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Biological effects of PAS TiO_2 sol on disease control and photosynthesis in cucumber (*Cucumis sativus* L.)

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Abstract

This paper reports the biological effects of TiO_2 sol derived from peroxotitanic acid solution (PAS TiO_2 sol) on the control of plant diseases and growth regulation. The PAS TiO_2 sol is a neutral, viscous aqueous colloid of TiO_2 containing 1.6% TiO_2 with a size of between 10-50 nm. Experiments were conducted under normal laboratory and greenhouse growing conditions. For the bactericidal effects of the PAS TiO_2 experiments, TiO_2 coated glass slides were inoculated with pathogenic bacteria in the laboratory. For the Greenhouse experiments on disease control and photosynthesis, all test plants (treatment block and control block) were inoculated with pathogenic bacteria. While plants in the treatment blocks were sprayed with PAS TiO_2, plants in the control blocks were sprayed with water. X-ray diffraction (XRD) results demonstrated that the crystal form of TiO_2 nano-particles was anatase. Results from greenhouse experiments using cucumbers showed that PAS TiO_2 sol formed a well-spread, transparent, and sticky layer of TiO_2 photo-catalyst on the plants' surface. The PAS TiO_2 photo-catalyst layer prevented pathogenic infection and improved photosynthetic performance. These results suggest that PAS TiO_2 sol can be used as a potentially environmentally friendly plant germicide and growth regulator.

Keywords: Nano-biomaterial, TiO_2 sol, disease control, photosynthesis.
Abbreviations: ANOVA – analysis of variance, CFU – colony forming unit, CRD – completely randomized design, LSD – least significant difference, PAS - peroxotitanic acid solution, TEM – Transmission Electron Microscope; UV – ultraviolet, XRD – X-ray diffraction.

Introduction

Synthetic germicides have been widely used to control crop diseases. However, application of synthetic germicides might cause problems such as environmental pollution, food safety, and human health issues. Therefore, it has become necessary to develop alternative methods to control crop diseases. PAS TiO_2 nano-particles are environmentally friendly photo-catalysts. They can be excited by near-UV light (360nm wavelength) to generate holes (h^+) and hydroxyl radicals (OH) in the valence band and electrons and superoxide ions (O_2^-) in the conduction band. The reactive oxygen intermediates generated by these PAS TiO_2 photo-catalytic reactions have a strong oxidizing power which can decompose and mineralize organic compounds through a series of oxidation reactions and cause damage to living organisms (Fujihira et al., 1981; Huang et al., 1997; Kikuchi et al., 1997; Sunada et al., 1998; Khan et al., 2002; Tamai et al., 2002). These findings suggested that PAS TiO_2 nano-particles may be a potential plant germicide. In 1985, Matsunaga et al. first reported the bactericidal effect of PAS TiO_2 photo-catalytic reactions. Since then, intensive research on the potential of using PAS TiO_2 photo-catalytic to kill pathogens has been conducted on a wide spectrum of organisms including viruses, bacteria, fungi, algae, and cancer cells (Matsunaga, et al., 1988; Cai et al., 1992; Ireland et al., 1993; Feng et al., 2004; Han et al., 2004; Hur et al., 2005; Peller et al., 2007;) to enhance photo-catalytic disinfection and photo-biological effect on plant growth and development (Choy and Chu, 2005; Zheng et al., 2007; Chu et al., 2009). Our research on the biological effects of PAS TiO_2 has demonstrated these nano-particles' beneficial effects and found that the photo-catalyst properties of PAS TiO_2 significantly prevented cucumber fungal/bacteria diseases and promoted photosynthesis (Zheng et al., 2007; Li et al., 2008; Zhang et al., 2008). A suspension of TiO_2 nano-particles usually has low adhesion to the surface of plant leaves which makes it difficult to fully and sustainably exert its biological effects. A synthetic method generating TiO_2 sol from peroxotitanic acid (PAS TiO_2 sol) to be used as a photo-catalytic film coating on ceramics was reported by Ichinose et al., 1996 and 1998. This form of TiO_2 sol is a neutral and viscous aqueous colloid of TiO_2 nano-particles. PAS TiO_2 sol as germicide or growth regulator may adhere better and sustain the germicide activity in plant than the suspension of TiO_2 nano-particles. Cucumber (*Cucumis sativus* L.) is the most consumed vegetable in China and ranks number one among all vegetable crops produced in China (Zhang, 2001). It also is very susceptible to a fungus disease caused by *Pseudoperonospora cubensis* and bacterial diseases caused by *Pseudomonas syringae pv. lachrymans* and *Xanthomonas campestris pv. vesicatoria* (Gao, 2005). These diseases significantly reduce cucumber plant growth, yield, and quality which impact the vegetable's availability, production costs, and profit margins to producers. To address the impact of these diseases, this research project

Table 1. The effect of PAS TiO_2 sol on control of cucumber disease pathogens.

