Antifungal Activity of Titanium Dioxide Photocatalysis Against *Fusarium oxysporum* f.sp.lycopersici.

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**ABSTRACT**

Studies were carry out to detect the efficacy of titanum dioxide (TiO2) photocatalysis combined with light (mercury lamp, 160 W) on number of colony forming unites (CFUs) and dry weight of biomass of fungus of *Fusarium oxysporum* f.sp. *lycopersici* Schlecht in photocatalytic reaction cell during different exposure periods of light. The results showed that TiO2 combined with light caused significantly reduced CFUs to 65.66, 12.66, 4 and 4.66 CFUs/0.5 ml after periods of time 30, 60, 90 and 120 min respectively compared with control in the dark without TiO2, while TiO2 alone didn’t effect on CFUs compared with control in the dark. When light was present along time with TiO2, it was found the survival ratio reduction into 1.93 and 2.2 % after 90 and 120 min., while rate of photo killing of TiO2 (slope) was 1.5531 CFUs /min. Also observed TiO2 combined with light was reduced significantly in dry weight of biomass of *F. oxysporum* f.sp. *lycopersici* to 42 and 50 mg /30ml after exposure it into periods 60 and 120 min respectively compared with control in the dark.

**INTRODUCTION**

The element titans (Titanium) was discovered in 1791 by William Gregor, in England. Martin Klaproth, Later named it titanium and he was only able to produce titanium dioxide. In nature its never occurrence pure. It found with contaminant metal such as iron (Higgin, 1973). Titanium dioxide (TiO2) is a white powder, occurs in three crystalline forms, anatase, rutile and brookite. Boiling point 2972 C in soluble, molecular weight 79.87 g /mol, density 4.23 g /cm³ (Fox and Dulay, 1993). It have important properties is photocatalysts when UV illuminated it with wave length less than 385 nm. Photocatalysts generate a strong oxidizing power and
could be decompose organic and inorganic compounds by oxidation or reduction (Higgin, 1973; Lee, 2004). The two crystalline forms of titanium dioxide, anatase and rutile have property photocatalysis the least it has been found to most active form (Higgin, 1973).

Titanium dioxide (TiO\textsubscript{2}) is a multifaceted compound, its the stuff that makes tooth paste white and paint opaque because non-toxic for human therefore its used in cosmetics products and in special pharmaceutics (Doll and Frimmel, 2005). Also titanium dioxide has been widely utilized as self-cleaning, self-sterilizing material for coating clinical tools, items for use in hospital (Fujishima et al., 1999) and in the purification of water and air on surfaces from microorganisms such as bacteria, viruses, protozoa and fungi (Lee, 2004; Lonnen et al., 2005).

In 1985 the first research work on the microbiocidal effect of titanium dioxide on microbial cell of Escherichia coli was found in water and it could be killed by contact with a TiO\textsubscript{2}-pt catalyst upon illumination with near-UV light for 60 to 120 min (Matsunaga et al., 1985). Since then sub sequently has been intensively conducted on a wide spectrum of organisms primarily with bacteria and tumor cells (Blake et al., 1999; Lonnen et al., 2005).

Saito et al. (1992) and Maness et al. (1999) have explained, that particles come into contact with the gram positive bacteria as Micrococcus luteus and Streptococcus sorbinus, when irradiation titanium dioxide. The microbial surface was the primary target of initial oxidative attack reactive oxygen species (ROS) such as hydroxyl radical (OH\textsuperscript{-}), superoxide (O\textsubscript{2}\textsuperscript{-}) and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) were generated on the irradiated titanium dioxide surface. Susceptibility four kinds of organisms (E. coli, Lactobacillus acidophilus, Saccharomyces cervises and Chlorella vulgaris) to killing by the photocatalytic effect was observed when it was compared with using platinized titanium dioxide and a metal halide lamp. The photocatalytic method has found its application also in the degradation of toxins secreted to water by bacteria and unicellular protozoa and degradation of algae, bacteria, viruses and protozoa which normally found in it (Matsunaga et al., 1988; Robertson et al., 1997; Lawton et al., 1999; Makowski and Wardas, 2001). Also its application to sterilize selected food borne pathogenic bacteria such as Salmonella choleraeuis, Vibro para haemolyticus, Listeria monocytogenes and Pseudomonas sp (Kim et al., 2006).

