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Green Synthesis of Nanoparticles from Leaves of *Cannabis sativa* L. and its effect on Seed Germination, Seedling Mortality and wilt incidence of Tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen

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ABSTRACT: Now-a-days, crop pest management totally depends on synthetic agro-chemicals and causes harmful residual effect on human and environmental health. There is a rising demand for eco-friendly non-toxic synthetic biological protocol for the synthesis of nanoparticles. The experiment's objective was to create silver nanoparticles from fresh leaves of *Cannabis sativa* L., characterise them, and test their antifungal properties under *in vitro* condition. The fungus *Fusarium,* which is mostly a soil-born pathogen but occasionally also appears on seeds and negatively impacts their viability and germination, is the cause of tomato wilt. Green synthetic nanoparticles greatly enhanced the germination rate and decreased seed mortality in tomato seeds. The highest germination 92.66% found in seed treated with silica nanoparticles at 100 ppm (T_{12}) compared to non-treated plants with the value of 54%. Rest all nanoparticles treated seed was representing superior than non- treated seeds. The minimum seedling mortality as 5.25% was found in T_{12} (silica NPs 100 ppm) than all treated and non-treated seeds. The disease incidence was decreased over control as 86.66%, 86.00% and 83.32% at 30, 60 and 90 days of pathogen inoculation, respectively was observed in treatment T_{12}. It was established that all plants treated with nanoparticles lower disease incidence than the control. In general, nanoparticles work to combat plant infections by altering root exudates and starting the release of stress hormones. Because of their varied and distinctive mechanisms of action, nanoparticles can suppress pathogens that cause wilt in sustainable manner.

Keywords: Nanoparticles, germination, seedling mortality, stress, wilt and pathogen.

INTRODUCTION

Currently, climate change is threatening food security, worldwide. Several countries facing this problem, India is also more vulnerable in view of the tropical monsoon climate and poor coping capacity, so, almost all kind of crops are growing but their production adversely affected by various biotic and abiotic factors. Tomato (*Solanum lycopersicon* L.) stands one of the most important vegetable crops growing worldwide because of its consumption and high nutritive values like vitamins, minerals, lycopene, fiber, and a dietary source of antioxidants (Yadav *et al.,* 2022). Many plant pathogens impose serious diseases that inflict economic yield losses to tomato plant. Among the diseases, Fusarium wilt is more devastating caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the most economically important diseases worldwide occur in mild to severe form in warm climate countries causing 10 - 50% yield loss to tomato production (Desai *et al.,* 2021). In India, up to 45% loss in yield of tomato has been reported due to *F. oxysporum* f. sp. *lycopersici* by Ramyabharathi *et al.* (2012). The pathogen is soil in habitant, therefore, its management have challenging task using through conventional methods likes cultural, chemical, biological, use of resistance varieties *etc*. However, each of these approaches has significant drawbacks and cannot completely control the disease. Therefore, there is need to serve an alternative method which can manage the disease in an appropriate and sustainable manner in near future (Worrall *et al.,* 2018). One of the new emerging approach nanoparticles, it ‘comes support to the search for alternative, eco-friendly and non-chemical tool for fusarium wilt disease management. Nanoparticles are materials that varying between the range of 10 to 100 nanometers (nm). Potentially use of a novel green synthesis nano-materials one of the need to increase motivation to develop such cost-efficient, eco-friendly, high performing pesticides that is biodegradable and least hazardous for human being and environment for plant disease management in future (Schiavi *et al.,* 2021). Nanoparticles have the potential for the protection against plant pathogens; reduce the disease development through direct application on seeds, foliage and roots. Therefore, specific antimicrobial mechanisms of silver nano-materials that can inhibit the expression of proteins associated with ATP production (Yamanaka *et al.*, 2005). Presently, the nanoparticles are using for the management of plant pathogens. Advanced nano-engineering is a useful technology for increasing agricultural output and ensuring sustainability in order to attain food security. The effectiveness of inputs, particularly those connected to plant protection methods, is increased by nanotechnology, which also minimises important losses (Karki *et al.*, 2023). Herbal nanoparticles are also used in anticancer, anti-diabetic, antimicrobial and antioxidant drugs (Kumari *et al.*, 2023). Nano-materials could provide a better coverage when applied on crops, enhancing antimicrobial properties of pesticides (Takehara *et al.*, 2023). Keeping all the points in view, the present investigation entitled "Green synthesis of nanoparticles from leaves of *Cannabis sativa* L. and its effect on seed germination, seedling mortality and wilt incidence of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen" was conducted.

