Synthesis of an organic-inorganic salt of \((\text{C}_2\text{H}_5\text{NO}_2)_2\text{H}_4\text{SiW}_{12}\text{O}_{40}\) and investigation of its anti-viral effect on the tobacco mosaic virus (TMV)

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**ABSTRACT**

Polyoxometalates (POMs) are important inorganic compounds that have been considered specifically in recent years due to abundant attributes and applications. Those POMs that have one central tetrahedral atom called keggin. The binding Amino-acid groups to keggin structure give the antivirus effect to these compounds. A new organic-inorganic hybrid structure, with formula \((\text{C}_2\text{H}_5\text{NO}_2)_2\text{H}_4\text{SiW}_{12}\text{O}_{40}\) was synthesized \((\text{C}_2\text{H}_5\text{NO}_2 = \text{Glycine} = \text{Gly})\). Investigation on Anti-viral effect of this compound showed the \((\text{Gly})_2\text{H}_4\text{SiW}_{12}\text{O}_{40}\) prevents infection of Tobacco Mosaic Virus (TMV) on the Nicotiana tabacum plants.

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1. Introduction

Polyoxometalates (hereafter, POMs) are category of inorganic bulk compound that are made from polymerization of oxometalates. Really, POM is a polyatomic anion that consists of three or more transition metal (such as, W, Mo, V, Nb, Ta and etc) oxoanions linked together by shared oxygen's to form a large, bulk and 3D structures. Positioning hetero atom (for example Si, P, As, B, Ge or Sb) into POM produces the heteropolyanions (HPAs). The hetero atom maybe located in the center of the anionin which case the HPA has keggin state. The keggin forms have tetrahedrally coordinated hetero atom like Si or P. Figure .1 shows the structure of keggin form.
HPAs have various applications in different sciences such as catalysts chemistry (Kozhenikov, 2012), analytical chemistry (Kim et al., 1999), medicinal chemistry (Anti-tumor, Anti-cancer, Anti-bacteria, Anti-microbial and anti-clotting) (Pope et al., 1991; Wang et al., 2003; Judd et al., 2001; Clercq, 2002), radioactive materials, gases absorbent (Coetzee et al., 1973) and food chemistry (Pope, 1983; Yamase et al., 2002). One of the most attractive applications of these compounds is their Anti-viral effect (Yamamot et al., 1992; Inouye et al., 1993; Rhule et al., 1998; Wang et al., 1998; Baker et al., 1998). HPAs have indicated that at experimental conditions of both in vitro and in vivo, they possess great antiviral activity with high efficiency (Pope, 1991). The studies have revealed that through binding amino-acid groups to POMs antiviral effect emerges in these compounds, hence POMs could act as antiviral compound.

Fig. 1. Structure of keggin form.

The keggin ions are the fully well-known anions which are also very much highly stable, arranged and bulk, being reducible by the electron attracting (Yamamot et al., 1992; Inouye et al., 1993; Rhule et al., 1998). One of the expanding researches in the POM chemistry is development of organic-inorganic hybrids by combining POM clusters with various amino acids like, glycine, tyrosine, ornithine, alanine, histidine, lysine, proline and cysteine (Kuntic et al., 2006; Bi et al., 2001; Wang et al., 2006; Li et al., 2004; Sanyal et al., 2005; Alizadeh et al., 2006; Alizadeh et al., 2008; Wang et al., 2003; An et al., 2006). While encountering this category of POMs with viruses, one chemical interaction is made between amino-acid groups and enzymes or proteins corresponding to the virus, understanding these interactions are very important to the interpretation and development of future compounds with selective affinity for particular proteins. Tobacco mosaic virus (TMV) is a positive-sense single stranded RNA virus infecting wide range of plants, especially tobacco. TMV infection causes discoloration, mottling and mosaic on leaves.

In this research Tungstosilicic acid (H₄SiW₁₂O₄₀) used as HPA compound which formed the keggin structure and Glycine with formula NH₂CH₂COOH used as an organic compound in the POM structure. We synthesize one organic-inorganic compound as (Gly)₂H₄SiW₁₂O₄₀ through chemically binding the keggin structure to Glycine, then the effectiveness of the POM on preventing TMV infection was investigated.