Pathogens	Treatment	Lesion			Disease incidences	Disease indexes	Control efficiency
		7^{th} leaves	8^{th} leaves	9^{th} leaves			
P. s. pv. lachrymans	Control	13.3 a	18.0 a	12.1 a	68.3 a	14.5 a	
	TiO_2-treated	3.9 b	6.5 b	5.6 b	42.7b	6.3 b	68.6%
X. vesicatoria	Control	18.8 a	26.43 a	33.5 a	76.2a	39.2 a	
	TiO_2-treated	4.5b	6.37 b	8.5 b	26.8 b	7.8 b	90.6%

Values for each pathogen in the same *column* followed by the same *letter* are not significantly different at $\alpha=0.05$.

Table 2. The effect of PAS TiO_2 sol on chlorophyll content of cucumber leaves.

Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control	1.1761	0.4094	1.5855
TiO_2-treated	1.7263**	0.5363 *	2.2626**

Significance levels are $\alpha=0.05$ (*) and $\alpha=0.01$ (**).

was designed to study the biological effects of PAS TiO_2 sol on disease control and promoting growth and production in cucumber. Specifically, we investigated PAS TiO_2 sol's bactericidal and fungicidal effects and its effects on chlorophyll content and therefore on photosynthesis to increase crop production as a potentially environmentally friendly plant growth regulator.

Results and discussion

Characteristics of PAS TiO_2 sol

TEM images (Fig. 1) indicated that PAS TiO_2 sol was a well-dispersed aqueous colloid of spindle-shaped TiO_2 nano-particles with an average size of 50 nm. The XRD patterns as dried powders from PAS TiO_2 sol (Fig. 2) revealed that TiO_2 nano-particles in PAS TiO_2 sol were anatase phase with photo-catalytic activity (Sridhar et al., 1999). The detailed preparation of TEM samples was as follows: a vial filled with nano-TiO2 sol was first subjected to ultrasonic vibration for 20 minutes, then was transferred to a carbon-coated copper grid for drying under room temperature overnight. TiO2 has different crystalline morphologies with anatase being a double cone, rutile being a short column, and brookite being a plate. TEM photographs clearly indicated that the particles were cone-shaped, dispersed in the sol samples with the measured fringe spacing of 0.35 nm for the particle lattice which fits exactly the anatase TiO2 (101) crystal.

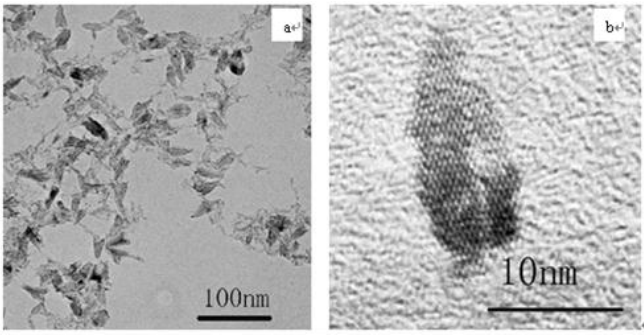

Fig 1. TEM images for shape characterization of TiO2 nano-particles in PAS TiO_2 sol.

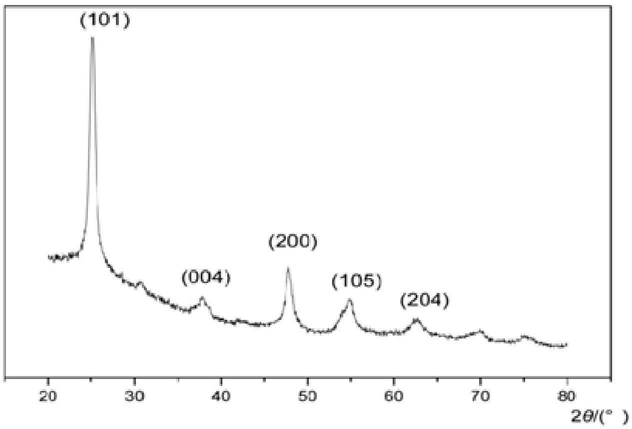

Fig 2. XRD pattern for crystal phase characterization of TiO_2 nano-particles in PAS TiO_2 sol.