MATERIALS AND METHODS

A. Isolation and purification of pathogen

Infected tomato plants which exhibiting wilting like symptoms followed by yellowing were collected from the Centre of Excellence, Department of Vegetable Science, C.S.A. University of Agriculture & Technology, Kanpur, and brought to the laboratory for further analysis. The infected vascular tissues of tomato (Navodaya cultivar) cut into small pieces, then surface sterilised with 1% sodium hypochlorite for 1 minute, subsequently washed three times in sterile distilled water, and placed on whatman filter paper to absorb excess moisture. Then, small bit placed on potato dextrose agar (PDA) medium containing plates and incubated in the B. O. D. incubator in the laboratory conditions for 6 days at 25 ˚C. The fungi were purified separately by transferring the tip of the mycelia into poured plates and maintained as stock cultures for further studies (Fig. 1).

Collection of specimen   Pure culture of *Fusarium*   Macro and Micro conidia

Fig. 1. Collection, isolation and purification of *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Snyder & Hansen.

B. Collection and green synthesis of nanoparticles (nanofungicides)

(i) Synthesis of silver nanoparticles (NPs) using through leaves of *Cannabis sativa* L. Namely, 0.2 M of $AgNO_3$ (212.996 g/mol) solution, 0.2 N ammonium hydroxide solution reagents are used during conducting our experiments. First of all, to take 5 g of fresh leaves of *Cannabis sativa*, and washes it thoroughly with distilled water. Then dry under shade inside room for 20-30 minutes. Leaf extract and heat it over hot plate or sand bath at 80-90°C for an hour. Filter the extract by Whatman No.1 filter paper. Using of capping and reducing agent in synthesis of silver nanoparticles. Prepare 0.2 M of $AgNO_3$ (212.996 g/mol) solution of 20 ml in a 250 ml conical flask as precursor. Yellowish colour of plant extract changes to black which indicates silver nanoparticles formation which can be the centrifuged at 5000 rpm for 20 minutes to get the nanosilver hydroxide particles. Then kept inside distilled water/80% Ethanol in suspended form and stored in refrigerator at 4 ˚C for characterization and further use.

Characterization. The characterization of the particles is an important part for proving whether it is a nanoparticle or not. If, particles size varies between the ranges of 10 to 100 nm means it is nanoparticles. Nano properties of green synthesized silver particles confirmed through scanning electron microscope (SEM) image Figure 2(a) from the Centre for Nanoscience, Indian Institute of Technology, Kanpur.

(ii) Collection of silica nanoparticles (NPs). Silica nanoparticles contain fungicidal activities known as silica nanofungicide collected from Department of Materials Science Programme, IIT, Kanpur. The characterization of silica nanoparticles was also confirmed through Scanning Electron Microscopic (SEM) image Figure 2(b) from the Centre For NanoScience, Indian Institute of Technology, Kanpur.

(iii) Collection of Agri-tech nanofungicides (NFs). Agri-tech nanofungicides a product (containing nanoparticles of silver + copper + zinc) was obtained from the market prepared by Nano Research Lab, Jamshedpur, Jharkhand, India.

After fungicidal activities confirmation of nanoparticles further investigations as nanofungicides against the *Fusarium oxysporum* f. sp. *lycopersici*. Three different nanofungicides *viz.*, silver nanoparticle, Agri-tech nanoparticle and silica nanoparticle with each four different concentrations as 25, 50, 75 and 100 ppm were used.

