular data provides strong evidence that T. ornata and S. vulgare act as bioelicitors, mitigating TMV infection by lowering viral replication and accumulation within host tissues.

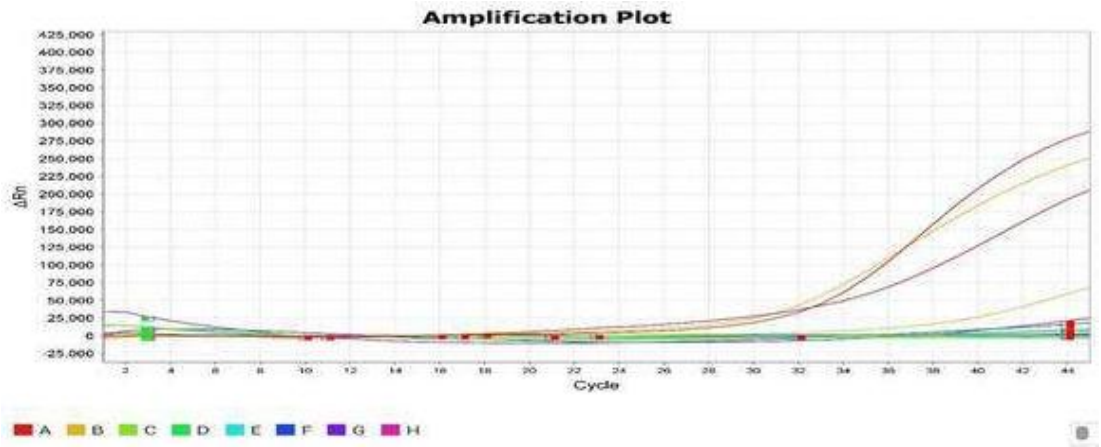

Figure 20. *Amplification plot from real-time PCR detection of Tobacco mosaic virus (TMV) in tested tomato leaf samples. Clear exponential amplification was observed in samples A (red) and B (yellow), indicating high viral RNA concentration, while other samples (C–H) showed minimal or no amplification above baseline*

Discussion

Plant viral diseases, especially those caused by Tobacco mosaic virus (TMV), remain a major constraint on tomato production worldwide. TMV is characterized by its remarkable stability, broad host range, and ease of transmission, making it a persistent challenge in both open-field and protected cultivation systems. Conventional management strategies often rely on resistant cultivars and sanitation practices, yet outbreaks continue to occur, highlighting the need for complementary, eco-friendly alternatives. The present study demonstrates that aqueous extracts of two brown marine macroalgae, *Turbinaria ornata* and *Sargassum vulgare*, can significantly suppress TMV infection in tomato plants under greenhouse conditions. By integrating serological, molecular, histological, and biochemical analyses, the findings provide strong evidence that these extracts exert antiviral effects through both direct interference with viral particles and induction of systemic plant defenses.

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Suppression of Viral Accumulation and Symptom Expression, the most compelling outcome of this study was the substantial reduction in TMV accumulation following treatment with macroalgal extracts. Both DAS-ELISA and qPCR assays confirmed significant declines in viral titers, with concentrations of 250 and 300 mg/L often resulting in undetectable viral loads. Treated plants exhibited little to no disease symptoms, whereas infected controls developed severe mosaic, necrosis, and stunting. These results are consistent with previous reports that marine algal extracts contain bioactive metabolites with antiviral properties (Nagorskaia et al., 2008b; El-Sheekh et al., 2014; Abdelkhalek et al., 2020). The near-complete suppression of visible symptoms at higher concentrations suggests that macroalgal-derived compounds may serve as effective bioelicitors capable of halting disease progression before it manifests.

Evidence for Direct Antiviral Activity, Transmission electron microscopy (TEM) provided further insights into the mechanism of action. Virions from treated plants appeared aggregated, shortened, or structurally deformed, in contrast to the intact rod-shaped particles observed in untreated controls. This morphological disruption supports the hypothesis that certain macroalgal constituents interfere with viral assembly or stability. Similar effects have been reported for sulfated polysaccharides isolated from seaweeds, which can destabilize viral capsids or inhibit adsorption to host cells (Pujol et al., 2002; Kim and Lee, 2008). Such direct effects complement the host-mediated responses, creating a two-pronged antiviral defense.

Induction of Systemic Acquired Resistance, equally important was the evidence for induction of systemic acquired resistance (SAR). Treatments with *T. ornata* and *S. vulgare* consistently enhanced peroxidase (POD) and polyphenol oxidase (PPO) activities, alongside higher levels of total phenolics and salicylic acid (SA). SA is a central regulator of SAR, orchestrating the activation of pathogenesis-related (PR) proteins such as PR-1a that fortify host immunity (Durrant and Dong, 2004). These biochemical profiles align with earlier findings where seaweed extracts triggered SAR in tomato plants against TMV and TYLCV (Ali et al., 2016; Abdelkhalek et al., 2020).

Structural Reinforcement of Plant Tissues, Histopathological analyses further substantiated the activation of defense pathways. Macroalgae-treated tomato plants displayed thickened epidermal and mesophyll tissues, enhanced lignin deposition, and increased trichome density. These structural modifications are recognized markers of primed resistance, as reinforced cell walls and surface barriers hinder viral movement and secondary infections (Mauch-Mani et al., 2017). The convergence of biochemical and histological evidence confirms that macroalgal extracts not only disrupt TMV directly but also fortify host tissues against viral invasion.

