Antifungal Activity of Titanum 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 TiO_2 ,it was found the survival ratio reduction into 1.93 and 2.2 % after 90 and 120 min., while rate of photo killing of TiO_2 (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.

فعالية التضاد الفطري للتحلل الضوئي لثاني اوكسيد التيتانيوم *Fusarium oxysporum* f.sp.*lycopersici* ضد الفطر

الخلاصة

نفذت دراسة لتحديد فعالية التحلل الضوئي لثاني اوكسيد التيتانيوم(TiO_2) متحدا مع الضوء (مصباح زئبقي , 160 واط) على عدد الوحدات المكونة للمستعمرات (CFUs) و الوزن الجاف للكتلة الحية للفطر *Fusarium oxysporum* f.sp. *lycopersici* Schlecht في خلية التفاعل الضوئي خلال فترات زمنية مختلفة للتعرض الضوئي . أظهرت النتائج ان ثاني اوكسيد التيتانيوم المتحد مع الضوء سبب انخفاض معنوي في عدد الوحدات المكونة لمستعمرات الفطر (CFUs) الى 65.66 و 12.66 و 4 و4.66 CFUs / 0.5مل بعد الفترات الزمنية 30 و60 و90 و120 دقيقه على التوالي مقارنة بمعاملة السيطرة في الظلام و بدون وجود ثاني اوكسيد التيتانيوم .بينما لم تؤثر معاملة ثاني اوكسيد التيتانيوم لوحده على عدد الوحدات المكونة للمستعمرات مقارنة بمعاملة السيطرة في الظلام . وجد ان نسبة البقاء اختزلت الى 1.93 و 2.2 % بعد 90 و 120دقيقة عند بقاء الضوء لفترة زمنية طويلة معTiO_2 بينما كان معدل القتل الضوئي لثاني اوكسيد التيتانيوم (Slope) 1.5531 CFUs / دقيقة . كما لوحظ ان TiO_2 المتحد مع الضوء سبب اختزال معنوي في الوزن الجاف للكتلة الحية للفطر *F. oxysporum* f.sp. *lycopersici* الى 42 و50 ملغم /30مل بعد فترات تعريض 60 و120 دقيقة على التوالي مقارنة بمعاملة السيطرة في الظلام .

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 (TiO_2) 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 /cm^3 (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

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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_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_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^-), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) were generated on the irradiated titanium dioxide surface . Susceptibility four kinds of organisms (*E coli ,Lactobacillus acidophilus , Saccharomyses cervices 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 *Sallmonella choleraseuis, Vibro para haemolyticus, Listeria monocytogenes* and *Pseudomonas sp* (Kim *et al*.,2006).

The fungicidal effect of the TiO_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_2 photocatalytic reaction in the form of TiO_2 powder and TiO_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^2 in the 300 nm -10mum range/ 200 W/m^2 in the 300-400 nm UV range, its reduced in viably against trophozoiote 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_2) of five wild strain of *Fusarium* which was successfully achieved (Sichel *et al*., 2007).

In this study ,we investigated the effect of TiO_2 powder and TiO_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;Dlgnanl and Anaissie ,2004) .This a study a first in Iraq about TiO_2 photocatalysis in fungi .

MATERIAL AND METHODS

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Isolation and preparation of the fungus

Isolates of *Fusarium oxyporum* f.sp. *lycopersici* Schlecht was isolated from infected stem of tomato *Lycoparsicum 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 purificated 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 (35ml)with quartz window (2cm^2)as reaction vessel ,oxygen pump .The light lamp was centered to illuminated properly the inner of vessel and the temperature was controlled at 25C° by using thermo-circulator (Desaga Frigostat) during the photocatalytic reaction .

