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Photocatalytic Activity of Zinc Sulphate Nano Material on Phytopathogens

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Abstract

Phytopathogens cause both qualitative and quantitative loss in all areas of agricultural practices. Phytopathogens are controlled by pesticides but it is associated with side effects. Most organisms develop resistance to pesticides and high concentration of pesticide is phyto toxic. Hence the present study is aimed to assess the potential application of nano dimensional zinc sulphate as a photo catalyst to control *Xanthomonas campestris, X.malvacearum, Pseudomonas solanacearum and P.syringae.* Nano material of zinc sulphate was produced by ball milling method. It was characterized by SEM and XRD. Antimicrobial activity of Zinc sulphate was evaluated by well diffusion method. 0.01%, 0.05%, 0.1% and 1.0% of zinc sulphate nano material was used to study its influence on growth and death kinetics of all phytopathogens. Size of zinc sulphatenano material was 100 nm and it showed sharp peaks in XRDcorresponding to zinc sulphate. Zinc sulphate at a concentration of 100 µg/100 µl exhibited microbicidal activity with an inhibition zone of 18mm, 14mm, 12mm, 10 mm against *P.solanacearum, P.syringae, X.malvacearum and X.campestris* respectively.In growth kinetics study, *P.solanacearum* without any photocatalyst had a lag phase up to 30 minutes and the presence of photocatalyst extended it upto 90 minutes. At 90min the cfu/ml was 38.5 x 10^8, 11.7 x 10^8, 10.73 x 10^8,10.22 x 10^8 ,1.93 x 10^8 in control,0.01%,0.05%,0.1% and 1.0 % respectively.1% of Zinc sulphatenano material was very effective in controlling and destroying all phytopathogens from one hour onwards. Hence the present study explores the possibility of applying zinc nano material as a photo catalyst to control phytopathogens.

Keywords: Nano zinc sulphate, *P.syringae, X.campestris*, Death kinetics

Healthy plants are essential to secure a safe food and energy supply for a growing global population, to sustain natural ecosystems and to promote quality of life through diverse and productive landscapes. But plants are widely infected by phytopathogens. Phytopathogens cause both qualitative and quantitative loss in all areas of agricultural practices. Especially *Pseudomonas* and *Xanthomonas* species causes serious defects in wide variety of plants.

Bacterial wilt, caused by *Pseudomonas solanacearum* is economically importance because it infects over 250 plant species in 50 families. (He *et al.*, 1983). This disease is also known as Southern wilt and brown rot of potato. Dicots are susceptible than monocots. Highly susceptible crops are potato, tomato, eggplant, chili, bell pepper and peanut. This disease has limited both commercial and domestic level productivity (Somodi *et al*., 1993). *P.*

syringae causes bacterial speck disease in tomato. It is difficult to control and cause significant economic loss. *P.syringae* also causes canker in plum and hazelnut which has a mortality rate of greater than 30% in Germany (Hinrichs-Berger, 2004) and even larger in Italy (Scortichini, 2002).This pathogen has the ability to kill both young and older trees.

The host of *Xanthomonas* genus is diverse and includes atleast 124 monocotyledonous and 268 dicotyledonous plants. It causes various diseases in plants, among which bacterial blight in rice, black rot disease in cabbage, and blight disease in citrus are the most serious. *X.campestris*is the most important member that causes a variety of plant diseases (Starr and Stephens, 1964). It causes different diseases in plant foliage by producing black rot, canker, leaf spot and blights. These diseases may destroy leaf, petiole and stem rendering infected plants unsightly and unusable (Assis *et al.*, 1997; Mariano *et al.*, 2001).Bacterial blight, caused by *X.malvacearum* is a serious disease in most upland cotton growing areas of the world (Bayles and Verhalen, 2007). 10% to 30% yield losses have been recorded in Africa and Asia (Verma, 1986).