Bactericidal effects of PAS TiO_2 sol

The results showed that PAS TiO_2 sol had good adhesive and film forming abilities to form continuous and stable films on the glass surface. The colony forming units (CFU) of *P. s. pv. lachrymans* and *X. vesicatoria* were 1.6±0.5 and 0 in PAS TiO_2 treated condition and 1944.0 ±45.6 and 1393.7±37 in the control, respectively. More than 99% of bacterial cells lost their viabilities after being illuminated for 12 hours on the surface of TiO_2-coated glass. The bactericidal rates of PAS TiO_2 sol against *P. s. pv. lachrymans* and *X. vesicatoria* were 99.9% and 100%, respectively. These findings indicated that when treated with PAS TiO_2 sol, cucumber plants should be able to maintain their vigor and productivity for a much longer time by preventing invasion of pathogenic microorganisms resulting in a much higher yield.

The effect of PAS TiO_2 sol on disease control

The results confirmed PAS TiO_2 sol possesses a perfect film-forming and effective bactericidal ability that help control plant bacteria. Spraying PAS TiO_2 sol on cucumber plants formed a well-spread, transparent and sticky PAS TiO_2 photo-catalyst layer on the leaf surface and prevented the infection of pathogens (*Pseudomonas syringae pv. lachrymans* and *Xanthomonas campestris pv. vesicatoria*) through PAS TiO_2 sol's photo catalytic disinfection (Table 1). The number of lesions, count of disease incidences, and disease index values in PAS TiO_2-treated blocks were significantly less on the treated cucumber plants than on the control plants (P=0.05). The control efficiency of PAS TiO_2 sol to *Pseudomonas syringae pv. lachrymans* and *Xanthomonas campestris pv. vesicatoria* was 68.6% and 90.6%, respectively. Our results indicated that PAS

TiO_2 sol acted as a germicide that could effectively prevent the invasion of bacterial and fungal pathogens to crops (Fig. 3).

The effect of PAS TiO_2 sol on photosynthesis

PAS TiO_2 sol significantly promoted photosynthesis (Figs. 4&5). Plants sprayed with 0.8% PAS TiO_2 sol displayed the highest photosynthesis activity and the net photosynthetic rate was increased by 30% compared with the control (Fig. 4). Net photosynthetic rate began to increase significantly after only one hour after the plant leaves had been sprayed with PAS TiO_2 sol (Fig. 5). At the third hour following the spraying with PAS TiO_2 sol, the net photosynthetic rate peaked with a gradual decline afterwards. The physiological mechanism of PAS TiO_2 sol by which it improves photosynthesis is not fully understood. Our data indicated that the increase of net photosynthetic rate might be attributed to the improvement of photochemical processes by photo-catalyst effect of TiO_2 and the increase of the synthetic pigment (chlorophyll) contents. This observation is similar to the findings of Zheng et al. (2007). PAS TiO_2 sol also significantly increased the content of chlorophyll a and b and the total chlorophyll in cucumber leaves (Table 2).

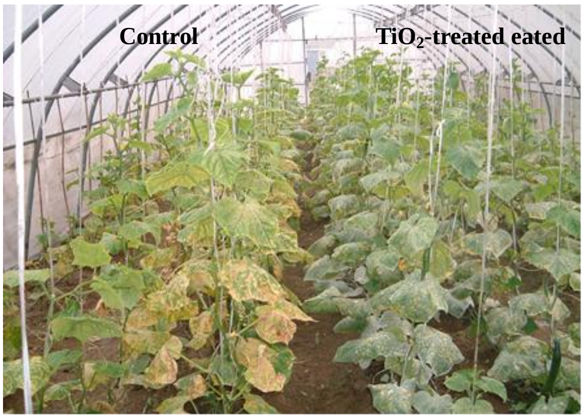

Fig 3. TiO_2 treated block (right) significantly reduced cucumber disease symptoms of *Pseudoperonospora cubensis*.