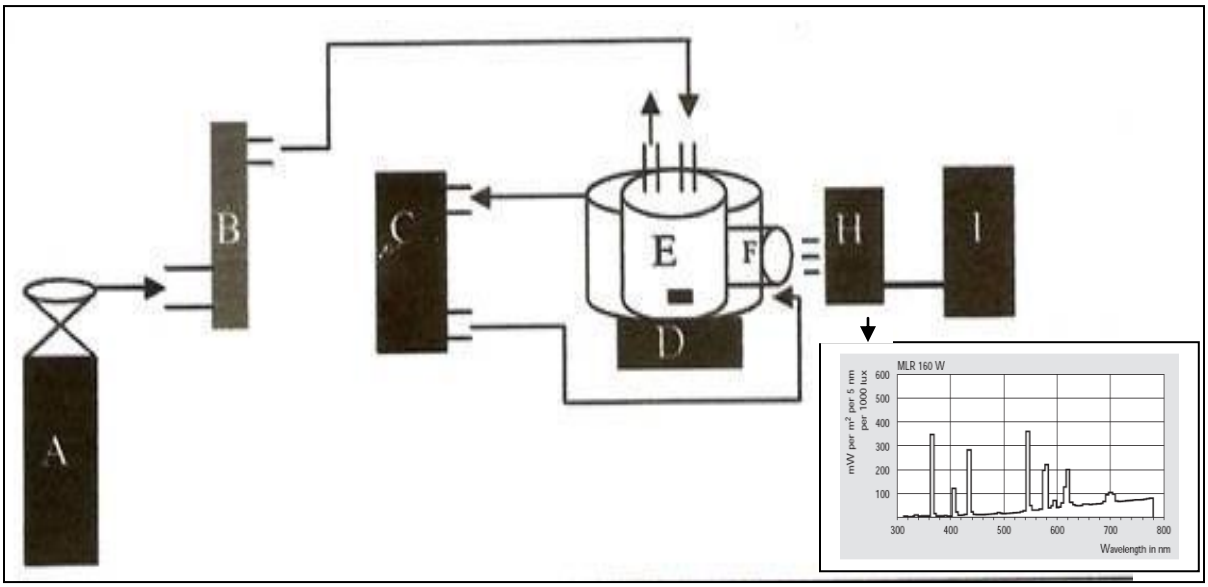

Fig. 1. Schematic diagram of the experimental apparatus for photocatalytic reaction .(A) gas container, (B) gas flowmeter,(C) circulating water thermostat ,(D) magnetic stirrer, (E) quartz photo cell, (F) windows quartz, (H) low pressure mercury lamp, (I) power supply unit .

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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 Photocatalytis, 120 ml spores suspension have 8×10^2 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 exposured 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° 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° ∓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° 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 treatmentse samples were centrifuged at 1000 rpm for 5 min to separate the solid catalyst,0.5 ml of suprnatant 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° ∓2 for 14 days. Dry weights of biomass of the fungus were obtained by filtrated cultured media then drying biomass at 70 C° 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.

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lycopersici were showed that TiO_2 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 TiO_2 alone didn't effect on numbers of colonies forming unites compared with control in the dark or light treatments (Table1 and Fig. 2).

