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Molybdenum and tungsten stimulate immune responses under biotic stress in Nicotiana abenthamiana infected with tomato bushy stunt virus

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Abstract

The present research examines how molybdenum and tungsten stimulate immune responses under biotic stress in Nicotiana abenthamiana. Plants are subjected to a wide range of environmental stressors that reduce and limit crop productivity. The primary response to any stress type is the production of reactive oxygen species (ROS) that cause oxidative stress, whose elimination by molybdoenzymes plays an active role. However, in the case of a molybdenum shortage in the soil or substrate, tungsten replaces molybdenum in the active centre of enzymes. Our study demonstrates the potential use of tungsten (W) and molybdenum (Mo) to stimulate the immune response of *Nicotiana abenthamiana* plants when interacting with *Tomato bushy stunt virus* (TBSV). The results indicate that the use of Mo and W metal salts activates the antioxidant system, particularly aldehyde oxidase (AO). Seed priming in metal solutions resulted in the appearance of the additional AO isoform. Furthermore, root length was high in the 1 mM Mo+W solution (4.05 cm, compared to 2.03 cm in the control). And seedling biomasses were significantly higher in infected plants in molybdenum and tungsten solutions at concentrations of 1 mM, 8.5 and 8.8 g, and about 7.6 g in control. The incubation of infected *N. benthamiana* plants in a solution of tungsten increased their resistance to TBSV. This is shown by a low level of accumulation of hydrogen peroxide (0.014), which is 23% less than the control infected plant. These results suggest the involvement of Mo and W in the mechanisms of resistance against viral infection and stimulation of the immune response of plants to biotic stress.

Keywords: Aldehyde oxidase, Molybdenum, N. benthamiana, Tungsten, Xanthine dehydrogenase.

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1. Introduction

Among the various environmental stresses, biotic stress is a significant factor that causes considerable loss in crop yield. Biotic factors impede the ability of plants to achieve their full genetic potential for vegetative and reproductive growth. Plants employ complex systems to overcome stress [1]. Reactive oxygen species (ROS) play an integral role in the stress response and the protection of plants from pathogenic stress [2].

Molybdenum (Mo) is one of the most significant trace elements in plants and is involved in the development of plant resistance to both abiotic and biotic stresses. Specific plant enzymes use Mo for reduction and oxidative reactions, just like they do with the majority of other metals necessary for plant growth. Molybdenum itself is not biologically active but is predominantly an integral part of a complex of organic pterins known as the molybdenum cofactor (Moco). Moco connects to molybdenum-needed enzymes (molybdoenzymes) that are found in many living things, like plants, animals, and prokaryotes [3]. These enzymes include primary nitrogen assimilation enzymes such as nitrate reductase (NR) and the nitrogen-fixing enzyme nitrogenase found in legume nodules of bacteroides. Other molybdoenzymes have also been identified in plants, including xanthine dehydrogenase/oxidase involved in purine catabolism and ureide biosynthesis in legumes, aldehyde oxidase (AO) involved in abscisic acid (ABA) biosynthesis, and sulfite oxidase, which converts sulfite to sulfate, an important step in ureide biosynthesis and catabolism of sulfur-containing amino acids [4].

Tungsten (W) is another element involved in enzymatic reactions, and its higher atomic number (Z = 74) makes it the most biologically active element. Although some bacteria and archaea utilize tungsten, eukaryotes do not. For example, in enzymes such as oxidoreductases, tungsten is used in a similar way to molybdenum, where it forms a complex with molybdopterin, despite its name not containing molybdenum. Living things that require either molybdenum or tungsten can use this complex. Tungsten-utilizing enzymes typically reduce carboxylic acids to aldehydes, and tungsten oxidoreductases also catalyse oxidation [5]. Molybdoenzymes are involved in eliminating oxidative stress during drought, salt stress, and low temperatures. However, plant viruses, which are a type of biotic stress, further complicate matters by aiding in rapid accumulation and spread from one plant to another. This leads to significant damage within a short period of time [6]. Researchers have found that Molybdenum (Mo) can boost the production of several enzymes, including catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). It can also raise the amount of proline in the body. Mononuclear Mo/W enzymes are the most common type of Mo/W enzymes right now. They have one Mo or W atom attached to an organic pyranopterin cofactor at their active site [7]. Nevertheless, the role of Mo and its action in viral infections remain poorly understood. The substitution mechanism of Mo metal for W during infection and the use of these metals by plants at different stages have not been studied. Thus, this study aimed to investigate the effect of Mo and W salts on the infection of *Nicotiana abenthamiana* plants with TBSV. It also investigated their impact on the activation of the enzymatic antioxidant system and phenotypic parameters during seed priming.

