







Management of Tobacco Mosaic Virus through Natural Metabolites

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Abstract: The viruses are one of the most threatening factors for plants resulting in gigantic economic losses. These utilize host internal machinery for reproduction and can spread through biological and non-biological means. Among the most hazardous plant viruses, Tobacco mosaic virus (TMV) is the most ancient virus which causes massive economic losses to tobacco, pepper, cucumber and ornamental crops globally. The problem can be reduced by minimizing the vector population through application of pesticides. Opposite to obtained success in virus control, rapid utilization of synthetic chemicals is disastrous for our ecosystem. Therefore, alternative approaches such as natural derivatives should be explored for eco-friendly management of TMV. So, here we have tried to take into account various natural metabolites which can be effectively and potentially used against TMV. We further explained about the derivatives from animals, fungi, bacteria and actinomycetes which are useful against TMV. The review imbibes the recent research findings regarding exploration of natural derivatives for management of TMV and concludes through highlighting the future prospects via hoping that future pesticides will be safer for human being and our planet.

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1. Introduction

Approximately 15% of global yield of economically important crops is being reduced every year by different plant diseases [1]. Plant viruses account for approximately 30 % of plant diseases [2, 3]. These are nucleic acid based single stranded (ss) or double stranded (ds) DNA or RNA pathogens

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packed in proteins (capsids) which survive within the host via acquiring host internal machinery and further utilize it for the intracellular movements and transmission [3-5]. Viruses attacking plants are categorized as the second largest culprits causing huge losses to vegetables, house hold plants, ornamentals and various field crops worldwide i.e. approximately 60 billion USD in financial terms [6]. According to International committee of taxonomy of viruses (ICTV), there are 950 different types of plant viruses so far reported on our planet [7,8].

TMV is the positive sensed single stranded (ss) RNA virus (Tobamovirus; Virgaviridae) producing mosaic"-like mottling discoloration symptoms on leaves. It is considered as the most ancient virus in plant virology as it was discovered in 1898 [9]. The particular virus causes massive damage to various crops including 125 plant species such as tobacco, cucumber, pepper and ornamentals [10]. TMV is a rod shaped virus with a capsid composed by 2130 coat protein (CP) molecules along with one ssRNA genomic molecule (6.3-6.5 kb) [11](Figure 1). The CP is self-assembled into the rod-like helical structure constituting 16.3 proteins per helix turn tight around the RNA forming a hairpin loop structure [12] TMV genome encodes 4 open reading frames (ORFs) [13] and is considered as thermo-stable virus tolerating up to 1200°F (50°C) up to 30 minutes with a refractive index of 1.57 [14].