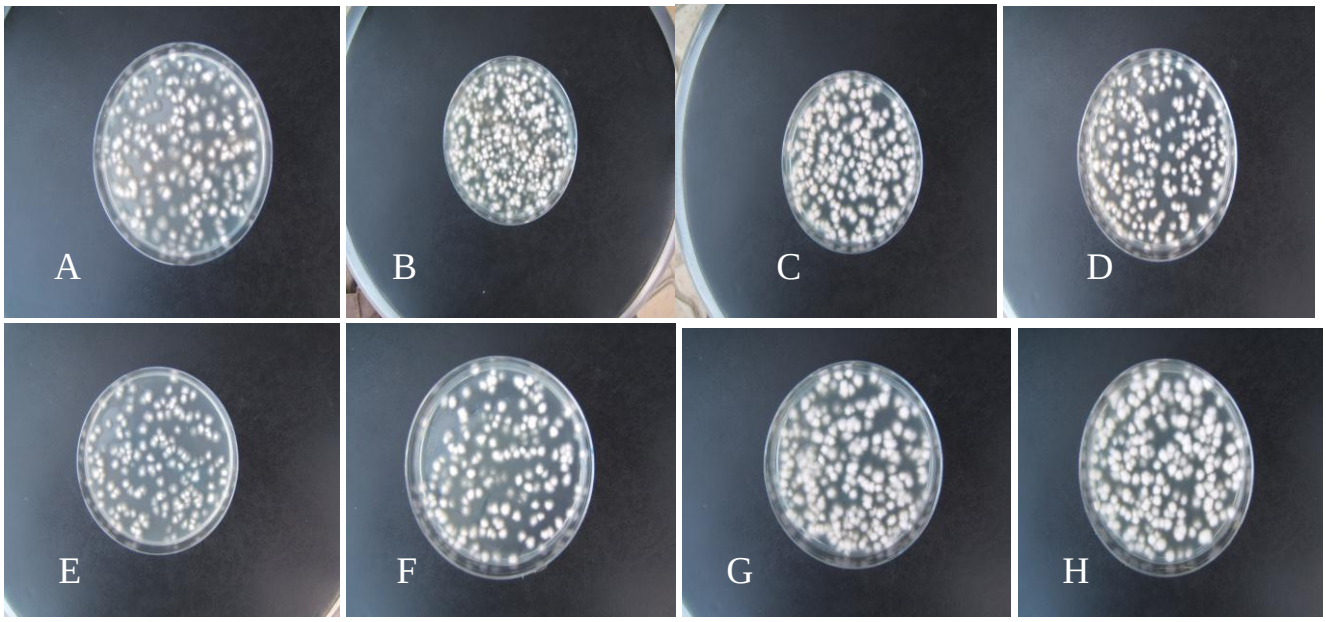
Table1. Effect of the TiO2 photocatalytic reaction on numbers of colony forming unites(CFUS) of *Fusarium oxysporum* f.sp.*lycopersici*.

Time(min)	Numbers of colony forming unites / 0.5 ml *			
	Treatments of TiO_2			
	Control in the dark	Light treatment	TiO2 alone	TiO2 with light
30	205.00	160.66	210.66	65.66
60	213.00	141.66	201.66	12,66
90	207,00	166.33	197.33	4.00
120	202.30	174.00	217.00	4.66
Means	206.80	160.66	206.66	21.75

L.S.D. 0. 01 for treatments of TiO_2 = 24.4

L.S.D. 0. 05 for Interaction between treatment of TiO_2 and time = 33.62

*** each number is mean of trireplicates**.



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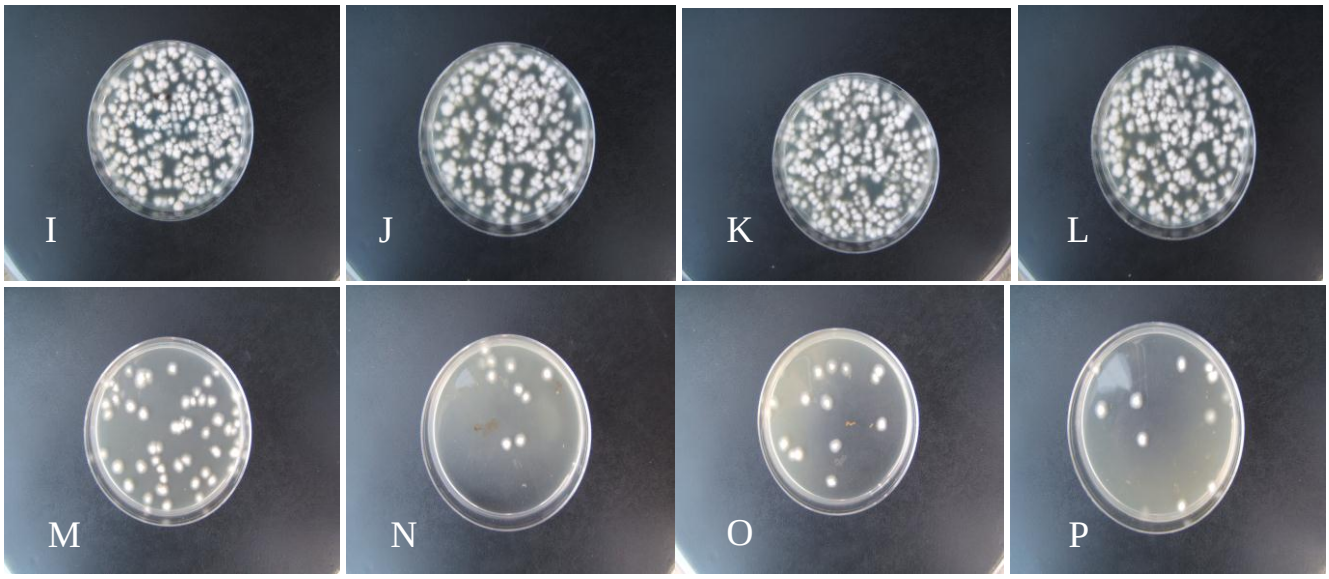