2. Materials and Methods

2.1. Plant Material

Nicotiana abenthamiana, a representative of the nightshade family *(Solanaceae)*, and TBSV were used as model plants in this study. Seed priming was performed by soaking *Nicotiana abenthamiana* seeds in sodium molybdate dihydrate and sodium tungstate dihydrate. Distilled water was used as the control. The seeds were kept at room temperature (19-22^{0}C) for 4-6 hours. After priming, one part of the seeds was homogenised to determine the change in aldehyde oxidase activity. The other part was transferred to Petri dishes with autoclaved 0.7% agar (Himedia BioSciences, Malaysia). The cultivation was performed in a light chamber with 16/8 daylight hours, 70% air humidity, and at 28 ºC. The ten-day-old seedlings were measured with a ruler (0.1 mm error) and then transferred to soil conditions. One month later, the grown plants were transferred to a hydroponic setup containing 0.1, 0.5-, and 1-mM molybdenum; 0.1, 0.5-, and 1-mM tungsten; 0.1, 0.5-, and 1-mM molybdenum and tungsten; or distilled water as a control.

2.2. Plant Infection

N. benthamiana plants were selected for infection after incubation in experimental and control solutions with the same phenotypic parameters. Infection with *TBSV wild type* virions was performed by mechanically damaging the leaves of the middle tier. The leaf plate was damaged by solid particles of silicon oxide, followed by rubbing in a suspension consisting of viral material and 10 mm PBS (pH 6.8) at a ratio of 1:3 (virions/phosphate buffer). The infected plants were grown separately from the healthy plants. On day seven post-infection, symptoms of viral infection were observed.

2.3. Determination of the Presence of Virions

The presence of virions in the inoculated plants was determined using a commercial kit, the TBSV - Double Antibody Sandwich Enzyme-linked immunsorbent Assay (DAS ELISA) / Detecting Conjugate and Alkaline Phosphatase (NanoELISA, USA).

2.4. Polyacrylamide Gel Electrophoresis (PAGE) in Native Condition

The samples were prepared for native PAGE following the method described by Batyrshina, et al. [8]. Native gel electrophoresis was performed for 3.5 hours, as previously described [9].

2.5. Determination of Enzyme Activity in PAAG under Non-Denaturing Conditions

Aldehyde oxidase (AO) was measured following the modifications made to the method of Sagi, et al. [9]. *Xanthine dehydrogenase (XDH)* was measured according to Nakagawa, et al. [10]. Catalase activity was evaluated as previously described [11].

2.6. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

When sodium dodecyl sulfate was added to a 12% polycrylamide gel, vertical electrophoresis was used to separate the denatured proteins from the samples [12]. The gel was stained for 2 hour in a solution comprising methanol, 70% acetic acid, and Coomassie Blue R. Destaining was performed in a solution consisting of methanol, 70% acetic acid, and distilled water for 8 h at 19-22℃.

2.7. Assays for Enzyme Activity and Determination of Protein Concentration

Activity of aldehyde oxidase (AO) was determined spectrophotometrically by using p-dimethylaminocinnamaldehydep-DMAC as a substrate and O2 as an electron acceptor, according to Kurth and Kubiciel [13]. The *activity of xanthine dehydrogenase (XDH)* was determined by using phosphate buffer. The reaction mixture contained 30 µM of xanthine as a substrate, according to Rajagopalan and Handler [14]. Protein concentration was determined by the Bradford [15], using the Bio-Rad protein assay kit and bovine serum albumin as a standard.

2.8. Spectrophotometric Analysis of Hydrogen Peroxide

The plants' leaves that were grown at the three different concentrations were mixed together in 50mM phosphate buffer, which is made up of monobasic dihydrogen phosphate and dibasic monohydrate (pH 7.5). The samples were then centrifuged twice for 10 minute at 14,000 rpm at 4°C. Supernatants were mixed (96 Well Polystyrene Cell Culture Microplates, Greiner Bio-One GmbH, Germany) with a reaction mixture containing 8.5 mM 4-aminoantipyrine (APP), 3.4 mM sodium 3,5-dichloro-2-hydroxybenzenesulfonate (BHS), and 45 U ml-1 horseradish peroxidase (HRP) in 50 mM Tris buffer (pH 7.5) at a ratio of 5:1:1:1 on a microplate. After 10 minutes, spectrophotometry was performed using the Infinite 200 PRO plate reader at a wavelength of 510 nm.

Statistical analysis of biochemical and morphological parameters was carried out according to the generally accepted methodology, determining the student criterion (t) and the reliability of differences (P) in Microsoft Excel 2010.

3. Results

We looked at what happened when Mo and Won seeds were primed before they were planted in solutions with 0.1, 0.5, and 1 mM of molybdenum, tungsten, or both. Phenotypic parameters were measured 10 days after germination, with exposure times of 4 and 8 hours. However, 8 hours treatment did not induce germination in any of the three solutions at all concentrations. Germination was observed only in seeds after 4 h of priming (Figure 1A, B).