The fungicidal effect of the TiO\textsubscript{2} photocatalytic ozonution reaction for control of Diaporthe actinidiae on kiwifruit and it was used to control post harvest storage rots in kiwifruit and decompose residual the fungicides (Hur et al., 2005). The antifungal activity of TiO\textsubscript{2} photocatalytic reaction in the form of TiO\textsubscript{2} powder and TiO\textsubscript{2} coated on plastic film against Penicillium expansum (air borne fungus) in vitro and in apple fruit was recorded (Maneerat and Hayata, 2006). When stimulated solar and solar photocatalytic exposure 870 W/m\textsuperscript{2} in the 300 nm -10nmum range/ 200 W/m\textsuperscript{2} in the 300-400 nm UV range, its reduced in viably against trophozoioite stage of protozoa of Acanthamoeba polyphage, Candida albicans, F. solani and Bacillus found in water (Lonnen et al., 2005). Also was obtained ability of solar only and solar photocatalytic(TiO\textsubscript{2}) of five wild strain of Fusarium which was successfully achieved (Sichel et al., 2007).

In this study, we investigated the effect of TiO\textsubscript{2} powder and TiO\textsubscript{2} photocatalysis on the fungus of F. oxysporum that as plant pathogen and producer to mycotoxin (fumonosin) that is cytotoxic effect to several mammalian cell lines (Abbas et al., 1998; Dlgnaln and Anaissie, 2004). This a study a first in Iraq about TiO\textsubscript{2} photocatalysis in fungi.

**MATERIAL AND METHODS**
Isolation and preparation of the fungus

Isolates of *Fusarium oxyporum* f.sp. *lycopersici* Schlecht was isolated from infected stem of tomato *Lycopersicum asculantium* by cultured some sections of infected parts after its surface sterile on Petri dish contain 20 ml potato dextrose agar media (PDA), the fungus was purified and identification by protocol Hansen and Smith (1932). Spores suspension were prepared from the fungus by suspended spores of *F. oxysporum* f.sp. *lycopersici* with sterile distilled water and it was counted this spores per ml by a hemocytometer (Keraly and Solymosy, 1974) for using in following experiments.

Titanium dioxide (TiO$_2$)

The photocatalyst titanium dioxide powder was supplied by Degussa company P-25 (Japan) particles with an average composition of 75% anatas and 25% rutil. Physical properties of TiO$_2$ crystallite were characterized by BET (Brunauer – Emmett – Taller) analysis, which is non-porous, with a surface area about of 55 m$^2$/g. It has a partial size of 0.03 micron and an average particles diameter of 21 nm (Gassim et al., 2004; Coleman et al., 2005). The P-25 titanium dioxide Degussa has become the standered for photo reactivity in water, air purification and bactericidal (Blake et al., 1999; Maness et al., 1999; Makowski and Wardas, 2001). This compound was used for all experiments and stored at room temperature.

Photocatalytic reaction cells

The photocatalytic reactor consisted of low pressure mercury lamp type Emkay (160 W) wave length between 360-750 nm was used as source of irradiation, photo cell contain the vessel (35 ml) with quartz window (2 cm$^2$) as reaction vessel, oxygen pump. The light lamp was centered to illuminated properly the inner of vessel and the temperature was controlled at 25°C by using thermo-circulator (Desaga Frigostat) during the photocatalytic reaction.