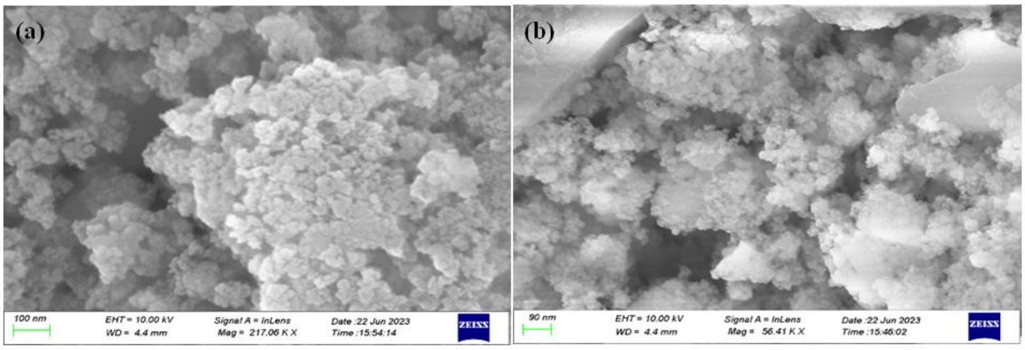


Fig. 2. Scanning electron microscopic image of (a) silver and (b) silica nanoparticles.

Treatment details

Treatment 1 = Silver NPs 25 (ppm); **Treatment 2** = Silver NPs 50 (ppm)
Treatment 3 = Silver NPs 75 (ppm); **Treatment 4** = Silver NPs 100 (ppm)
Treatment 5 = Agri-tech NFs 25 (ppm); **Treatment 6** = Agri-tech NFs 50 (ppm)
Treatment 7 = Agri-tech NFs 75 (ppm); **Treatment 8** = Agri-tech NFs 100 (ppm)
Treatment 9 = Silica NPs 25 (ppm); **Treatment 10** = Silica NPs 50 (ppm)
Treatment 11 = Silica NPs 75 (ppm); **Treatment 12** = Silica NPs 100 (ppm)
Control = Untreated

C. Sowing and Seed treatment procedure

Seed are soaked in different concentrations (25, 50, 75 and 100 ppm) of three nano-materials (silver, Agri-tech and silica based) for 30 minutes. After that, remove excess moisture and shade dried. Nanoparticles treated seed sown on plastic tray filled with soil and spray water time to time according to need.

D. Seed germination (%)

Fourteen days after sowing (DAS), germinated number of seeds in each treatment was observed and counted. Germination percentage was calculated by given formula:

$$\text{Germination \%} = \frac{\text{Number of germinated seed}}{\text{Total number of sown seed}} \times 100$$

E. Effect of nanoparticles on Fusarium wilt incidence (%) of tomato

Nanoparticles and nanofungicides were used as seedling treatment and single foliar application (30 days after transplanting), to find out the most effective nanoparticles/fungicides against Fusarium wilt incidence (%) of tomato. The disease incidence was recorded after 30, 60 and 90 days of pathogen inoculation. The disease incidence (%) was measured by using following scales as given by Weitang *et al.* (2004):

0 : No infection
1 : Slight infections, one or two leaves became yellow (25% leave wilted)
2 : Moderate infection, two or three leaves wilted (50% of leaves became wilted)
3 : Extensive infection, 75% of leaves become wilted and growth is inhibited (all leaves became yellow)
4 : Complete infections, 100% of leaves become wilted and the plants die (whole plant leaves become yellow)

The percentage of disease incidence was determined using the formula given by Weitang *et al.* (2004):

$$\text{Disease incidence (\%)} = \frac{(\Sigma\text{scale} \times \text{number of plants infected})}{(\text{Highest scale} \times \text{total number of plants})} \times 100$$

F. Statistical analysis

The Completely Randomized Design (CRD) used during conducting our experiment with the mean of 3 replications. All the examined was carried out at 5% (five) level of significance.