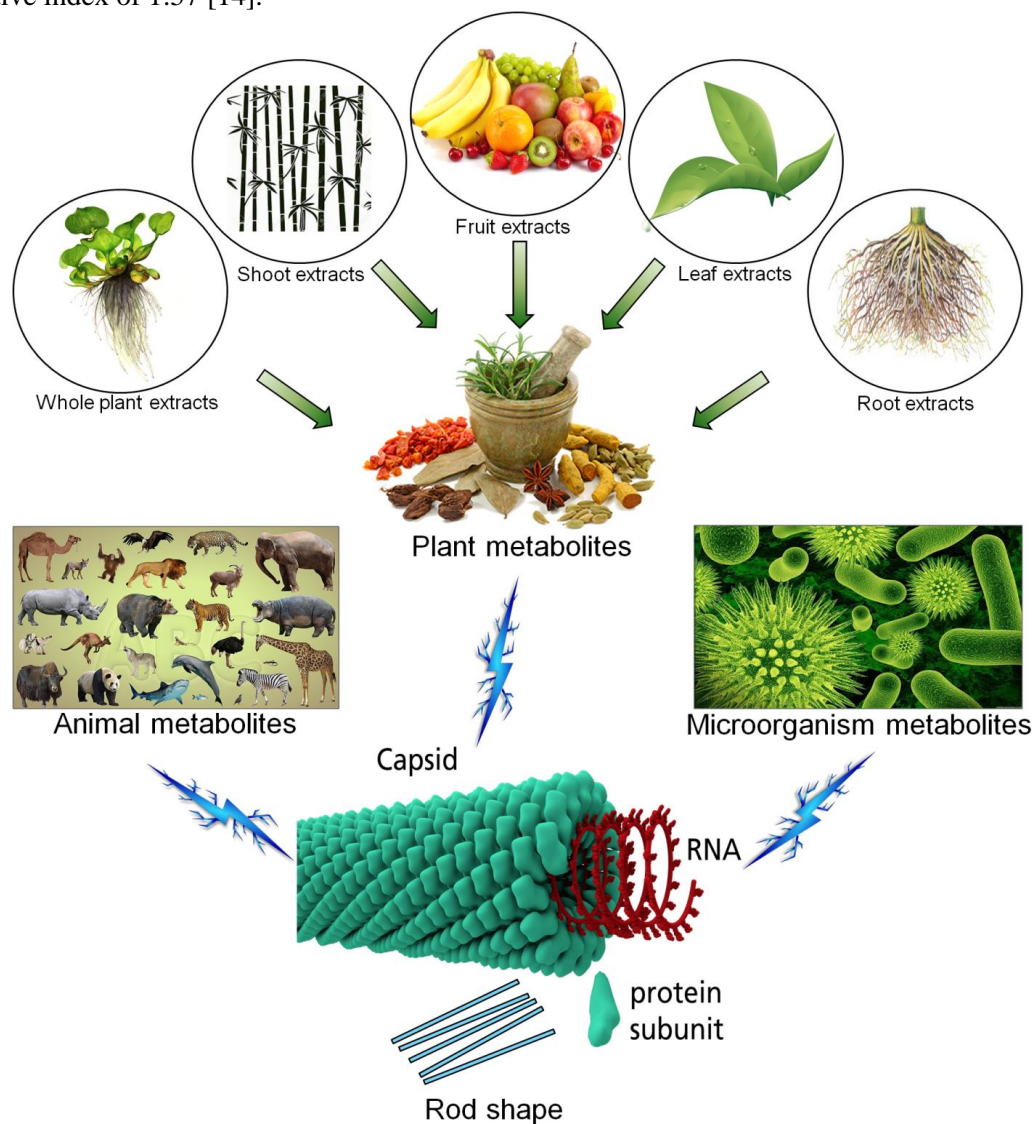


Figure 1. Structure of TMV and natural metabolites which have positive impacts against TMV

Although impact of virus diseases can be reduced by minimizing the vector population by application of pesticides [8] but such treatments cannot completely eliminate the virus infections as they are not directly hitting upon the viruses [15]. Furthermore, pesticides have severe adverse effects upon human beings and our ecosystem [8,16-19]. For example, organophosphates and dichlorodiphenyl-trichloroethane (DDT) which were launched in early 1930s for pest control against various pests proven severely lethal towards human health after their continuous usage of three decades [20-23]. Moreover, Carson extensively revealed about the injurious effects of synthetic chemicals towards human health and surrounding environment in his book named *Silent spring* [24]. Afterwards, awareness regarding preserving the ecosystem from pesticides and finding the alternatives enhanced rapidly. But the developing countries are yet not taking up the matter seriously. In the start of 21st century, researchers focused to find the natural compounds which had the potential to be used as natural pesticides due to relatively less toxicity threat and least residual effects to surrounding environment [25-28]. Considering the environmental safety and human health, researchers shifted their thoughts towards the ancient times when people utilized the herbals and natural products for treatment of various infectious diseases. This led them towards the management of plant viruses through natural extracts from plants, animals and microorganisms [8, 29] through further commercialization of bio-pesticides [30]. These bio-pesticides are environmentally safer having least residual effects and more target specificity. Furthermore, these are not susceptible to viruses for attaining quick resistance against them thus encourage their large scale commercialization [30, 31]. This can be proved simply, as production of bio-products is increasing sixteen percent every year which is the three times more than synthetic pesticides i.e. 5.5% per year [32]. Since 2001, researchers have made considerable progress in identification and evaluation of various plants, animal and microorganism based products against TMV as they have found several metabolites such as alkaloids, essential oils, flavonoids, phenols, polysaccharides and proteins [33-38]. So here we have reviewed about the research progress made in recent years regarding efficacy of plant metabolites against TMV. We further explained about the metabolites found in animals and microorganisms having anti TMV properties. We also have highlighted the important aspects, concerns and limitations via describing future prospects by hoping that upcoming pesticides utilization against TMV and other plant viruses will be safer for human beings and our ecosystem.