Fig. 2. Effect of TiO_2 and light on numbers of colony forming unites(CFUs) of *Fusarium oxysporum* f.sp. *lycopercici* . (A) control in the dark after 30 min , (B) control in the dark after 60 min., (C) control in the dark after 90 min. , (D) control in the dark after 120 min., (E) light exposure after 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).

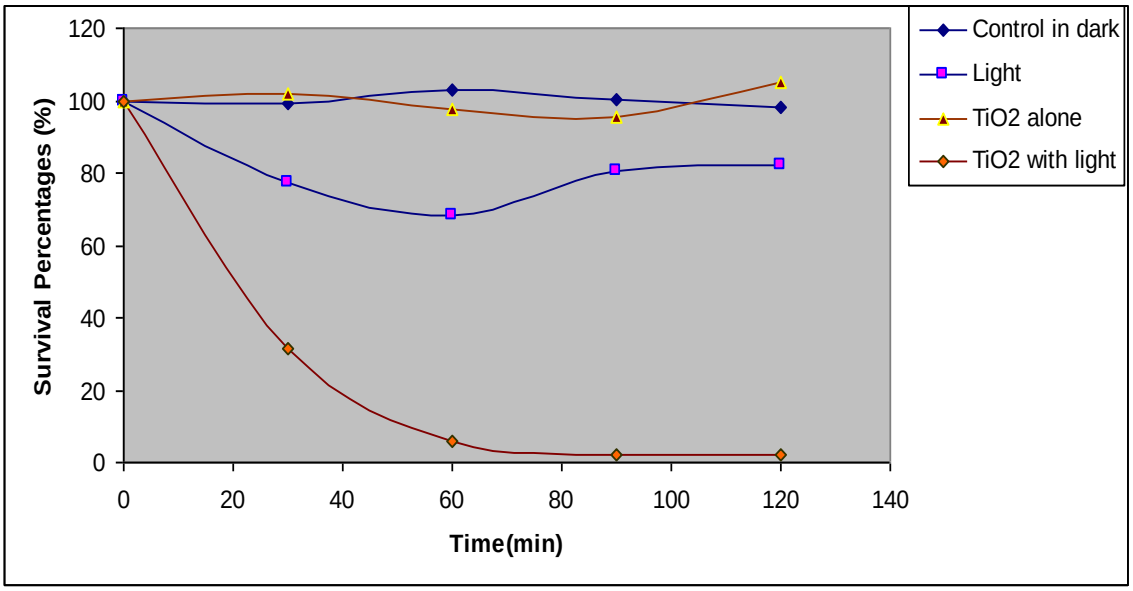

Fig. 3. Effect of the TiO_2 photocatalyst on the survival ratio (%) of *F.oxysporum* f.sp. *lycopersici* in aqueous solution at 25 C°.