Preservation of Photosynthetic Function, one of the characteristic outcomes of TMV infection is chlorophyll degradation, which compromises photosynthesis and reduces yield (Liu et al., 2017). In this study, treated plants retained significantly higher levels of chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll than infected controls. This preservation of photosynthetic pigments suggests that macroalgal treatments mitigate viral stress, possibly by stabilizing chloroplast membranes and preventing the breakdown of pigment–protein complexes. The protective effect on chlorophyll integrity is consistent with improved photosynthetic efficiency and plant vigor under viral pressure.

Role of Mineral Nutrition, mineral nutrition also played a supportive role in enhancing resistance. Treated plants accumulated greater amounts of calcium and magnesium compared with controls. Calcium acts as a secondary messenger in plant defense

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signaling, activating transcription factors and enzymes associated with pathogen resistance. Magnesium, as the central atom in chlorophyll, is indispensable for photosynthetic processes (White and Broadley, 2003). The combined increases in these minerals further explain the improved physiological stability of treated plants.

Pigments, Proline, and Stress Mitigation, analysis of pigments and proline revealed additional evidence of protective effects. Both macroalgal extracts elevated pigment concentrations and proline content relative to infected plants, with pre-inoculation treatments generally producing stronger effects. Proline is a multifunctional osmolyte that stabilizes proteins, membranes, and redox balance under stress conditions (du Jardin, 2015; Battacharyya et al., 2015). Its accumulation in treated plants indicates enhanced stress tolerance and resilience. The coordinated increase in pigments and proline reflects the biostimulant activity of seaweed extracts, which improve osmotic adjustment, antioxidant defenses, and photosynthetic stability under both biotic and abiotic stresses (Rouphael and Colla, 2018; Shukla et al., 2019).

Dual Mode of Action and Practical Implications, taken together, these findings support a dual mode of action for *T. ornata* and *S. vulgare* extracts: (i) direct inactivation of TMV virions, and (ii) activation of SAR through biochemical and structural pathways. This combined action provides a robust and resilient defense mechanism, offering stronger protection than either strategy alone. Such dual-action agents are particularly valuable in integrated pest management (IPM), where minimizing reliance on synthetic agrochemicals is critical for environmental sustainability and economic viability.

Broader Implications for Sustainable Agriculture, the broader significance of these results extends beyond TMV management in tomato. Marine macroalgae are renewable, widely available, and environmentally benign resources, aligning with the goals of sustainable agriculture and the circular bioeconomy. By reducing dependence on synthetic pesticides, seaweed-based biostimulants contribute to eco-friendly crop protection strategies while lowering risks of environmental contamination. In addition, the development of seaweed-based agricultural products can generate socio-economic benefits, supporting coastal economies through harvesting and processing industries.

Despite these promising outcomes, several areas warrant further investigation. First, the precise bioactive compounds responsible for antiviral activity must be identified and structurally characterized. While fucoidans and phenolics are strong candidates, comprehensive chemical profiling would enable more targeted applications. Second, the durability and reproducibility of these effects under open-field conditions should be validated, as environmental variability may influence efficacy. Third, molecular studies using transcriptomics and metabolomics could elucidate the signaling pathways modulated by macroalgal extracts, providing deeper insights into plant–elicitor interactions. Finally, testing across other crops susceptible to tobamoviruses and related pathogens would clarify the broader applicability of these treatments.

In summary, this study highlights the potential of *Turbinaria ornata* and *Sargassum vulgare* extracts as eco-friendly, multifunctional bioelicitors against TMV in tomato. Their ability to combine direct antiviral activity with induction of systemic acquired resistance, while also preserving photosynthetic integrity and enhancing stress tolerance, positions them as valuable tools for sustainable crop protection. By bridging antiviral efficacy with environmental sustainability, marine macroalgal extracts represent promising candidates for integration into next-generation IPM strategies.

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Conclusion

This study demonstrated that aqueous extracts of the brown marine macroalgae *Turbinaria ornata* and *Sargassum vulgare* effectively suppressed Tobacco mosaic virus (TMV) infection in tomato plants under greenhouse conditions. Both serological (DAS-ELISA) and molecular (qPCR) assays confirmed a significant reduction in viral accumulation, particularly at concentrations of 250 and 300 mg/L, where complete suppression of visible symptoms was achieved. Transmission electron microscopy revealed fragmented and malformed virions in treated plants, suggesting a direct antiviral effect in addition to host-mediated resistance. Biochemical analyses further indicated enhanced activities of peroxidase and polyphenol oxidase, increased total phenolics, and elevated salicylic acid levels, all of which are hallmarks of systemic acquired resistance. Histopathological changes, including thickened epidermal tissues, lignin deposition, and increased trichome density, supported the structural reinforcement of host defenses. Moreover, treated plants retained higher chlorophyll contents, accumulated more calcium and magnesium, and exhibited greater proline levels, reflecting improved photosynthetic performance and stress resilience under viral challenge. Overall, the results highlight the dual role of marine macroalgae extracts as both direct antiviral agents and potent inducers of plant immunity. Their eco-friendly, renewable nature underscores their potential as sustainable alternatives to synthetic agrochemicals within integrated pest management (IPM) and sustainable agriculture frameworks.

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