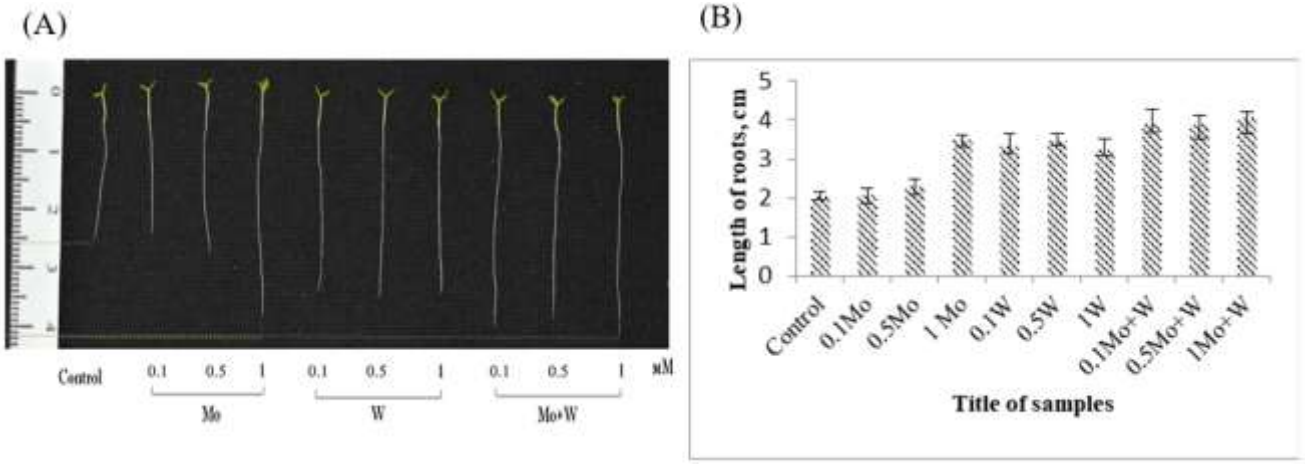

Figure 1.
Effect of molybdenum, tungsten and combined concentrations on the growth of *N. benthamiana* seedlings (A). Results of phenotypic measurements (B). Metal concentrations (Mm) are represented by numbers at the bottom of the figure.

An increase in growth stimulation was observed in plants after metal priming at concentrations of 0.5 ($p < 0.01$) and 1 mM ($p < 0.001$) of molybdenum and at concentrations of 0.1 ($p \geq 0.05$), 0.5 mM ($p < 0.05$), and 1 mM ($p < 0.001$) of tungsten, as well as with 0.1 mM of molybdenum and 0.5 and 1 mM of tungsten ($p < 0.05$). Seedlings grown in combined concentrations showed a higher average length compared to those grown in tungsten alone by 15%, control by 48%, 0.1 mm by 47%; and 0.5 mM at 42% molybdenum concentrations. The longest seedlings were grown from seeds incubated in 1 mM Mo ($p < 0.001$), as well as at 0.1, 0.5, and 1 mM ($p < 0.001$) Mo+W concentrations.

These data are supported by studies by Wu, S, who demonstrated that extrinsic (e.g., environmental stress) and intrinsic factors (e.g., signaling molecules) regulate root growth. Nitric oxide (NO) is a signaling molecule catalyzed by the Mo enzyme nitrate reductase [16], which in turn regulates a number of growth modules, including primary growth [17],

lateral root formation [18], the development and formation of adventitious roots, the development of root hairs, and the growth of the root meristem. These root growth processes are essential for plant adaptation to regulate water deficits and nutrient uptake, as the effects of root morphology and anatomy are ultimately combined [19].

To make sure that seed priming with metals had an effect on growth in virus-infected conditions, we checked the fresh weight of seedlings whose seeds had been treated with Mo and W metal solutions before they were planted (Figure 2).

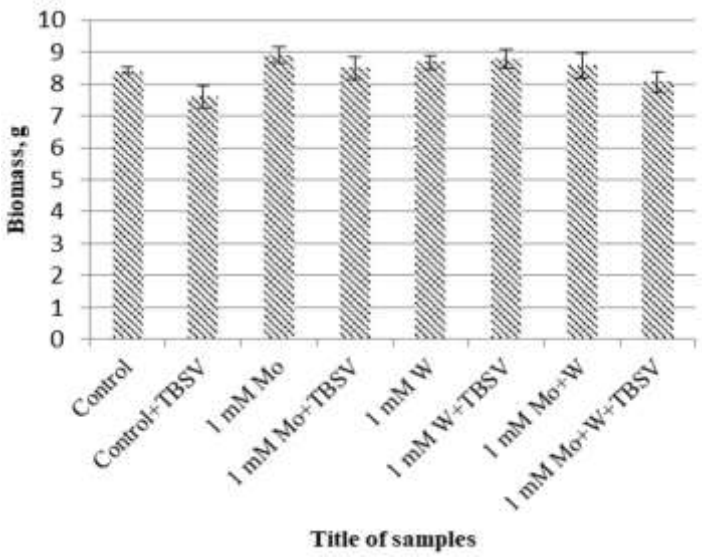

Figure 2.
Indicators of biomass of seedlings (p≥0.05).