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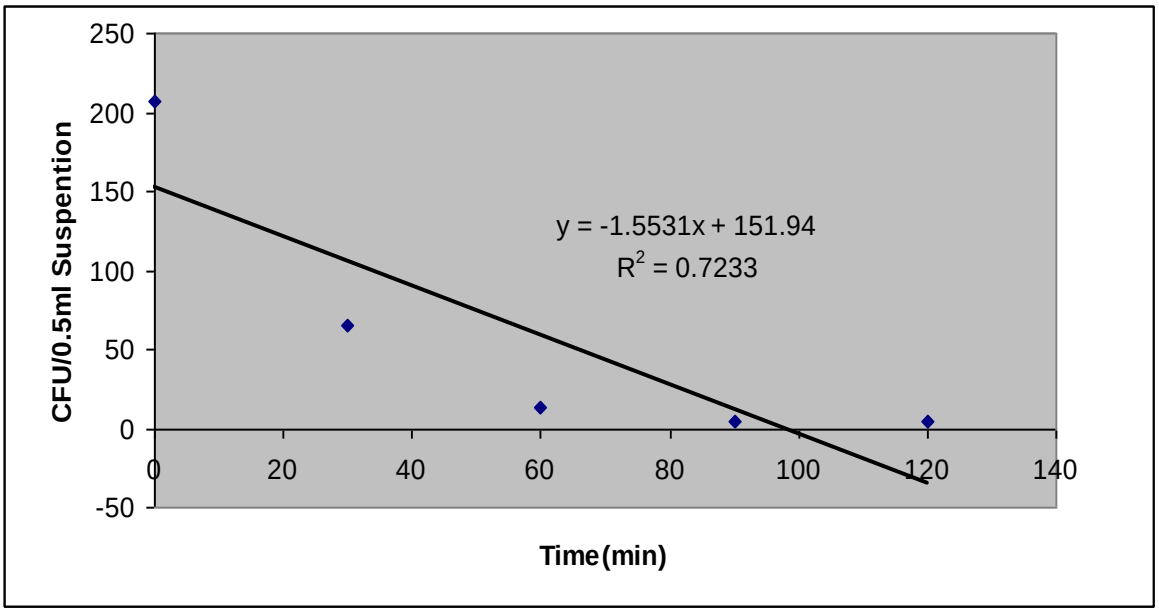

Fig . 4. The relationship between colony forming units (CFU) / 0.5 ml with time of irradiation of TiO_2 at 25 C°.

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 were168 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*.

Time(min)	Dry weight of biomass (mg)/ 30 ml *				
	Control in the dark	Light treatment	TiO_2 alone	TiO_2 with light	means
60	168.0	156.3	143.0	42.0	127.32
120	168.3	143.3	125.0	50.0	121.65
Means	168.1	149.8	134.0	46.0	124.47

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, Saccharomyses cervesiae* were observed (Matsunaga *et al*.,1985,1988; Saito *et al*.,1999; Maness *et a*l., 1999). Also that killing property was found to positively correlate with time, type of source was used irradiation , type of TiO_2 and organisms .

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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 .A natase 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 Dualy ,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) .