As these diseases are widely distributed, it is difficult to control with chemicals and cultural practices. (Grimault *et al.*, 1993).Because the use of chemical compounds has failed to control plant diseases due to resistance, environment pollution, and damage to human health. Different approaches may be used to prevent, mitigate or control plant diseases. However the use of chemical has been proved efficient and economical in controlling blight diseases (Cuthbertson *et al*., 2005).

Photo catalytic oxidationis an alternative antimicrobial technology .It has been studied using wide target microorganisms such as viruses, bacteria, fungi, algae, and cancer cells or protozoa (Zheng *et al*., 2000). It has been exploited for disinfection with TiO2 coated filters. Photo catalytic toxicity on bacterial, bacterial spores, fungi and fungal spores were studied (Grinshpum *et al*., 2007). An ideal photo catalytic material should have high efficiency of solar energy conversion, non-toxic, biologically and chemically inert, stable over long periods, readily available and easily processable. Light irradiation determines the activity of the photocatalyst. Wavelength in the range of 300–370 nm was widely used in experimental studies. (Goswami *et al*., 2007). Solar light was also applied for photo catalytic activity. (Dobkin and Zuraw, 2003). Researchers are also focused on the extend application of photo catalyst to non –UV region. (Khan *et al*., 2002).

Nanotechnology generates, manipulates, and deploys nano materials and represents an area holding significant promise for the improving agricultural productivity (Navrotsky, 2000; Baruah and Dutta, 2009). The benefits of nanomaterial based formulations are the improvement of efficacy due to higher surface area. In addition to the use of NPs for pesticide delivery, the use of nanosized aqueous dispersion formulations was suggested to enhance the bioavailability of pesticides).

Nanocrystalline semiconductor particles like zinc have attracted considerable attention because of their special properties and unique catalytic and optical properties as compared to those of the bulk materials. Some methods for the preparation of nanocrystalline Zn are sonochemical , microwave irradiation (Zhu *et al*.,2001), thermal evaporation (Wang *et al*.,2002) etc. Zinc sulfate is a cheap, nontoxic and highly efficient photo catalyst and is employed in environmental application and purification process. Both the technological and economic importance of photocatalysis has increased considerably. (Byrne *et al*., 1999).Application of zinc in the photocatalytic destruction of human pathogens was reported and nearly no work is cantered towards phytopathogens.

Hence the present work is aimed to study the application of zinc sulphate nano material as a photo catalyst for the control of phytopathogens.

Materials and Methods

Zinc sulphate nano material was produced by ball milling method. The synthesized zinc sulphate Nano material was characterized by UV-Vis 1601 Hitachi spectrophotometer operated at a resolution of 1nm. The Nano material was characterized by SEM and XRD. *X. campestris*, *X.malvacearum*,*P.solanacearum and P.syringae*.were donated as a gift from Tamilnadu Agricultural University,Madurai,India. Antimicrobial activity of Zinc sulphate against these pathogens was evaluated by well diffusion method. Growth kinetics of phytopathogens in the presence and absence of photo catalyst in darkness and solar illumination was assessed at 0.01%, 0.05%, 0.1%, and 1% of zinc sulphate nano material.

Results and Discussion

Wide range of organic and inorganic substrates can be photo oxidized by photo catalysts. But less is studied about the application of divalent cation zinc. The application of zinc ferrite as a catalyst for the oxidative dehydrogenation of n-

butane to butanes was studied (Peral, 1997). SEM image of the zinc sulphate nano material is given in Figure 1. The size was 100 nm.Various methods for the production of II–VI nanocrystals with narrow size distributions havebeen reported (Pileni, 2000).

XRD Spectrum of zinc sulphate nano material is represented in Figure 2.it showed sharp peaks in XRD corresponding to zinc sulphate. Similar pattern of peaks were also reported by Anderson *et al. (*2005). Orthorhombic structure of $ZnSO_4.7H_2O$ was studied by Saha and Podder,(2004). The physical properties such as packing density, agglomeration and re-dissolution mainly depend on the shape of the crystal (Cano *et al.* 2001).