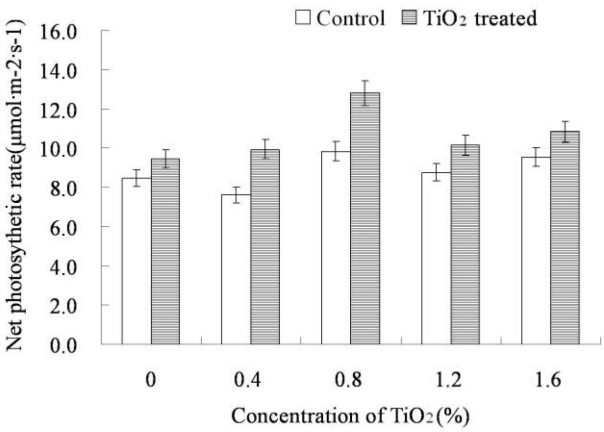

Fig 4. Effects of PAS TiO_2 sol concentrations on photosynthesis of cucumber leaves.

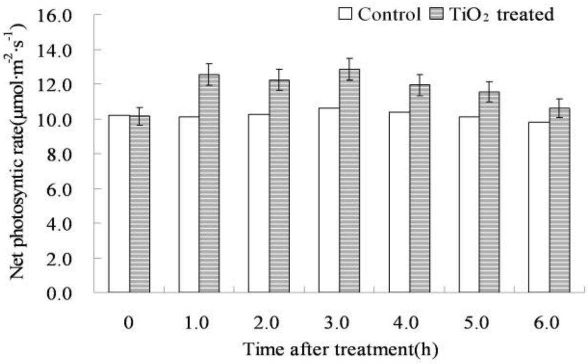

Fig 5. Effects of time (h) of spraying PAS TiO_2 sol on photosynthesis of cucumber leaves.

Materials and methods

Preparation and characterization of PAS TiO_2 sol

PAS TiO_2 sol was prepared from peroxotitannic acid solution according to the Ichinose method using $TiCl_4$ and H_2O_2 as the main components (Ichinose et al., 1996 and 1998). The resultant PAS TiO_2 sol was a yellow, viscous aqueous colloid of 1.6% TiO_2 with pH7.8, and characterized by X-ray diffraction (XRD, D/max-IIIA, Rigaku Corporation, Japan) and Transmission Electron Microscope (TEM, JEOL-100CXII, Nippon Electric Company, Japan).

Evaluation of bactericidal effects

Two pathogenic bacteria: *Pseudomonas syringae pv. lachrymans* and *Xanthomonas campestris pv. vesicatoria* were used for evaluating TiO_2's bactericidal effects. TiO_2 coated glass slides (100 mm × 50 mm) were prepared by dipping them into the PAS TiO_2 sol and heating the coated slides at 160°C for 3 hours. The photocatalytic bactericidal effects of the PAS TiO_2 sol were evaluated by inoculating the slides with the PAS TiO_2 coating with an aqueous suspension of the bacteria followed by UV irradiation 20 cm underneath black light (40 watts, 360 nm) for 12 hours with an intensity of 3.0 mW/cm^2. Standard microbiology colony forming units (CFU) were counted for the PAS TiO_2 sol treatment and control. CFU is a measure of viable bacterial or fungal numbers, which is different from the direct microscopic counts where all cells (dead and living) are counted. CFU results are typically represented as CFU/ml for liquids or CFU/g for solids.

Greenhouse experiment

Cucumber is a very important vegetable crop worldwide. Cucumber is very susceptible to bacterial and fungal diseases, such as bacterial angular leaf spot, downy mildew under high humidity greenhouse environment. To effectively study the effect of PAS TiO_2 sol on disease control, a susceptible cucumber cultivar, Z*hongnong* No.118, was chosen in this research. Cucumber plants were cultivated under normal greenhouse conditions. Solutions of disease causing pathogens were prepared at the concentration of 10^8 $CFU{\cdot}ml^{-1}$. The pathogenic solutions were then sprayed onto the cucumber seedlings at the 5 leaf stage counting from the bottom first true leaf as the artificial inoculation. Inoculated cucumber seedlings were kept in a growth chamber for 48 hours at 25°C and 95% relative humidity before being transplanted into treatment blocks and control blocks in a greenhouse for further testing under normal cultivation conditions. At the 7-8 leaf stage of the cucumber seedlings, counting from the bottom first true leaf, cucumber leaves in the treatment blocks were sprayed with 0.8% PAS TiO_2 sol using a nanometric sprayer.