The biomass of seedlings grown with various concentrations of metals and seedlings inoculated with the virus showed insignificant differences between the concentrations. A significant fluctuation in biomass was noted between the control samples without virus and those infected with virus at 0.8 g ($p < 0.05$). The remaining samples, when inoculated with the virus, did not show significant differences in biomass ($p \geq 0.05$) and ranged from 0.1-0.5 g in other variants. These results suggest that the use of metal solutions during seed priming reduces the effect of TBSV infection without reducing the biomass of *N.benthamiana* plants.

3.1. Determination of AO Activity in Native Gel after Seed Priming

To determine the effect of metal priming duration and concentration on AO enzyme activity in seeds, enzymatic staining was analysed after native electrophoresis for 4-hour and 8-hour priming periods. Although the seeds did not germinate after 8 hours of priming, the isoform composition of AO was different.

For 8-hour priming, the presence of three isoforms of the AO enzyme (AO1, AO2, and AO3) was noted compared to two isoforms (AO1 and AO2) found in the seeds after 4-hour priming (Figures 3a and b).

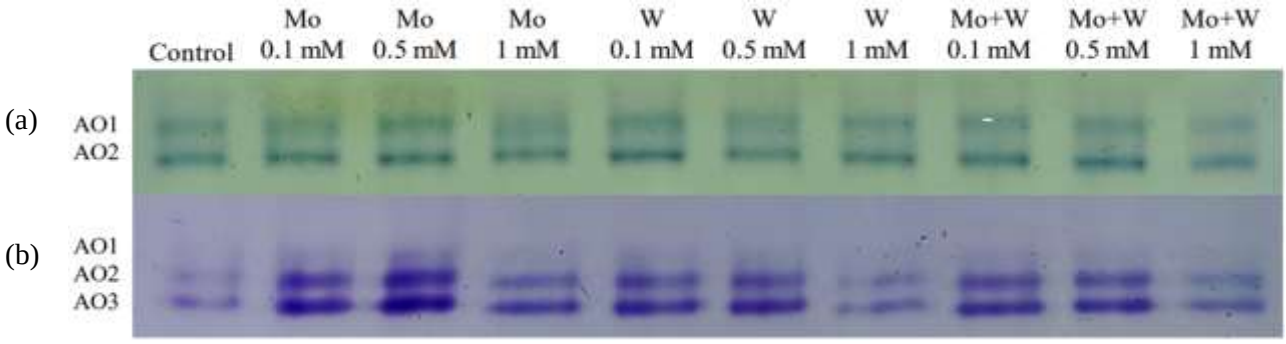

Figure 3.
Detection of AO activity of *N. benthamiana* seeds after 4 h (a) and 8 h (b) priming in gel after electrophoresis under non-denaturing conditions.
Note: (a) after *4 h priming* in gel after electrophoresis under non-denaturing conditions.
(b) after *8 h priming* in gel after electrophoresis under non-denaturing conditions.

Canavalia Okla, et al. [20] conducted a study to compare the effects of Mo seed priming on seedlings of *Canavalia* species or cultivars. Their findings showed that Mo enhances photosynthetic pigments, resulting in improved growth and increased biomass. They also noted a 0.2–0.3-fold increase in nitrogen content. The increase in metabolism resulted in increased activity of the antioxidant (2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and free radical scavenger 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and iron, which reduced the antioxidant capacity (FRAP-Ferric Reducing Antioxidant Power Assay) of *Canavalia spp*. Furthermore, the antioxidant activity increased by 8–20% [20].

3.2. Morphological Signs of Infection of Plants Incubated in Hydroponics

After studying the effects of metals on plant development after seed priming, the optimal concentration for the three variants was 1 mM. Also, to find out what role Mo and W play in *N. benthamiana* plants that have TBSV, the plants were grown in 1 mM solutions of Mo, W, and Mo with W.

A commercial NanoELISA kit (USA) was used to detect viral infection in the hydroponically grown inoculated plants (Table 1).

Table 1.
Confirmation of viral infection in *N. benthamiana* plants on day seven after inoculation with the TBSV wild type by ELISA.