2. Anti-TMV Metabolites from Plants

Plant extracts always guide our thoughts on the basis of the historical utilization of herbs and herbal medicines for curing all type of human, mammalian and plant diseases [39-42]. In 1914, antiviral activity of pokeberry juice laid the research foundation for searching more plants with similar action [43]. Approximately, only 10% of 250,000 plants species on our planet [21] had been chemically characterized till the end of 20th century [44]. These plants have played their vital role in human life through their industrial applications via providing nutrition, chemicals, medicines, cosmetics and much more [45]. Moreover, around 2400 plants have been successfully identified having anti-bio-organismic properties [46]. Whole plant extracts or extracts from different plant parts such as roots, shoots, leaves, bulbs, rhizomes and fruits have been found quite effective against various plant viruses (Figure 1; Table, 1). Correspondingly, looking into the history, Chinese herbals were very famous [47]. But in the beginning of this decade, researchers inspired to find the plant based extracts and products for their antiviral activities [48-50]. These efforts further clarified that plant based primary substances like proteins have better antiviral properties [51,52]. Similarly, 0.4 million secondary metabolic compounds i.e. alkaloids, anthocyanins, caratins, flavonoids, phenolics and certain type of oils, which play vital role during biotic and abiotic stresses, also have been found to have anti-bacterial, anti-fungal and antiviral characteristics [79, 80]. These primary and secondary metabolites are commercially being utilized to synthesize various drugs to cure human, mammalian and plant diseases [30,31]. Some of these metabolites are actively being used in producing anti TMV bio-products, are explained here.

Table 1. Natural metabolites and extracts which successfully inhibit TMV infection

Name	Family	Organism	Extract / Metabolite	% TMV inhibition	Concentration	Reference
<i>Celosia cristata</i> L.	Amaranthaceae	Plants	7-deoxytrans-dihydnarciclasine	>90	20-30 µg/mL	[53]
<i>Cynanchum komarovii</i>	Asclepiadaceae	Plants	Whole plant extract	65	1.0 mg/mL	[54]
<i>Pleurotus citrinopileatus</i>	<u>Pleurotaceae</u>	Fungi	Protein	50	0.24 µg/mL	[55]
<i>Sambucus williamsii</i>	Caprifoliaceae	Plants	Essential oil and phenolic compounds	43	--	[56]
<i>Strobilanthes cusia</i>	Acanthaceae	Plants	Leaf extract	100	50 nM	[34]
<i>Hosta plantaginea</i> Aschers	Liliaceae	Plants	Whole plant extract	91.4	50 µg/mL	[57]
<i>Bougainvillea xbuttiana</i>	Nyctaginaceae	Plants	Protein, root extract	94	50 µg/mL	[58]
<i>Brucea javanica</i> (L.) Merr.	Simaroubaceae	Plants	Leaf extract	78.9	200 mg/L	[59]