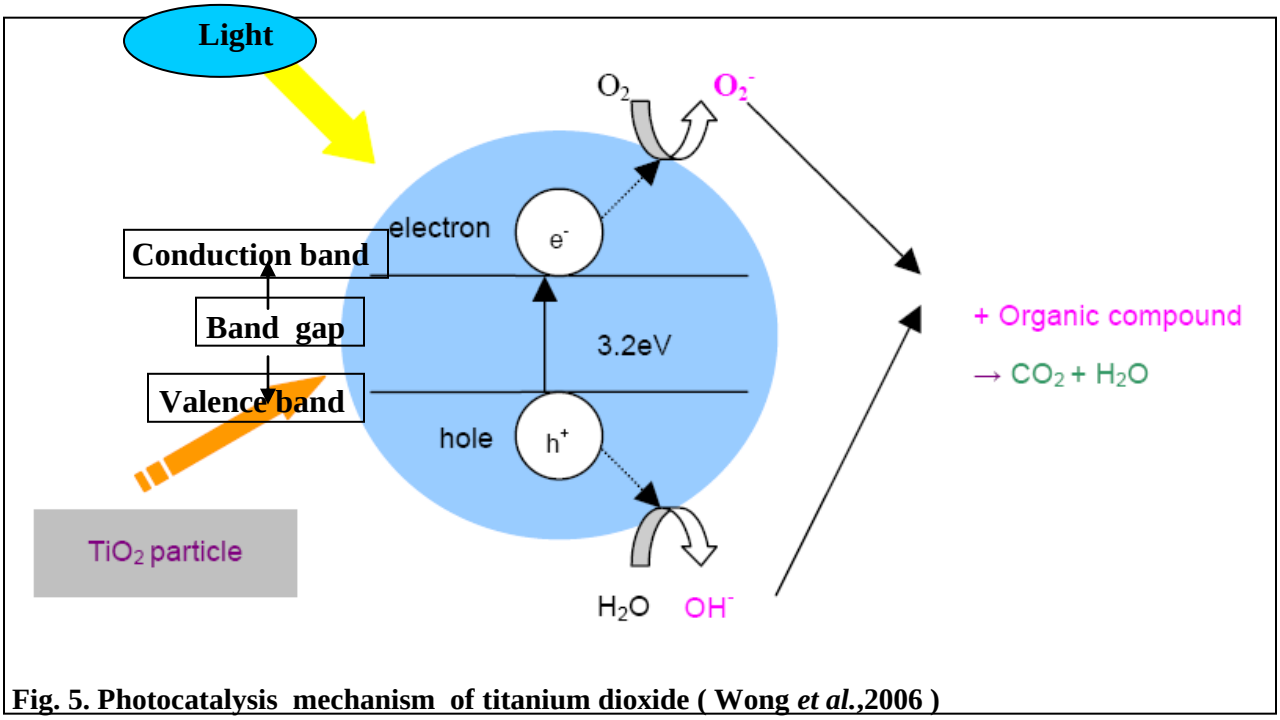

Fig. 5. Photocatalysis mechanism of titanium dioxide (Wong *et al*.,2006)

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

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molecule(absorbs on the surface of TiO_2 particles) to form super oxide ions .These hydroxyl radicals contact with each other to produce hydroxyl peroxide(H_2O_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.

$$TiO_2 \longrightarrow TiO_2 (e_{cb}^- + h_{vb}^+) \quad (1)$$

(conduction band electron and valence band hole)

Electron removal from the conduction band

$$H_2O + h_{vb}^+ \longrightarrow OH^{\cdot} + H^+ \quad (2)$$

$$H^+ + e_{cb}^- \longrightarrow H^{\cdot} \quad (3)$$

$$O_2 + e_{cb}^- \longrightarrow O_2^{\cdot -} \longrightarrow HO_2^{\cdot} \quad (4)$$

Nonproductive radical reactions

$$2HO_2^{\cdot} \xrightarrow{H} O_2 + H_2O_2 \qquad {}^{\cdot}OH + OH^- + O_2 \quad (5).$$