Sample	OD[1]	Positive control	Negative control	Pos.
Control	0.053±0.01	0.048±0.03	0.058±0.03	–
1 mM Mo	0.781±0.02	0.788±0.07***	0.058±0.03	+
1 mM Wo	0.877±0.03	0.967±0.08***	0.058±0.03	+
1 mM Mo+Wo	0.918±0.03	0.964±0.03***	0.058±0.03	+

Note: [1]OD – Optical density; ***p<0.001.

The severity of symptoms directly depended on the metal with which the plants were incubated (Figure 4).

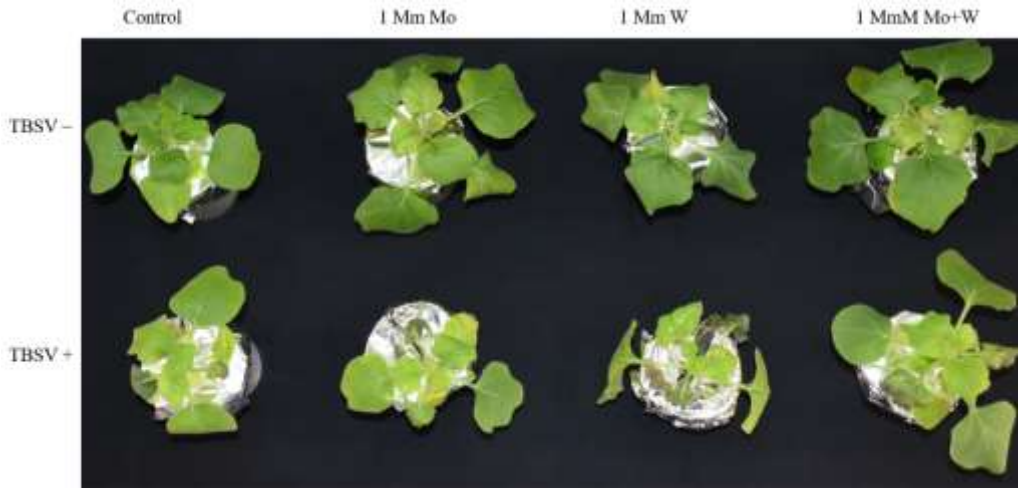

Figure 4.
Monthly plants of *N. benthamiana* incubated in 1 mM concentrations of molybdenum, tungsten and molybdenum with tungsten, on the 7th day after inoculation with TBSV wild type.

The control plants, which were in distilled water after infection, showed typical symptoms on day seven: growth retardation, twisting of the upper tier of leaves, and the appearance of side shoots. Infected plants in a 1 mM Mo solution showed mild symptoms in the form of slight twisting of the leaves in the upper tier. In contrast, the opposite effect was seen in an infected plant in a W solution. When plants were infected with 1 mM W, they showed severe symptoms like slower growth, leaves in the upper layer curling, and leaves in the lower layer losing their chlorosis. The infection culminated in the systemic necrotic collapse of the *N. benthamiana* plant in W approximately 9–11 days after inoculation. The combined concentration of Mo + W also indicated the presence of a viral infection, with stunting and curling of the leaves in the upper tier. However, the general condition of the plant was satisfactory. The collapse of the plants incubated in combined concentration occurred on the 11–12th day, whereas in distilled water, it occurred on the 12–14th day. Nevertheless, the complete collapse of plants in a 1 mM Mo solution occurred only on the 16-17th day after infection. This suggests that this metal may mitigate the effect of viral infection more effectively.

Based on a study by Datnoff, et al. [21], molybdenum, like other heavy metals, can deactivate viruses by denaturing their protein shell, making it a particularly effective metal or metalloid for this purpose. Leo, et al. [22] assessed the effects of different concentrations of Mo on four hybrids of tomato F1 grown using a soilless system with varying Mo contents. The results showed that tomato plants grown with a Mo concentration of 2.0 µmol L-1 yielded significant improvements in total yield (21.7%), marketable yield (9.1%), aboveground biomass (16.7%), plant height by 50 (days after treatment), DAT (6.5%), polyphenol content (3.5%), ascorbic acid content (1.0%), SSC (3.5%), number of fruits (24.8%), Mo content in fruits (20.0%), and Fe content in fruits (60.5%).

3.3. Determination of the Activity of AO, XDH and CAT in Plants Incubated in Metal Solutions

To determine the effect of TBSV infection on protein levels when plants were incubated with various concentrations of TBSV, enzyme activity was detected spectrophotometrically (Table 2).

Table 2.
AO and XDH enzymes activity, mM/1 g of wet weight.