RESULTS

A. Effect of green nanoparticles on seed germination (%) and seedling mortality (%) of tomato

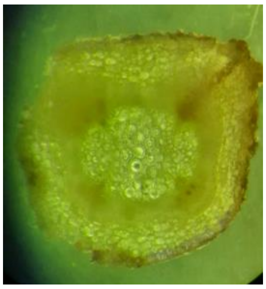
The four different concentrations (25, 50, 75 and 100ppm) of nano-materiales (silver, Agri-tech and silica) increased the rate of tomato seed germination and significantly reducing the seedling mortality (%). The highest germination % was observed in T_{12} (silica 100 ppm) treated seed about 92.66 %, followed by T_8 (Agri-tech 100 ppm), T_{11} (silica 75 ppm) and T_4 (silver 100ppm) with the values of 89.32, 83.72 and 80.66%, respectively, against control about 54 % which was least than all treated seeds. On the other hand, the minimum seedling mortality with 5.2% was also recorded from seeds treated by silica 100 ppm (T_{12}), followed by T_8 (Agri-tech 100 ppm) representing 10%, over the control. The mortality of seedling decreased as 96.20 % against control recorded from seeds treated with silica 100 ppm (T_{12}), followed by T_8 (Agri-tech 100 ppm) with the value of 93.21%. Among the treatments, the least decreasing percentage of seedling mortality recorded from seeds treated with silver nanoparticle 25 ppm (T_1) indicating as 43.2%. It is evident that all treated plants with nanoparticle have ability to increase germination per cent over control.

Table 1: Effect of seed treatment with nanoparticles on their consortial germination (%) and seedling mortality (%) in tomato.

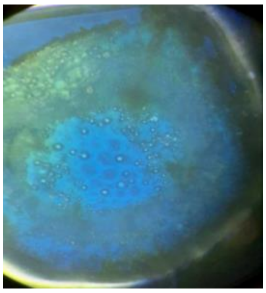
Treatment details		Tomato seed infected with *Fusarium*	
Name of nanoparticles	**Concentrations (ppm)**	**Germination (%)**	**Seedling mortality (%)**
Silver nanoparticles	T_1 = 25	62.66	43.2
	T_2 = 50	67.32	40.0
	T_3 = 75	70.00	37.2
	T_4 = 100	80.66	15.2
Agri-tech nanofungicides	T_5 = 25	74.66	27.2
	T_6 = 50	75.32	22.6
	T_7 = 75	77.32	16.6
	T_8 = 100	89.32	10.0
Silica nanoparticles	T_9 = 25	72.66	32.0
	T_{10} = 50	77.33	19.2
	T_{11} = 75	83.72	12.6
	T_{12} = 100	92.66	5.2
Control	T_{13} (Untreated)	54.00	58.6
C.D.		4.782	2.469
SE(m)		1.311	0.906
C.V.		5.493	3.408

B. Effect of nanoparticles on disease incidence (%) and transverse sections of root and shoot of treated and non-treated tomato plants.

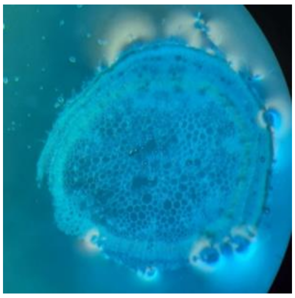
The effect of seedling treatment and single spray with same concentration of nanoparticles is significantly reduced disease incidence as compared to control under wire house condition. Among the treatments, highest minimizing the disease incidence was observed in plant treated with silica 100 ppm (T_{12}) treatment, representing 86.66%, 86.00% and 83.32% at 30, 60 and 90 days of pathogen inoculation, respectively, followed by plants treated with Agri-tech 100 ppm (T_8), representing as 86.00%, 84.66% and 79.32% at 30, 60 and 90 days of pathogen inoculation, respectively. Among the treatments, the least reducing rate of disease incidence was found in plants treated with silver 25 ppm (T_1) with the value of 60.00%, 57.32% and 51.32% at 30, 60 and 90 days after pathogen inoculation, respectively. From the Table 2, it is cleared that nanomateriales tested plant have ability to minimized the Fusarium wilt incidence of tomato. The transverse section of root and shoot of diseased (non-treated) and healthy (treated) plant are representing in Fig. 3 (a- transverse section of treated root; b- non-treated root and c- transverse section of treated stem and d- diseased stem, seen under Stereo Zoom Microscope at 10x magnification). Histochemical staining of the root section of tomato at 8 days after pathogen inoculation showed significant variation of lignin deposition between nanoparticles treated and non-treated plants. But in case of Fusarium wilt infected stem transverse section Fig. 3(d) clearly indicated that the discoloration of tissues.