<i>Bacillus cereus</i>	Bacillaceae	Bacteria	ZH14	94.2	--	[60]
<i>Picrasma quassioides</i>	Simaroubaceae	Plants	Bruceine D	60.4	50 µg/mL	[61]
<i>Trichoderma pseudokoningii</i> SMF2	Hypocreaceae	Fungus	Antimicrobial peptide	54	100 nM	[62]
<i>Sambucus williamsii</i>	Caprifoliaceae	Plants	Essential oil and phenolic compounds	34.3	1 mmol/L	[63]
<i>Lithospermum erythrorhizon</i>	Boraginaceae	Plants	Bark extract	63.6%	2–10 µg/mL	[64]
<i>Munronia unifoliolata</i>	Meliaceae	Plants	Protein	64.2	30 µg/mL	[65]
<i>Rhodiola eurycarpa</i>	Crassulaceae	Plants	Whole plant extracts	54.55	10 µg/mL	[48]
<i>Achnatherum splendens</i>	Poaceae		Stem extracts	60.36		
<i>Lactuca tatarica</i>	Asclepiadaceae		Whole plant extracts	50.92		
<i>Syneilesis aconitifolia</i>	Asclepiadaceae		Whole plant extract	71.67		
<i>Chaenomeles sinensis</i>	Rosaceae		Fruit extract	94.57		
<i>Rubus flosculosus</i>	Rosaceae		Whole plant extract	60.00		
<i>Thermopsis lanceolata</i>	Leguminosae		Leaf extract	53.78		
<i>Cotinus coggygia</i>	Anacardiaceae		Leaf extract	93.52		
<i>Rodgersia podophylla</i>	Saxifragaceae		Stem extract	98.25		
<i>Pulsatilla chinensis</i>	Ranunculaceae		Leaf, root, stem extract	61.25		
<i>Thlaspi arvense</i>	Brassicaceae		Whole plant extracts	50.00		
<i>Rhodiola eurycarpa</i>	Crassulaceae		Whole plant extracts	53.19		
<i>Achnatherum splendens</i>	Poaceae		Stem extracts	60.39		
<i>Pseudomonas chlororaphis</i>	<u>Pseudomonadaceae</u>	Bacteria	Peptide	95	1 mg/mL	[66]
<i>Arundina graminifolia</i>	Orchidaceae	Plants	Munronoids K	48.2	20 µM	[67]
<i>Zingiber officinale</i>	Orchidaceae	Plants	Gramniphinol G	50.00	100 µg/mL	[68]
<i>Chenopodium album</i>	Chenopodiaceae	Plants	Leaf extract	98.2	60 g	[69,70]
<i>Lentinus edodes</i>	<u>Marasmiaceae</u>	Fungus	Lentinan	83.2	10 µg/mL	[71]
<i>Cassia fistula</i>	Papilionaceae	Plants	Whole plant extract	31.3	32.2 mg	[36]
<i>Momordica charantia</i>	Cucurbitaceae	Plants	Protein	67.21	500 µg/mL	[72]
<i>Eupatorium adenophorum</i>	Asclepiadaceae	Plants	Fructo oligosaccharide	79.69	50 mg/mL	[73]
<i>Pseudomonas fluorescens</i>	<u>Pseudomonadaceae</u>	Bacteria	Protein	88.3	49.8 × 10 ¹⁰ cful/mL	[74]
<i>Schisandra rubriflora</i>	Schisandraceae	Plants	Whole plant extract	78.00	0.15 mM	[50]
<i>Coriolus versicolor</i>	<u>Polyporaceae</u>	Fungus	Polysaccharide peptide	71.5	500 µg/mL	[37]
<i>Cephalotaxus sinensis</i>	<u>Cephalotaxaceae</u>	Plants	Drupacine and cephalotaxine	50.76-53.41	100 µg/mL	[75]
<i>Boerhaavia diffusa</i>	<u>Nyctaginaceae</u>	Plants	Root extracts	100	0.2 mg/mL	[76]
<i>Phyllanthus emblica</i>	<u>Phyllanthaceae</u>	Plants	Root extracts emblirol A (1) and B (2)	62.1-79.6	1 mg/mL	[77]
<i>Tithonia diversifolia</i>	<u>Asteraceae</u>	Plants	Tagitinin C (Ses-2) and 1β-methoxydiversifolin-3-0-methyl ether	60.27-62.86	100 µg/mL	[78]