Name	Control	Control+TBSV	1 mM Mo	1 mM Mo+TBSV	1 mM W	1 mM W+TBSV	1 mM Mo+W	1 mM Mo+W+TBSV
AO	166±0.1	207.5±0.04	255.6±0.3	340.3±0.2	23.2±0.1	26.6±0.2	185.9±0.05	210.8±0.17
XD	158.7±0.2	296.8±0.28	628.5±0.04	593.5±0.18	38.01±0.3	99.9±0.17	311.1±0.3	298.4±0.3

The activity of the AO enzyme in infected plants incubated in 1 mM Mo solution was the highest compared to other samples, by more than 200% compared to the control. Conversely, plants grown in tungsten solution demonstrated almost complete inhibition of AO activity by 86% below control. A similar situation was observed with the XHD enzyme: plants incubated with Mo showed high levels of xanthine dehydrogenase accumulation, more than 3 times higher than the control, while when using tungsten, the opposite effect was observed, 76% less than the control. Similar to Mo, the combined concentration resulted in an increase in the activity of XDH compared to the control.

Plants infected with the wild-type *TBSV* showed increased catalase (CAT) activity compared to healthy plants. High CAT activity was characteristic of plants infected with Mo and combined concentrations. In contrast, tungsten showed the weakest CAT activity in both healthy and infected plants (Figure 5).

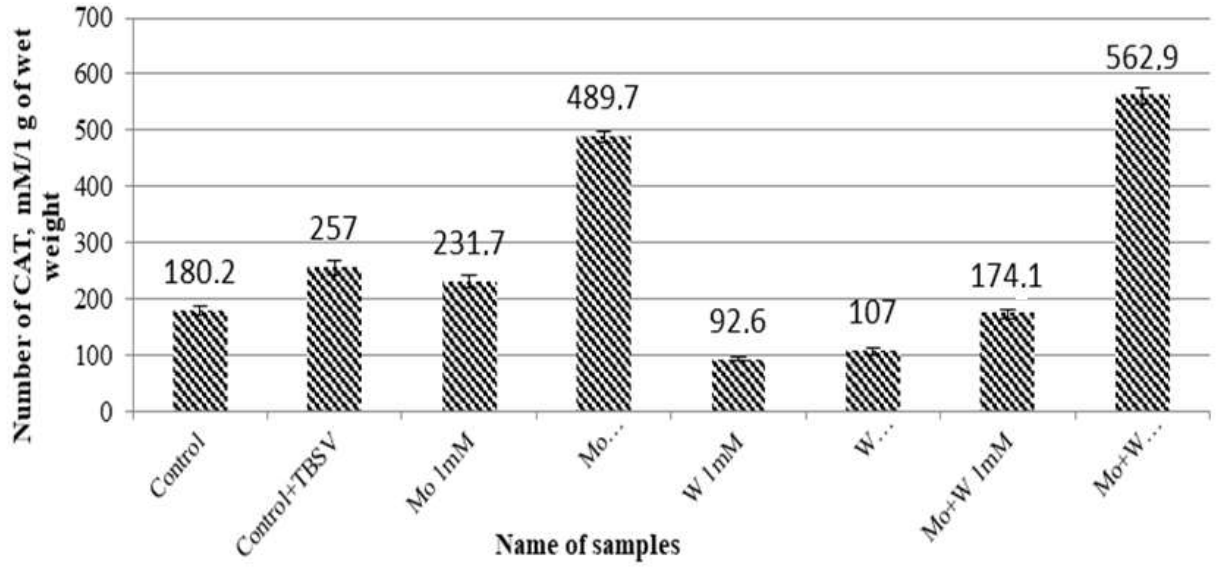

Figure 5.
Catalase activity in *N. benthamiana* plants.
Note: p<0.001.

From these results, it can be concluded that TBSV infection promoted an increase in the activity of AO and CAT in infected plants. The addition of molybdenum and its combined concentrations led to an increase in the activities of AO, XDH, and CAT. In contrast, tungsten exhibited the opposite effect, almost completely inhibiting the activity of these enzymes. According to data, the level of catalase increases in tissues in the presence of H2O2, which also characterizes the level of plant resistance to viral infection.

The level of accumulation of hydrogen peroxide, as the main product of oxidation, was measured in the same experimental and control samples (Figure 6).