Zinc sulphate nano material at a concentration of 100 µg/ 100 µl exhibited microbicidal activity with an inhibition zone of 18mm, 14mm, 12,10mm against *P. solanacearum, P. syringae,X. malvacearum and X. campestris respectively.*

Zinc sulphate nano material at concentrations of 0.01%,0.05%,0.1% , 0.5% and 1.0 % were used to evaluate their action on growth kinetics of phyto pathogens.The influence of zinc sulphate nano material concentration on the growth of *P. solanacearum* is represented in Figure 3.It is evident from the graph that the growth of organism is significantly reduced by the photo catalyst. *P.solanacearum* without any photocatalyst (control) had a lag phase upto 30 minutes. But photo catalyst added cultures had lag phase upto 90 minutes. After lag phase, the cfu/ml was 38.5 x 10^8, 11.7 x 10^8,10.73 x 10^8 ,10.22 x 10^8 ,1.93 x 10^8 in control,0.01%,0.05%,0.1% , 0.5% and 1.0 % respectively.1% of Zinc sulphatenano material was very effective in controlling and destroying all phytopathogens from one hour onwards. All organisms were destroyed within 150 min. Bacterial death can also be caused by the damage of cell wall by photocatalyst (Saito *et al*.,1998) It alters the cell permeability.The disordered cell membrane allows free efflux of intracellular constituents. The photo catalyst also disturbs the structure of proteins (Fujishima and Zhang, 2006).

The influence of zinc sulphate nano material concentration on the growth of *P. syringae* is represented in Figure 4. The cfu/ml at 30th minute was 11.2 x 10^8 in control and it was nearly similar in 0.01 and 0.05 % of zinc nano material. It was markedly reduced to 6.15 x 10^8 in 1%.of zinc sulphate nano material. At 30 minutes. After 90 minutes of growth significant difference among different concentration of zinc nano material was noted. The cfu/ml was 20.54x 10^8, 15x 10^8, 13.97x 10^8, 10x 10^8 and 1 x 10^8 in control, 0.01%, 0.05%, 0.1% and 1% of zinc nano material respectively. The mechanisms of photo catalytic antimicrobial process have been proposed as the oxidation of the intracellular coenzyme. This automatically inhibits cell respiration and subsequently death of cell (Zheng *et al.*, 2000)

The influence of zinc sulphate nano material on the growth of *X.campestris* is represented in Figure 5.Above pattern of growth inhibition with respect to concentration of zinc sulphate nano material was recorded. 18.17x 10^8 and 3.54 x 10^8cfu/ml was noted in control and 1% of zinc nano material. Compared to *Pseudomonas, Xanthomonas* is susceptible to photo catalytic oxidation and destructions.

Similar pattern was observed with respect to *X.malvecerum* also as shown in Figure 6. They are susceptible to the photo catalytic destruction even at a lower rmost concentration of 0.01% of zinc nano material. In *X.malvaecerum* the cfu/ml was nearly close to the control during 30thminute of growth.The data depicts the different response of organisms to photocatalyst at different time intervals. The present results are consistent with the reported mechanism (Marugan *et.al.*,2008 ;Chen *et al*.2009) .

Photo catalytic microbial degradation process is described with Survival number curve versus illumination time. It states that three different regions can be identified in the process:

1. A smooth decay at the beginning of the reaction, usually called ‘‘shoulder’’,
2. A log-linear inactivation region that covers most part of the reaction and
3. A deceleration of the process at the end of the reaction, usually called ‘‘tail’’

The presence of the shoulder can be justified by a single-hit multiple-target or a series event phenomenon in which the damage to the cell is cumulative.

Photo oxidation by photo catalyst depended strongly on the light or the photon flux on the surface of the catalyst. Commonly used light are metal-halide light (Matsunagat,1985), with the wavelength of 300–370 nm studies. Solar light which included UV light irradiation was also applied (Goswami *et al.,19*97). Low light intensity with long exposure time was more effective to inactivate microbes than high light intensity with short exposure time with the same UV dosage, which was defined as the product of intensity and irradiation time (Chen *et al*., 2009).