2. Experimental

2.1. Geometric structure of [SiW₁₂O₄₀]⁺ and preparation of (Gly)₂H₄SiW₁₂O₄₀

In structure of [SiW₁₂O₄₀]⁺, the tungsten (W) was considered as transition metal and central atom of silicon (Si) was considered as hetero and tetrahedral atom. General form of this anion is α-keggin. The central Si atom is surrounded by a tetrahedron whose oxygen vertices are each linked to one of the four W₃O₁₃ groups. Each W₃O₁₃ consists of three W₇O₆ octahedral linked in a triangular arrangement by sharing edges and the four W₇O₁₃ are linked together by sharing corners (Figure 2).
In order to prepare the (Gly)$_2$H$_4$SiW$_{12}$O$_{40}$ salt that is obtained from interaction between Gly and H$_4$SiW$_{12}$O$_{40}$, at first 0.25 g Glycine dissolved in 5 ml of 1.0 M HCl solution, then 2.5 g of solid H$_4$SiW$_{12}$O$_{40}$ (Merck, code: 602336) gradually was added to the solution and stirring for an hour. The transparent solution kept in air at room temperature. The transparent crystals were isolated from the solution seven days later. The final solid material is (Gly)$_2$H$_4$SiW$_{12}$O$_{40}$ in which Gly units has been bounded to W=O oxygen's through hydrogen bonding (Figure 3) (Bi et al., 2001).

2.2 antiviral property of POM and experimental on TMV prevention

Antiviral property of the POM was studied by spraying of the POM solution on Nicotiana tabacum var Turkish followed by Tobacco mosaic virus (TMV) infectivity assay. Two days before TMV inoculation, POM solution was sprayed onto the tobacco plants. Then the plants were divided into three groups include,

(1) Healthy control which spray with POM solution without TMV inoculation.
(2) POM-A group treated with the solution first then extract of TMV infected tobacco rubbed on leaves.
(3) POM-B likes as group A but POM sprayed once a day post inoculation.

Negative control plants sprayed with distilled water, whereas positive control inoculated with the virus without any spraying. Plants were kept in the greenhouse for symptom development up to four weeks post
inoculation (WPI). The plants were checked for TMV infection by indirect ELISA using TMV specific antibody according to Clark and Adams 1988.

3. Results and discussion

Results showed all plants within virus control showed mottling followed by sever mosaic symptoms 10 days post inoculation (dpi), but no symptoms were observed in POM-A and POM-B groups as healthy control up to 21 dpi. One out of three plants in POM-A group expressed mottling in 3 WPI. No symptoms were observed in negative control, but healthy control as all POM treated leaves had pale yellow color. ELISA results indicated the presence of TMV antigen in virus control plants. Also the plant with mottling symptom in POM-A group was infected with the virus. No TMV contamination was found in POM treated plants (data not shown). There was no difference between results of POM-A and POM-B groups indicated spraying after virus inoculation is not necessary for virus control. POM was effective in concentration of 50 and 100 ppm against TMV infection, however side effect of POM spraying; pale yellowing; was greatly reduced in 50 ppm concentration of the solution. The results are summarized in Table-1.

<table>
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<th>Table 1</th>
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<td>Effects of POM on TMV infectivity.</td>
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<td>Group</td>
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<td>Healthy control</td>
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<td>POM-A group</td>
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<td>Virus control</td>
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<td>Negative control</td>
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Here, we synthesized a new organic-inorganic hybrid salt of 12-silicotungstate, with formula (Gly)$_2$H$_4$SiW$_{12}$O$_{40}$. It exhibited Anti-viral property and prevent infection of Tobacco Mosaic Virus in Nicotiana plants. Similarly Bi et al, reported anti-TOMV properties of 12-silicomolibdate salt (Bi et al., 2001). The antiviral activity of POMs was reported as early as 1971(Rhule et al., 1998). Most of the POMs showed good inhibitory activity with low cytotoxicity (Rhule et al., 1998). POMs have been shown to inhibit the replication of paramyxo-, flavi-, and several herpes viruses (Rhule et al., 1998). The molybdenum salt of polyoxometalates seems to exhibit a more potential antitumor activity while the tungsten series have more potential antiviral activity. Mechanism for the antiviral and antitumoral activity of POMs has remained elusive (Rhule et al., 1998; Jun et al., 2004; Mirabelli et al., 1980). However, the tungsten series of polyoxometalates bind to nucleic acids and affect it cleavage by a hydrolysis mechanism (Jun et al., 2004).

4. Conclusions

In this paper, the procedure of preparing an organic-inorganic salt by formula (C$_2$H$_5$NO$_2$)$_2$H$_4$SiW$_{12}$O$_{40}$ was provided for which a validated experiment had been carried out about coping with the virus. Given to our objective observations of tobacco leaves during experiments, it is revealed that the applied concentrations of this salt for killing the virus is a critical parameter such that the low concentrations than usual lead to low effectiveness of the salt against the virus and the excess concentrations than usual lead to burn the tobacco leaves and subsequently to death the plant. The results of the study indicated that this compound (organic-inorganic salt) could considerably cope with TMV with lowest damage to the leaf.

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References