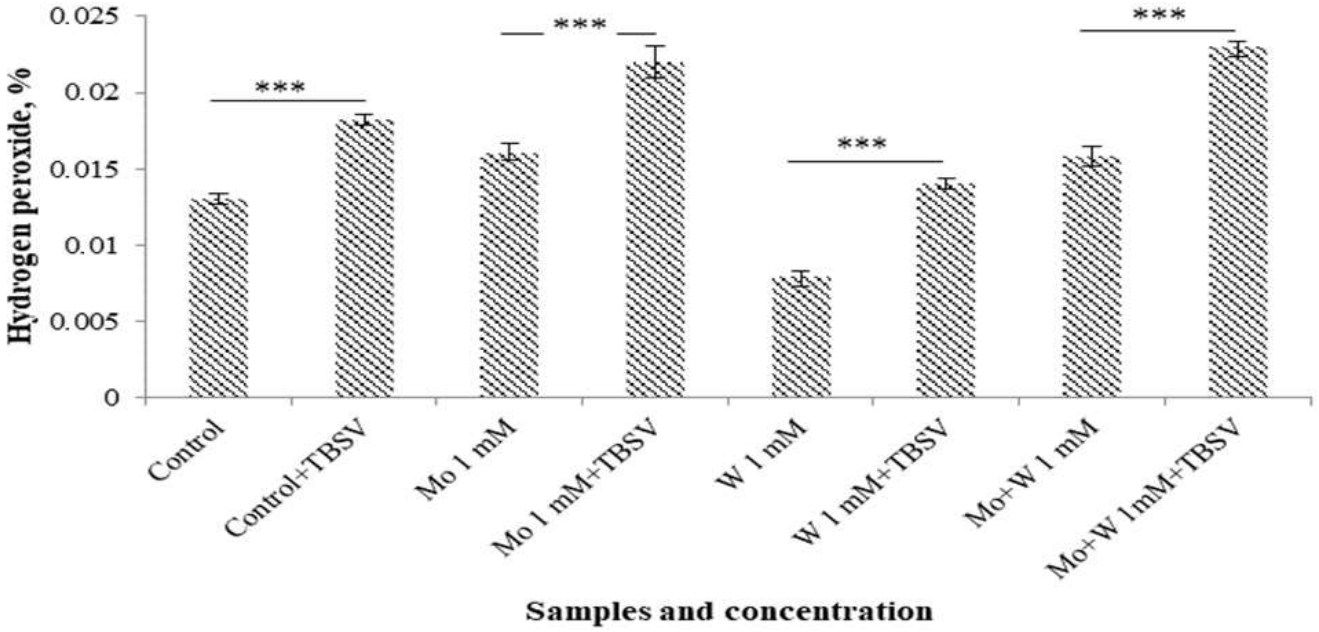

Figure 6.
Spectrophotometric analysis of hydrogen peroxide of plants incubated in molybdenum and tungsten, combined concentration and distilled water.
Note: ***p<0.001.

As per the results of catalase activity and peroxide level, it can be noted that both substances have a high level of accumulation in the test variants of Mo 1mM+TBSV and Mo+W 1mM+TBSV. The amount of catalase in the W 1mM, W 1mM+TBSV, and Mo+W 1mM variants is lower than in the control variant by 87.6, 73.2, and 6.1 mM/1 g of wet weight, respectively. This means that less peroxide builds up in these variants. It can be concluded that in the presence of W and Mo, both under conditions of infection and in the native state, the level of H2O2 production slightly increases. If processing W 1 mM (0.07%) is lower compared to the nil treatment (0.13%), while the catalase level is also not high (92.6±1.34 mM/1 g of wet weight). Thus, a decrease in stress was noted when W 1mM was exposed.

According to Hayyawi, et al. [23], the H2O2 content in *Vigna radiata L.* beans slightly increased with an increase in Mo concentration to 30 mg L−1, followed by a significant decrease with an increase in Mo concentration to 45 mg L−1. Thus, Mo is a reliable agent for activating the defense system in mung beans. Lei, et al. [24] investigated the accumulation of H2O2 in tobacco plants inoculated with cucumber mosaic virus strain M (M-CMV), which increased in leaves after inoculation and then decreased after 4 days. For systemically infected leaves with symptoms of chlorosis, the accumulation of H2O2 was always higher than for healthy leaves. Incubation in a solution with Mo and W may not have been long enough to show a decrease in hydrogen peroxide; however, in the 1mM variant, W in healthy plants showed a value of H2O2 less by 38.5%, while inoculated tobacco plants in the same solution had the same peroxide level as in the control variant. At the same time, the catalase level was 40% lower than in the control, which may indicate a low level of stress in tobacco plant cells in the presence of the TBSV virus. However, it is necessary to further study the incubation time of both the virus and exposure to a metal solution. So Shetty, et al. [25] showed that the presence of hydrogen peroxide in cells makes cells more resistant to the pathogen. For example, in the 1 mM Mo+W+TBSV variant, the accumulation of peroxide was 77% higher than the control, and catalase was more than 2 times higher than the control. When, as in the Control+TBSV variant, catalase activity was 118% lower, which may indicate a low level of immune response to the invasion of the virus in the absence of metals.