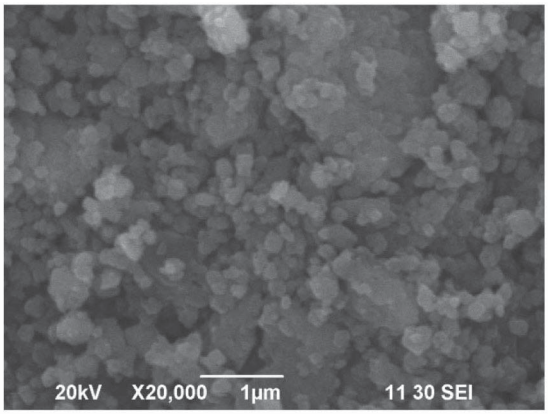

Figure 1: SEM Image Of Zinc Sulphate Nano Material

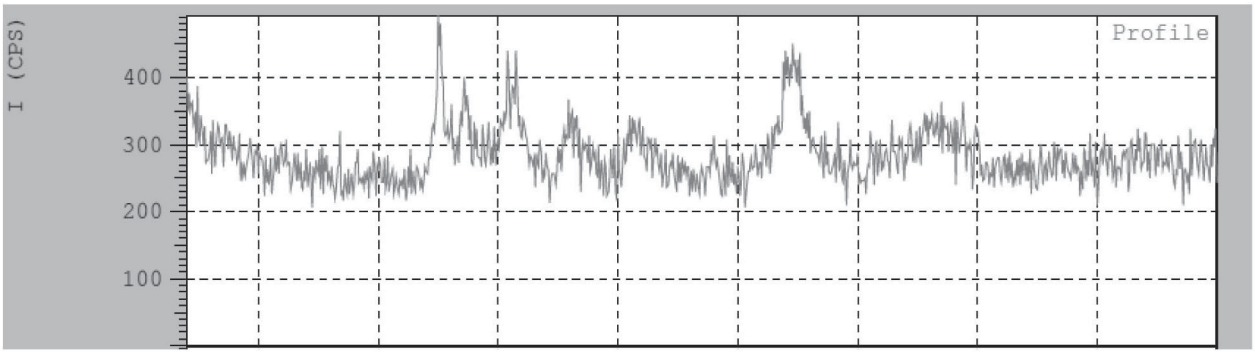

Figure 2: XRD of Zinc Sulphate Nano Material

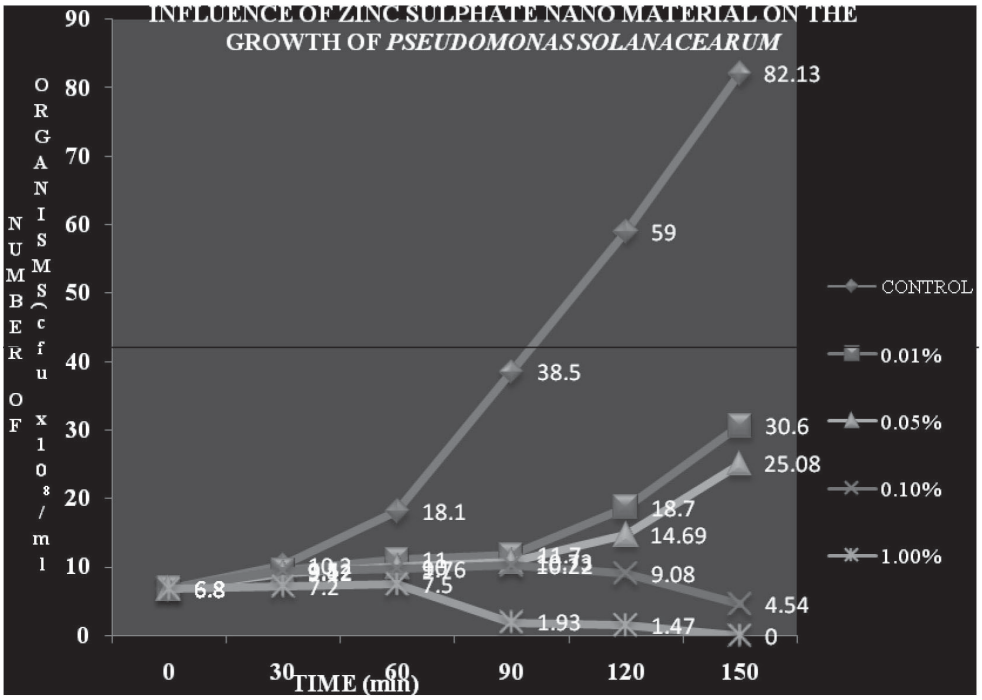