Fig. 1. Schematic diagram of the experimental apparatus for photocatalytic reaction. (A) gas container, (B) gas flowmeter, (C) circulating water thermostat, (D) magnetic stirrer, (E) quartz photo cell, (F) windows quartz, (H) low pressure mercury lamp, (I) power supply unit.
Effect photocatalytic reaction of TiO$_2$ on numbers of colony forming unites (CFUs) of *F. oxysporum* f.sp. *lycopersici*

To investigate the antifungal activity of TiO$_2$ Photocatalyts, 120 ml spores suspension have $8 \times 10^2$ spore /ml were freshly prepared with sterile distilled water for all treatments, aliquots of 30 ml from spores suspension per each treatment, four treatments were carried out (i) the control treatment in the dark by adding 30 ml of spores suspension to the photocatalytic reaction cell, quartz window were covered with black cover without TiO$_2$. (ii) treatment in the light, spores suspension was prepared by the same method for the first treatments but photocatalytic reaction cell were exposed to the light without TiO$_2$. (iii) the TiO$_2$ alone treatment was prepared with 10 mg of TiO$_2$ adding into 30 ml spores suspension, quartz window were covered with black cover. (iv) the last treatment 10 mg of TiO$_2$ adding into 30 ml of spores suspension and its added into the photocatalytic reaction cell then its exposure to the light (Maneerat and Hayata, 2006). Spores suspensions of all treatments were stirring by using magnetic stirrer. Oxygen gas was passed with rate 10 cm$^3$ / min to the photocatalytic reaction cell. Temperature was controlled in 25 C$^\circ$ using the thermo – circular during the photocatalytic reaction cell in all treatments. The treatments samples were collected from the reaction cell every subsequent 30 minute. For each sampling, 2 ml of the suspension was draws by using a syringe with along pliable needle from the reaction cell for all treatments after 0,30,60,90 and 120 min, then the treatments samples were centrifuged at 1000 rpm for 5 min to separate the solid catalyst, 0.5 ml of supernatant immediately added into Petri dishes (9cm diameter), than 20 ml of PDA media poured into Petri dish with trireplicates per each treatments.

The Petri dishes were incubated in the dark at 30 C$^\circ$ ± 2 for 48 hours. The numbers of colony forming unites of *F. oxysporum* per each plate were counted (Leonard and Blackford, 1949; Ohmori and Gottlieb, 1965). The survival ratio (%) of *F. oxysporum* f.sp. *lycopersici* in aqueous solution and rate of photokilling of TiO$_2$ (slope) were calculated.

Effect photocatalytic reaction of TiO$_2$ on dry weight of *F. oxysporum* f.sp. *lycopersici*

The experiment was carried out with four treatments as well as previous experiment (control in the dark, light, TiO$_2$ alone, and TiO$_2$ with light). Spores suspensions of all treatments were stirring by using magnetic stirrer. Oxygen gas was passed with rate 10 cm$^3$ /min to the photocatalytic reaction cell. Temperature was controlled in 25 C$^\circ$ using the thermo – circular during the photocatalytic reaction cell in all treatments, 2 ml of the suspension were draws by using a syringe with along pliable needle from the reaction cell for all treatments after 60 and 120 min, then the treatments samples were centrifuged at 1000 rpm for 5 min to separate the solid catalyst, 0.5 ml of supernatant immediately added into bottle have 30 ml PDB(potato dextrose broth) with trireplicates per each treatment, the treatments were incubated in the dark at 30C$^\circ$ ± 2 for 14 days. Dry weights of biomass of the fungus were obtained by filtrated cultured media then drying biomass at 70 C$^\circ$ for 24 h (Singh et al., 1980).

Statistical analysis

All experiments were designed complete randomized design and data analyzed by using least squares analysis of variance (ANOVA), least significant difference (L. S. D.) test was used at the 1% and 5% level of significance (Steel and Torrie, 1960).

RESULTS

Results effect of treatments (control in the dark, the light treatment, TiO$_2$ alone and TiO$_2$ combined with light) on number of colonies forming unites(CFUs) of *Fusarium oxysporum* f.sp.
lycopersici were showed that TiO₂ combined with light caused significantly reduced the colonies forming unites (CFUS) to 65.66, 12.66, 4 and 4.66 CFUs /0.5 ml after exposure periods of light 30, 60, 90 and 120 min respectively compared with dark treatment. Also observed reducing in the numbers of colony forming unites in the light treatments compared with dark treatment. While TiO₂ alone didn’t effect on numbers of colonies forming unites compared with control in the dark or light treatments (Table1 and Fig. 2).