2.1. Primary Metabolites

Certain plant primary metabolites include polysaccharide carbohydrates, lipids and proteins. Of these, only polysaccharides and proteins have been noticed for their antiviral properties. The widely found plant polysaccharides have diverse biological functions [81-83]. These possess antiviral actions based upon their great target specificity, low residual effects and lower toxicity levels with broader action e.g. anti-aging, anti-cancer, anti-oxidation [71, 84-86]. Researchers have documented anti TMV performance of polysaccharides isolated from diverse plant species like *Lycium barbarum* [87], *Chuanminshen violaceum* [88], *Caesalpinia ferrea* [89], *Achyranthes bidentata* [90], *Eupatorium adenophorum* [73], *Portulaca oleracea* [91] and *Astragalus propinquus* [92]. Moreover, polysaccharides derived from root extracts of *Arctium lappa* appeared quite effective against TMV as it amplifies the transcription levels of multiple defense related proteins (DRPs) and enzymes as compared to control treatment within 24 hours post inoculation [93].

On the other hand, the plant proteins are actually DRPs synthesized in response to the pathogen attack e.g. bacteria, fungi and viruses [94]. These DRPs are classified into 17 different families. Recently, *beetin27* i.e. a DRP produced in response to virus attack in sugar beet (*Beta vulgaris*) leaves is believed to possess strong capacity against various phyto-pathogens as well. The protein like *beetin27* also responds to the signals produced by salicylic acid (SA), RNA polynucleotides and hydrogen per-oxide (H₂O₂) generated due to viral infection [95,96]. Likewise, anti TMV properties due to DRPs productions have been observed in *Bougainvillea xbuttiana* [58]. In addition, some DRPs have also been identified in elderberry [56]. Correspondingly, DRPs i.e. *CCP25* and *CCP27* from *Celosia cristata* extracts also proved target specific and effective in minimizing TMV infections at a concentration of 30 µg/mL [53]. Researchers have evaluated DRPs from Pokeweed with respect to their antiviral activity against human, mammalian as well as plant viruses like Human immune deficiency virus (HIV), Influenza virus, cytomegalovirus, TMV and several others. DRPs effectively suppress and inhibit virus replication [97-100].

2.2. Secondary Metabolites

Plant secondary metabolites include multiple kinds of substances involved in metabolism and also have anti-viral capacity [80]. These metabolites are alkaloids, essential oils, flavonoids and phenolics [101, 102]. Pharmacological as well as medicinal effects of alkaloids on living organisms have been well documented. Alkaloids possess diverse structures having numerous bio active substances [103-105]. Ancient Chinese herbs reportedly possess 18000 alkaloids with anti-viral attributes [106]. Few years back, five diverse alkaloids were obtained from *Hosta plantaginea* and an alkaloid *7-deoxytrans-dihydronarciclasin* was separated that exhibited anti-TMV activity even in least inhibitory concentration i.e. 1.80 µM [57]. In the same way, *Brucea javanica* extract have *Bruceine-D* displaying inhibitory effects against TMV [59]. Likewise, 17 quassinoids with anti TMV infection characteristics were identified having 3.42-5.66 µM IC₅₀ value [107]. Chen and his fellow researchers [61] investigated *Picrasma quassioides* wood extract for anti TMV activities. They identified some *β-carboline* alkaloids and a quassinoid with moderately positive results. Furthermore, the extracts revealed synergetic effects when applied in combination with *nigakilactone B*. The combined supplement of these alkaloids and quassinoid improved the inhibition from 36.4 to 68.4 %. Similarly, An et al. [54] explained about *Cynanchum komarovii* derivatives embedding two alkaloids (*7-demethoxytylophorine* and *7-demethoxytylophorine N-oxide*) with approximately 60 and 65% anti TMV activity at a concentration of 500 µg/mL and 1.0 µg/mL.

Essential oils are complex mixtures of lower molecular weight [108, 109]. Utilization and processing of essential oils have exponentially increased over the years [110]. They are normally used in cosmetics, drinks, food flavors and perfumes [111]. These oils can be found in resin ducts, various glands and oil ducts inside plant body [112]. Besides, these oils show anti-bacterial, anti-fungal, anti-insects and anti-viral characteristics [113]. It is interesting to note that over 50% TMV inhibition was observed when essential oils from ginger, lemon, tea tree, tangerine peel, artemisia and lemongrass were applied at a

concentration of 100 µg/mL [68, 114]. Equally, essential oils i.e. carvacrol and thymol from *Satureja montana* performed 34.3 % suppressing activity against TMV at 2 mmol/L concentrations [63]. Talking about flavonoids extracted from herbs reveals interesting facts [115]. It is estimated that approximately 10000 flavonoids are on record [116]. For their antiviral roles, 28.5% and 31.3% TMV repression was recorded by *fistula B* and *fistula C* from *Cassia fistula* at a concentration of 20 µM [36].

Phenolics also have anti-viral characteristics [117], and their higher concentration have been reported in tea, cottons seeds and other medicinal plants [114, 118, 119]. Phenolics further include important compounds like *anthocyanins*, *ellagitannins*, *hydroxycinnamates* and *procyanidins* [120]. *Arundina graminifolia* possess three diverse phenolics (*Gramniphénol C*, *Gramniphénol F* and *Gramniphénol G*). These compounds have respectively shown 48.2, 35.8 and 32.1 percent TMV inhibition after application at 20 µM concentration [67]. In addition, *gossypol* from cotton seed presented up to 54.4% TMV inhibition with concentration of 500 µg/mL [38]. These data supported in commercial development of anti TMV product called *Zai-xi-chun* containing active *gossypol* and several other bio components. Likewise, another commercial anti-viral bio-product (*Ningnanmycin*) has now been improved after addition of *Schisandra rubriflora* extract possessing phenol called *schisanhenol*. This new composition presented 78.00-83.5% inhibition of TMV [50].