Figure 3: Influence of Zinc Sulphate Nano Material Concentration On The Growth Of *Pseudomonas Solanacearum.*

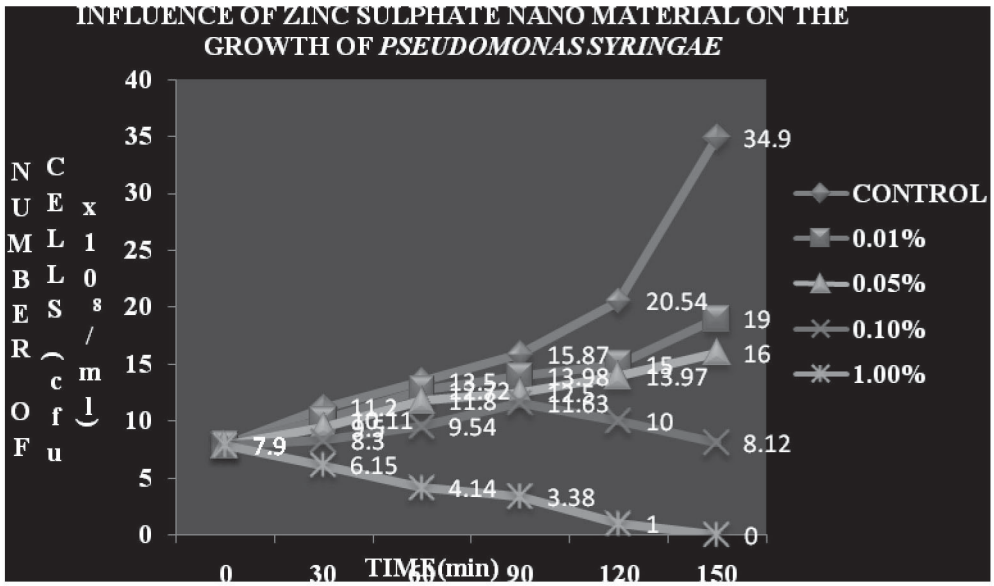

Figure 4: Influence Of Zinc Sulphate Nano Material Concentration On The Growth Of *Pseudomonas Syringa*