Table1. Effect of the TiO₂ photocatalytic reaction on numbers of colony forming unites (CFUS) of Fusarium oxysporum f.sp.lycopersici.

<table>
<thead>
<tr>
<th>Time( min )</th>
<th>Numbers of colony forming unites / 0.5 ml *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatments of TiO₂</td>
</tr>
<tr>
<td></td>
<td>Control in the dark</td>
</tr>
<tr>
<td>30</td>
<td>205.00</td>
</tr>
<tr>
<td>60</td>
<td>213.00</td>
</tr>
<tr>
<td>90</td>
<td>207.00</td>
</tr>
<tr>
<td>120</td>
<td>202.30</td>
</tr>
<tr>
<td>Means</td>
<td>206.80</td>
</tr>
</tbody>
</table>

L.S.D. 0.01 for treatments of TiO₂ = 24.4
L.S.D. 0.05 for Interaction between treatment of TiO₂ and time = 33.62
* each number is mean of trireplicates.
Fig. 2. Effect of TiO$_2$ and light on numbers of colony forming units (CFUs) of *Fusarium oxysporum* f.sp. *lycopersici*. (A) control in the dark after 30 min., (B) control in the dark after 60 min., (C) control in the dark after 90 min., (D) control in the dark after 120 min., (E) light exposure after 30 min., (F) light exposure after 60 min., (G) light exposure after 90 min., (H) light exposure after 120 min., (I) TiO$_2$ alone in the dark after 30 min., (J) TiO$_2$ alone in the dark after 60 min., (K) TiO$_2$ alone in the dark after 90 min., (L) TiO$_2$ alone in the dark after 120 min., (M) TiO$_2$ combined with light exposure after 30 min., (N) TiO$_2$ combined with light exposure after 60 min., (O) TiO$_2$ combined with light exposure after 90 min., (P) TiO$_2$ combined with light exposure after 120 min.

Also TiO$_2$ photocatalytic causes reduced survival ratio of *F. oxysporum* f.sp. *lycopersici* to 31.7, 6.12, 1.93 and 2.2 % after exposure periods of light 30, 60, 90 and 120 min respectively (Fig.3). While ,rate of photo killing of TiO$_2$ (slope) was 1.5531 CFU/min (Fig.4).

Fig. 3. Effect of the TiO$_2$ photocatalyst on the survival ratio (%) of *F. oxysporum* f.sp. *lycopersici* in aqueous solution at 25°C.
Fig. 4. The relationship between colony forming units (CFU) / 0.5 ml with time of irradiation of TiO$_2$ at 25 °C.

Results of dry weight of biomass of *F. oxysporum* f.sp. *lycopersici* showed that TiO$_2$ combined with light caused significantly reduced of biomass to 42 and 50 mg / 30 ml after exposure period 60 and 120 min respectively compared with control treatment in the dark were 168 and 168.3 mg / 30 ml and light treatment were 156.3 and 143.3 mg /30 ml. While didn’t found any significant difference between exposure periods of time and treatments of TiO$_2$ (Table 2).

**Table 2. Effect of TiO$_2$ photocatalytic reaction on dry weight of biomass of *Fusarium oxysporum* f.sp *lycopersici*.**

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Dry weight of biomass (mg)/ 30 ml *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control in the dark</td>
</tr>
<tr>
<td>60</td>
<td>168.0</td>
</tr>
<tr>
<td>120</td>
<td>168.3</td>
</tr>
<tr>
<td>Means</td>
<td>168.1</td>
</tr>
</tbody>
</table>