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_2 surface ,both hydroxyl radicals and super oxide would have to interact immediately with the outer surface of an organism unless the TiO_2 particle has penetrate into the cell (Neiland ,1982 ;Blanco - Gàlvez *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 :

$$Fe^{+2} + H_2O_2 \longrightarrow OH^{\cdot} + OH^- + Fe^{+3} \quad (6)$$

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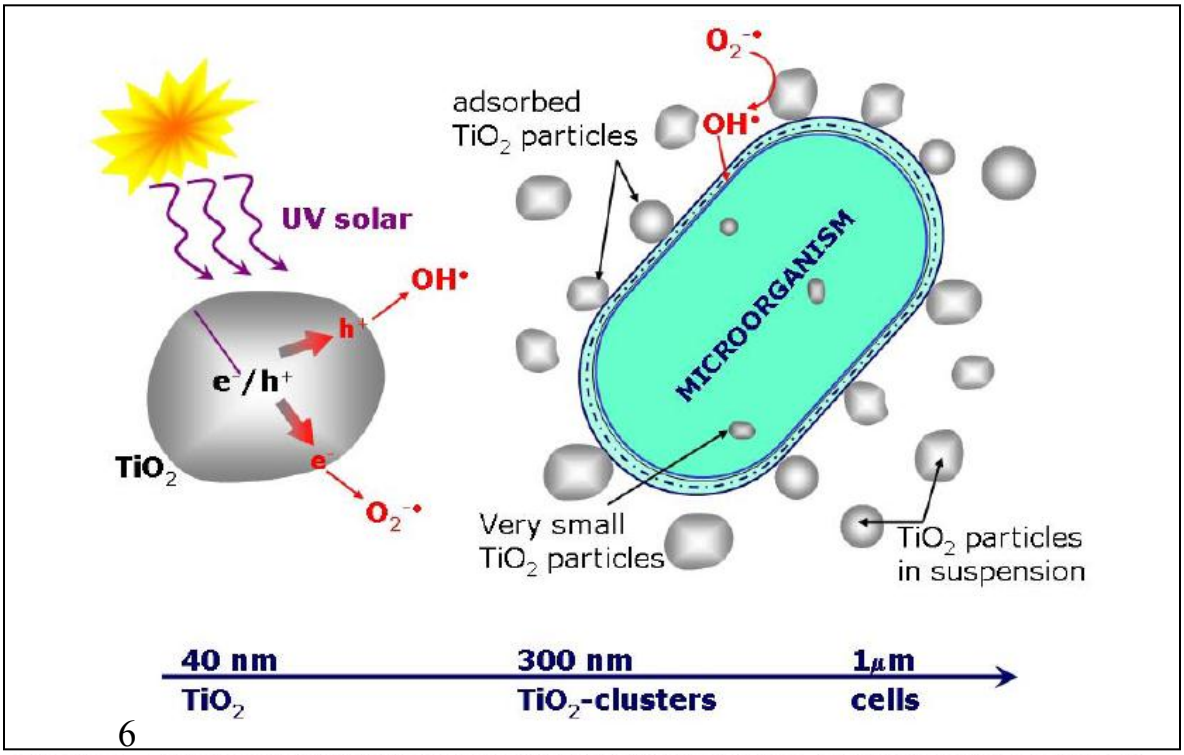

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Figure 10. Schematic illustration of solar photocatalytic process foe bacteria inactivation in the presence of an aqueous suspension of TiO2 (relative size of each element is schematically represented at the bottom) (Blanco, Malato, Fernández-Ibáñez,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

The photokilling of illuminated TiO_2 because the reactive oxygen space (ROS),such as OH- ,O_2 and H_2O_2 generated on the irradiated TiO_2 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 TiO_2 is being illuminated to produce H_2O_2 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 generated additional hydroxyl radicals through the Fenton reaction . When both H_2O_2 and $O_2^{\cdot-}$ are present , the iron – catalyzed Haber –Weiss reaction can provide a second pathway to from addition hydroxyl radicals (Youngman ,1984) .

$$Fe^{+3} + O_2^- \longrightarrow Fe^{+2} + O_2 \quad (7)$$

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH^- + OH^{\cdot} \quad (8)$$

Therefore the lipid peroxidation reaction that causes a break down 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 TiO_2 photocatlytic reaction in the form of TiO_2 powder and TiO_2 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

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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 photoactiviated semiconduct or powder but Hammel *et al*.(2002) noted the degradation of poly saccharides by $OH^{\cdot}$ 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 .

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