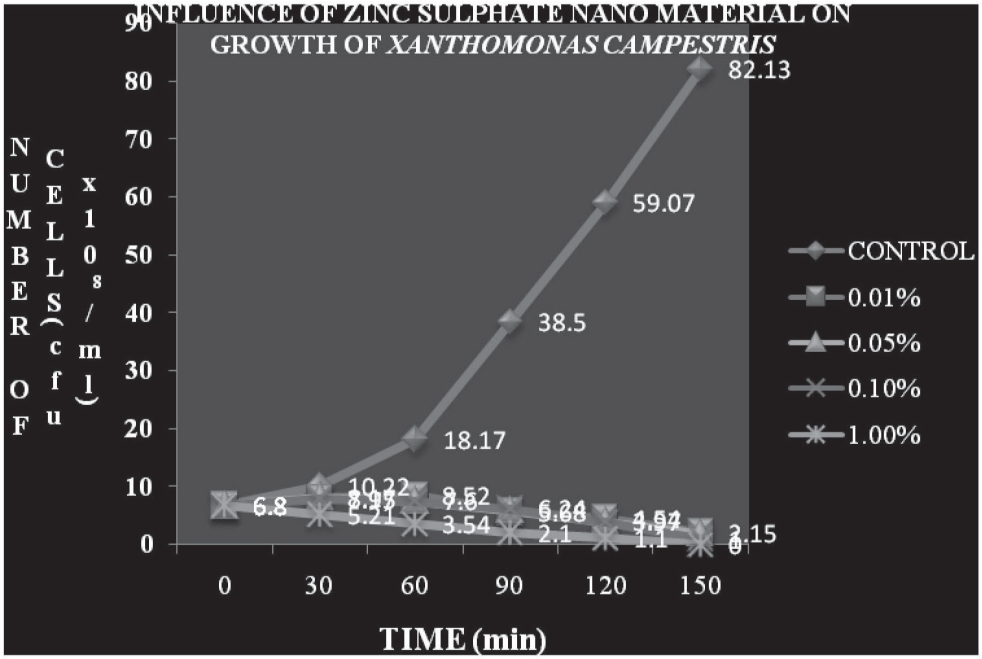

Figure 5: Influence Of Zinc Sulphate Nano Material Concentration On The Growth Of *Xanthomonas Campestris*

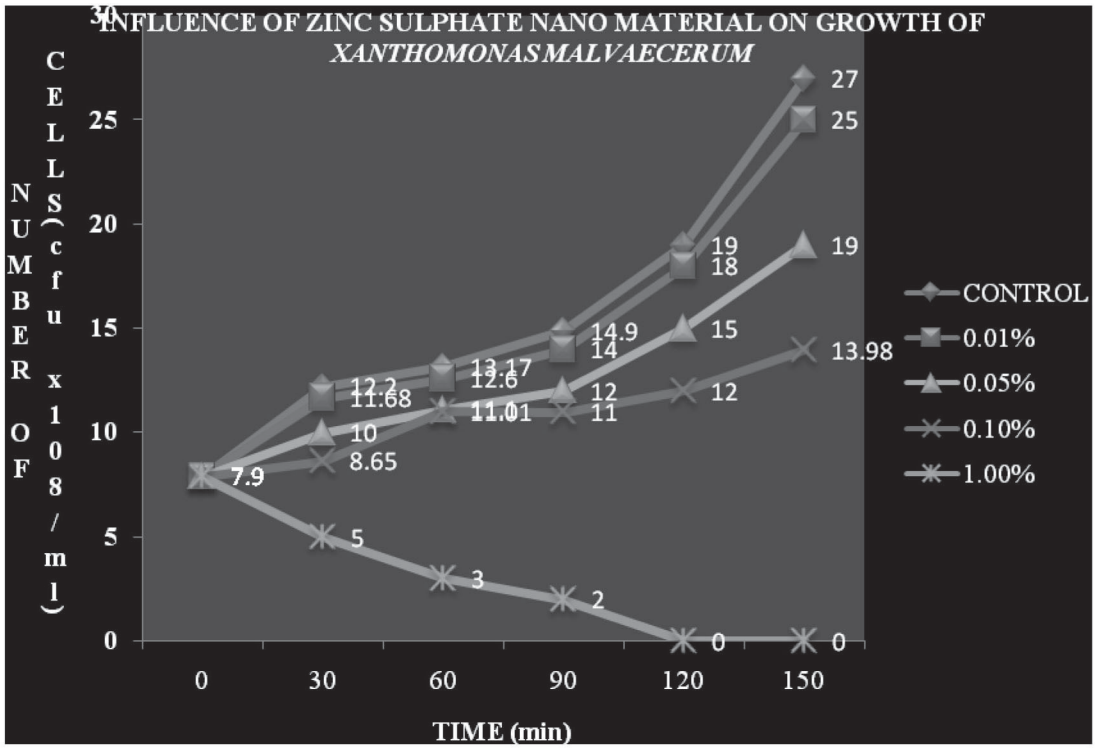

Figure 6: Influence Of Zinc Sulphate Nano Material Concentration On The Growth Of *Xanthomonas Malvaecerum*

Conclusion

The present study clearly reveals the possibility of controlling the growth of phytopathogens by zinc sulphate nano materials.

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