L.S.D. 0.01 for treatments of TiO$_2$ = 48
*each number is mean of trireplicates

**Discussion**

Our results of illuminated TiO$_2$ photocatalyst effect conformed previous researches showed that killing property of illuminated TiO$_2$ on other microorganisms such as *E. coli*, *Streptococci*, *Lactobacillus*, *Salmonella*, *Candida albicans*, *Saccharomyces cerevisiae* were observed (Matsunaga *et al.*, 1985, 1988; Saito *et al.*, 1999; Maness *et al.*, 1999). Also that killing property was found to positively correlate with time, type of source was used irradiation, type of TiO$_2$ and organisms.
Significant effect in light treatment compared with control treatment in the dark may be attributed to inhibited effect of light on fungal growth (Grow and Gadd, 1995) while TiO$_2$ alone didn’t caused any effect on colonies forming unites and dry weight because its non toxic effect on human therefore, its using in cosmetic products and pharmaceutics (Blake et al., 1999; Doll and Frimmel, 2005). The significant reduction of TiO$_2$ combined with light treatments of biomass may be due to low colonies from unites led to reduce biomass.

The mechanism of photokilling is, when photocatalyst titanium dioxide (TiO$_2$) two crystalline forms of TiO$_2$ have photocatalytic activity, anatase and rutile. Anatase has a forbidden band gap 3.2 eV and rutile 3.0 eV. Anatase has been found to be the most active form. The action spectrum for anatase shows a strong reduction of activity in wavelengths higher than 385 nm. The photocatalytic process includes chemical steps that produce reactive species in principal can cause fatal damage to structure or functions of microorganisms cells (Fox and Dually, 1993). So the photocatalytic TiO$_2$ in aqueous solution it was absorbed Ultraviolet radiation from sunlight or illuminated light source (fluorescent lamps), it will produce pairs of electrons and holes. The electron of the valence band of titanium dioxide becomes excited when illuminated by light. The excess energy of this excited electron promoted the electron to the conduction band of titanium dioxide therefore creating the negative – electron ($e^-$) and positive – hole ($h^+$) pair. This stage is referred as the semiconductor’s (photo-excitation) state. The energy difference between the valence band and the conduction band is known as the’ Band G’ (Fig. 5)(Wong et al., 2006; Hoffmann et al., 1995).

Fig. 5. **Photocatalysis mechanism of titanium dioxide** (Wong et al., 2006)

The positive – hole of titanium dioxide breaks the apart of water molecule to from hydrogen gas (H$_2$) and hydroxyl radicals (OH$^-$). The negative – electron reacts with atmospheric oxygen.
molecule (absorbs on the surface of TiO\textsubscript{2} particles ) to form super oxide ions. These hydroxyl radicals contact with each other to produce hydroxyl peroxide (H\textsubscript{2}O\textsubscript{2}) this cycle continues when light is available of the photocatalytic system, there can also be direct photochemistry as there would be from any UV source. Mechanism of a photocatalytic process on irradiated titanium dioxide (Barbeni et al., 1987):

Electron – Hole Pair Formation.

\[
\text{TiO}_2 \rightarrow \text{TiO}_2^+ (e_{cb}^- + h_{vb}^+) \quad (\text{1})
\]

( conduction band electron and valence band hole )

Electron removal from the conduction band

\[
\begin{align*}
\text{H}_2\text{O} + h_{vb}^- & \rightarrow \text{OH}^- + \text{H}^+ \\
\text{H}^+ + e_{cb}^- & \rightarrow \text{H} \\
\text{O}_2 + e_{cb}^- & \rightarrow \text{O}_2^- \\
\end{align*}
\]

Nonproductive radical reactions

\[
2\text{HO}_2^- + \text{O}_2 + \text{H}_2\text{O}_2 \rightarrow \text{OH}^+ + \text{OH}^- + \text{O}_2 \quad (\text{5})
\]

For a cell or virus in contact with the titanium dioxide surface these also be direct electron or hole transfer to the organism or one of its components. If titanium dioxide particles are small size, they may penetrate into the cell and these processes could in the interior. Since light is an essential component of the photocatalytic system (Kamat, 1993; Blake et al., 1999). Hydroxyl radicals are highly reactive and therefore short–lived. Superoxide ion are more long-lived; however, due to the negative charge they cannot penetrate the cell membrane. Upon their production on the TiO\textsubscript{2} surface, both hydroxyl radicals and super oxide would have to interact immediately with the outer surface of an organism unless the TiO\textsubscript{2} particle has penetrate into the cell (Neiland, 1982; Blanco–Gálvez et al., 2007) (Fig.6).