All plants in the control blocks and in treatment blocks were grown under the same greenhouse condition. The only difference between the control blocks and the treatment blocks was the spraying of PAS TiO_2 sol. While plants in the control blocks were sprayed with water, plants in the treatment blocks were sprayed with PAS TiO_2 sol. Each treatment had three blocks (2.7 × 2.0 m) and each block had 20 plants. Each block was sprayed either with PAS TiO_2 sol or water three times with a 7-day interval between sprayings. Samples were collected for analyzing the effect of PAS TiO_2 sol on disease control and photosynthesis 18 days after spraying PAS TiO_2 sol.

The effect of PAS TiO_2 sol on cucumber diseases control

Twenty randomly selected seedlings from each of the treatment and control blocks (N = 3) were analyzed according to the standard methods mentioned in *GB/T* 17980.26—2000 for the effectiveness of PAS TiO_2 sol on controlling two cucumber pathogenic bacteria (*Pseudomonas syringae pv. lachrymans* and *Xanthomonas campestris pv. vesicatoria*) (Iwasaki et al., 2002). Cucumber downy mildew index was calculated according to the Chinese National Standards (GB/T17980.26-2000). The grading standards for cucumber downy mildew progression included: Level 0: no lesion; Level 1: lesion area accounted for less than 5% of the total leaf area; Level 3: lesion area accounted for 6-10% of the total leaf area; Level 5: lesion area accounted for 11-25% of the total leaf area; Level 7: lesion area accounting for 26-50% of the total leaf area; and Level 9: lesion area accounted for more than 50% of the total leaf area. Disease index indicated the degree of disease at the population level and was calculated according to the following equation. Disease index (%) = ∑（disease level × number of diseased leaves）/ (total number of diseased leaves × 9) ×100%.

The effect of PAS TiO_2 sol on photosynthesis

The photosynthetic rate of cucumber leaves was measured by portable photosynthesis system (Li-cor 6400). Chlorophyll was extracted according to the protocol described by Zhou (2000). The content of chlorophyll of leaves was analyzed by UV-VIS spectrophotometer (UV-2250, Shimatsu). PAS TiO_2 sol at four concentrations (0.4%, 0.8%, 1.2%, 1.6%) was sprayed on cucumber seedling leaves. Each concentration treatment had twenty (20) seedlings. Net photosynthetic rate of the 6^{th} leaf of each seedling was measured at the same one hour period and thirty (30) minutes after spraying with PAS TiO_2 sol. One (1) leaf per plant (the 6^{th} leaf for each plant) and 20 plants per treatment were assayed. The light intensity (when photosynthetic rate was assayed) was 1000 uMol/m^2/s provided by cool white fluorescent light.

Experimental design and statistical analysis

A completely randomized design (CRD) was used for this research. Data was statistically analyzed accordingly using SAS (SAS Institute, Cary, NC, USA). Results on evaluating bactericidal properties of PAS TiO_2 sol were displayed as ($\bar{x} \pm SE$). Significance of differences between control and treated samples was determined by analysis of variance (ANOVA) followed by t-test (α=0.01). Greenhouse experiment results were analyzed using Duncan's multiple range test (α=0.05). The difference in chlorophyll contents between the TiO_2 sol treatment and the control was separated using the Least Significant Difference (LSD, α=0.05).

Conclusions

Our research confirmed that PAS TiO_2 sol has the following biological actions: (1) Ability to form a successive, good adhesive and transparent film on the surface of leaves that enables it to exert photo catalytic and photo-biological effects; (2) Obvious bactericidal effects to prevent pathogens from penetrating cucumber plants; and (3) Promotes chlorophyll synthesis and photosynthesis. Based on these findings we conclude that PAS TiO_2 sol has potential for being used as an environmentally friendly germicide and plant growth regulator to control plant diseases and increase the yield by enhancing photosynthesis. Further studies using different plant species and a wider range of bacteria are needed to explore the applicable potential of TiO_2.

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