3. Anti-TMV Metabolites from Animals

Majority of animal metabolites with anti-plant virus activities have not been much explored. However, it is documented that a couple of oligosaccharides such as chitin and chitosan have anti-plant virus characteristics [81]. These are hydrolysed products from chitosan polymers which have potential to activate plant defenses against invading viruses [121]. Particularly against TMV, chitosan have been found to have 50.41% inhibition rate at a concentration of 50 µg/mL [122]. Further exploring the chitosan, researchers have found that chitosan inhibitory effects are modulated via production of nitric oxide, hydrogen peroxide, protein kinase, phenylalanine ammonia-lyase activity and co-regulated through a signaling pathway i.e. Ca²⁺ [123-127].

4. Anti-TMV Metabolites from Micro-organisms

These may include various pathogenic and non-pathogenic organisms such as actinomycetes, algae, bacteria and fungi. Anti-TMV metabolites from these organisms have been explained further. Various peptides, proteins and polysaccharides are the famous metabolites found in various fungi which have anti-viral properties [128,129]. Extracts from *Coriolus versicolor*, *Coprinus comatus*, *Lentinus edodes*, *Pleurotus ostreatus*, *Flammulina velutiper* and several other fungi have been characterized to have anti TMV properties (Table 1). For fungal polysaccharides from *Coriolus versicolor*, it has been observed that the particular polysaccharide exhibits 85.4% inhibition @ 500 µg/mL concentration while the inhibition rate of the disease was reduced to 64.8% at 100 µg/mL concentration, respectively [37]. Exploiting the same fungus, another metabolite called *Lentinan* recorded 58.7% TMV inhibition at an application of 10 µg/mL concentration [71]. Researchers has explained that *Lentinan* actively increase the anti TMV tolerance in plants via increasing the host plant resistance by generating per oxides and DRPs [37, 130, 131]. In a similar context, some bacterial metabolites have been recently found to have anti TMV properties. For example, ZH14 bacterial strain which produces some proteins, have inhibitory effects against TMV infection [60]. Similarly, various strains from pseudomonas have anti TMV properties [66,74]. Moreover, Actinomycetes which have great importance regarding commercial production of medicines [132,133] also have anti-plant virus characteristics such as Ningnanmycin extracted from *Streptomyces noursei* [134] and Cytosinepeptidemycin isolated from *Streptomyces ahysroscopicus* [135, 136]. Further investigating these two metabolites clarified that Ningnanmycin inhibited TMV infection up to 58.1% when applied at a concentration of 500 µg/mL [38] while Cytosinepeptidemycin showed 80% TMV infection inhibition at the concentration of 1 mg/mL [137].

5. Conclusion and Future Prospects

Although plant virus diseases results in gigantic economic losses worldwide but it is still not fair to apply synthetic chemicals for minimizing their impact upon global agriculture. To cope with the situation and to find alternative anti-viral substances, it is more feasible to search the natural sources of least residual effects along with overwhelming anti-viral activities. Although, we have witnessed an increased rate of commercialization of bio-products but when we talk specifically about anti TMV bio-products, we find only few efforts made in this research field. Metabolites from plants, animals and micro-organisms can be utilized to prepare commercial bio-products and limitations and constraints must be reduced. It is not easy to obtain natural extracts directly as several technical themes are necessary to fulfill for their effective preparation and formulation [8,138]. International federation of organic agriculture movements (IFOAM) standards must be followed for extraction of any active ingredient from natural herbs [139]. We have witnessed that higher concentration of natural extracts are normally required for quick and efficient minimization of TMV infection thus requiring the larger population of herbs, animals or micro-organisms locally can be challenging sometimes. To address this problem, particular organisms should be isolated and their population should be increased via breeding strategies. Similarly, complete characterization of anti TMV natural metabolites has not yet been achieved. Researchers must maintain a gene pool regarding all anti TMV metabolites. Such characterization is also necessary for the insect vectors. Therefore, rapid screening of natural metabolites should be carried out regarding their anti-virus-host interaction effects. Likely screening efforts will amplify the research regarding structural and functional relationships among the several primary and secondary active components in natural extracts. Detailed analyses and utilization of these substances will open new horizons in finding the similar components in marine, fossils and algal communities. Finally, we recommend accelerated field experimentation regarding efficacy of natural metabolites to identify plant species with new anti-viral properties. We believe that future pesticides will be safer for human health as well as for our ecosystem.

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