4. Discussion

Mo and W are redox transition metals that participate in various reactions within enzymes, including substrate oxidation using water and oxygen Hagen [26]. Wu, et al. [16] did a study on how nitric oxide NO and molybdenum Mo affect the shape of roots, how much water they take in, the expression of aquaporin, and the levels of minerals and nutrients in water wheat that is stressed by drought. It was demonstrated that the NO signal was involved in Mo-regulated root growth, mainly reflected in the supply of Mo. NO donors improved root morphology, whereas the rate of water uptake by roots increased under the influence of Mo, which was positively correlated with root tips and lateral roots' length. Mo also improves the expression of aquaporins, particularly TaPIPs. This suggests that improved root growth and aquaporin expression are involved in increased Mo water uptake by wheat roots during drought [16, 26].

Studies have shown that MO can increase frost resistance in wheat by increasing the expression of the transcription factor Cbf, as reported by Al-Issawi, et al. [27]. Therefore, Mo can be useful in situations where increased frost resistance is required.

The application of Mo significantly increased chlorophyll, dry matter, grain yield, and biomass while reducing water loss in wheat under drought conditions. These findings suggest that the application of Mo improved the water use efficiency of wheat. In addition, Wu, et al. [16] reported that the use of Mo under stress conditions resulted in increased activity of antioxidant enzymes, including superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase. Furthermore, it increased non-enzymatic antioxidant content, such as ascorbic acid, reduced glutathione, and carotenoids. Moreover, the levels of protein, proline, and soluble sugar increased, while the content of malondialdehyde decreased with the use of Mo.

The influence of molybdenum was investigated by Vigani, et al. [28] in conjunction with iron supplementation, to determine the overall absorption and distribution of each element in tissues and at the mitochondrial level. The nutritional status of Fe is more important than Mo homeostasis, and it changes how much Mo is available for molybdoenzymes in the form of Moco. Fe deficiency triggers Moco biosynthesis and affects molybdoenzymes, with the main effect on nitrate reductase and xanthine dehydrogenase involved in nitrogen assimilation and mobilization, and on the mitochondrial amidoxim-reducing component.

In another study examining the effect of Mo on leguminous plants under drought conditions, Hayyawi, et al. [23] introduced Mo as a foliar exogenous top dressing and seed soak at concentrations of 0, 15, 30, and 45 mg L^{-1}. Scientists found that when mung bean leaves are under a lot of water stress, the levels of malondialdehyde (MDA) and hydrogen peroxide (H2O2) rise. These are signs of lipid peroxidation and the build up of reactive oxygen species (ROS). However, the application of Mo improved some growth and yield indicators and strengthened the defense system by increasing the expression of antioxidants such as proline, catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD).

Recent studies by Siddiqui et al. have shown that hydrogen sulfide (H2S) is a potential gas transmitter in plants and plays a useful role in stress reduction. This study demonstrated the role of Mo and H2S in reducing arsenate (AsV) toxicity in faba bean (*Viciafaba L.*) sprouts. AsV-stressed seedlings treated with exogenous Mo and/or NaHS (H2S donors) showed resistance to AsV toxicity, as manifested by reduced apoptosis, reduced reactive oxygen species (ROS), reduced NADPH oxidase and GOase activities, and the upregulation of antioxidant enzymes in the leaves. Under AsV toxicity, seedlings exposed to Mo + NaHS exhibited an increased rate of nitrogen metabolism. The increased activity of glutamine synthetase, nitrate reductase provided evidence for this. Using both Mo and NaHS together also improved the production of cysteine and hydrogen sulfide, both when ManzerAsV stress was present and when it wasn't present [29].

Thus, the use of Mo and W under biotic stress can significantly reduce the viral load on plants, using *N. benthamiana* as an example. This is done by activating the antioxidant system while maintaining biomass levels. Further research is needed to explore the involvement of tungsten and molybdenum in the defense system against biotic stress.

5. Conclusion

In this study, Mo, W, and Mo+W salts were used to reduce the effects of TBSV *on N. benthamiana* plants. Seed priming in the experimental solutions resulted in the activation of the antioxidant enzyme AO. After 4-hour incubation, there were 2 isoforms of AO, while in seeds after 8-hours, there were 2 isoforms. The seedling biomass showed slight differences in the case of infected and healthy plants in the presence of metals, in particular, in solutions with 1 mm Mo, W, Mo+W, the difference was 0.4 g, 0.1 g, 05 g, respectively. The root length was higher in the 1 mM Mo and W solutions and was 8,5 g и 8,8 g, in infected plants, respectively. The results showed that the treatment of infected plants in solutions of molybdenum and tungsten metals in both cases reduces the viral load on plants, does not reduce biomass, and does not affect the reduction of root length. At the same time, seed priming stimulates the antioxidant system. The obtained data can be used for further searches for the treatment of biotic stresses in plants and for a better understanding of the mechanisms of interaction between molybdenum and tungsten.

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