Compared to hydroxyl radical and super oxide ions, hydrogen peroxide is less detriment. However, the important part for killing hydrogen peroxide can enter the cell and be activated by ferrous ion via the Fenton reaction:

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \text{OH}^+ + \text{Fe}^{3+} \quad (\text{6})
\]
Anbeaki et al.


Figure 10. Schematic illustration of solar photocatalytic process for bacteria inactivation in the presence of an aqueous suspension of TiO2 (relative size of each element is schematically represented at the bottom) (Blanco, Málato, Fernández- Ibáñez, 2007).

The photokilling of illuminated TiO2 because the reactive oxygen space (ROS), such as OH-, O2-, and H2O2 generated on the irradiated TiO2 surface have been proposed to attach with polyunsaturated phospholipids in cell membrane of E. coli. Iron levels on the cell surface, in the periplasmic space or inside the cell, either as iron clusters or in iron storage proteins (such as ferritin) are significant and can serve as a source of ferrous ion. Therefore, while the TiO2 is being illuminated to produce H2O2, the Fenton reaction may take place in vivo and produce the more damaging hydroxyl radicals (Neiland, 1982; Cai et al., 1991; Maness et al., 1999).

When the light is turned off, any residual hydrogen peroxide would continue to interact with the iron species and generate additional hydroxyl radicals through the Fenton reaction. When both H2O2 and O2− are present, the iron-catalyzed Haber–Weiss reaction can provide a second pathway to form additional hydroxyl radicals (Youngman, 1984).

\[
\begin{align*}
\text{Fe}^{3+} + \text{O}_2^{-} & \rightarrow \text{Fe}^{2+} + \text{O}_2 & \quad (7) \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \text{OH}^{-} + \text{OH} & \quad (8)
\end{align*}
\]

Therefore the lipid peroxidation reaction that causes a breakdown of the cell membrane structure and its associated functions is the mechanism underlying cell death. Because all life forms have cell membrane. Thus, the proposed killing mechanism is applicable to all cell type (Saito et al., 1992; Maness et al., 1999). Apart from the cell wall, there exists another possible cause of death, this one is the destructive effect of oxidative photocatalysis on RNA and DNA molecules, mainly due to hydroxyl radicals (Tachikawa et al., 2008).

The antifungal activity of TiO2 photocatalytic reaction in the form of TiO2 powder and TiO2 coated on plastic film against Penicillium expansum (air born fungus) and Diaporthe actinidiae a major fungal pathogen of kiwifruit (Hur et al., 2005; Maneerat and Hayata, 2006). The mechanisms for antifungal effect presented by Matsunaga et al. 1988, they were evidenced for the
An oxidation of coenzyme A (COA) in *S.cereviace*, a yeast when exposed to light and platinized TiO$_2$ for 120 minutes under a metal halide lamp, more than 97% of intracellular COA content was lost and 42% of respiratory actively was decreased led to cell death and observed the cell membrane would have to be oxidized first under go its semi permeability. Same authors failed to detect and destruction of cell wall by photoactivated semiconductor or powder but Hammel *et al.* (2002) noted the degradation of poly saccharides by OH$^-$ has also recently by them that the OH$^-$ Abstracts hydrogen atoms from sugar subunits of polysaccharides, resulting in cleavage of the polysaccharide chain. We suggestion for its effected on spores suspension of *F.oxysporum* may be the reactive (ROS) lead to breakdown the contains such as protein, lipid and polysaccharide (cellulose) for thin cell wall of spores first and degradation of cell membrane then effected its on other chemical compounds activates such as respiratory or, and TiO$_2$ particles may be affection on structures of DNA and RNA for *F.oxysporum* spores all or some factors led to photokilling it and prevent the germination of spores therefore didn’t formation of fungal colonies.

**REFERENCE**