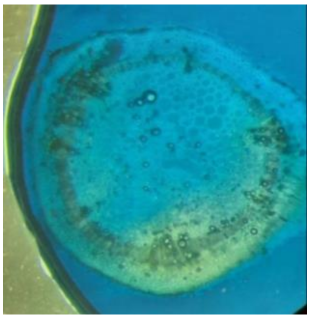
a. Section of treated root



b. Section of non-treated root



c. Section of treated stem



d. Section of non-treated stem

Fig. 3. Transverse section of **(a)** treated root **(b)** non-tretaed root **(c)** Healthy shoot **(d)**.non-tretaed shoot.

Table 2: Effect of nanoparticles on fusarium wilt incidence (D.I.) of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*.

Treatment details		Observations in days		
Name of nanoparticles	**Concentrations (ppm)**	**after 30 days**	**after 60 days**	**after 90 days**
Silver Nanoparticles	$T_1 = 25$	60.00	57.32	51.32
	$T_2 = 50$	63.32	58.66	56.00
	$T_3 = 75$	66.00	63.32	58.66
	$T_4 = 100$	80.66	80.00	76.66
Agri-tech Nanofungicides	$T_5 = 25$	72.00	70.66	68.00
	$T_6 = 50$	73.32	73.32	69.32
	$T_7 = 75$	80.00	78.66	75.32
	$T_8 = 100$	86.00	84.66	79.32
Silica Nanoparticles	$T_9 = 25$	68.66	65.32	63.32
	$T_{10} = 50$	76.66	74.00	72.00
	$T_{11} = 75$	83.32	81.32	78.00
	$T_{12} = 100$	86.66	86.00	83.32
Control	(Untreated)	53.32	46.00	42.66
C.D.		3.728	2.479	2.259
SE(m)		1.217	0.572	0.482
C.V.		2.493	3.365	4.265

DISCUSSION

For the establishment of plants, germination is one of the most crucial stages and plays a vital role in crop production. In the present study, all treated seed with nanoparticles exhibited better germination. The highest germination % was found in T_{12} (silica 100ppm) treated seed about 92.66 % with least seedling mortality as 5.2 %. All nanoparticles treated seed were statistically increased germination and reducing the percentage of seedling mortality. Lahiani *et al.* (2015) have been also reported that the hastening of seed germination in different crops *viz*. rice, maize, soybean and tomato has been observed when treated with carbon containing nano-materials. The observations is in this study agreement with Rhaman *et al.* (2022) who reported that the seed treatment with nono-material’s is an economical and most efficient physio-chemical technique which enhanced the seed germination.

The plant treated with silica 100 ppm (T_{12}) treatment decreased disease incidence over control as 86.66%, 86.00% and 83.32% at 30, 60 and 90 days after inoculation of pathogen, respectively. Since the Table 2, it is cleared that the all tested plant with nanoparticles recorded efficient as disease minimizing agents. Giannousi *et al.* (2013) had proved that the foliar application of copper oxide nanoparticles (150-350 mg/l) on tomato decrease leaf lesions by 61%, 7 days after application. Kumar *et al.* (2023a) also reported that the leaves extract of Hemp (*Cannabis* sp.) reduces the radial growth of *Fusarium* causing pathogenic activities in plants. The current results also supported by the finding of Joshi *et al*. (2021), has been concluded that the selenium nanoparticles primed tomato seeding and pathogen challenged plants shows a significant protection of 72.9 % against wilt disease of tomato. Ahmad and Kalra (2020) also supported to current work, using nano-materials against the management of *Alternaria mali, Botryosphaeria dothidea and Diplodia seriata* in apple orchards.

CONCLUSIONS

On account of eco-friendly, biodegradable, non-chemicals more efficient in nature and the increasing trends of green synthesis nanoparticles in near future may be prove best substitute instead of chemical pesticides against plant disease management. The seed treated with green synthesized nanoparticles was positively raising germination (%) and reduces the mortality percentage of tomato seedling. All plant treated with nanoparticles significantly increased the root, shoot length and total biomass of the plant. The Treatment T_{12} (silica 100 ppm) treated plant reduces 86.66%, 86.00% and 83.32% disease incidence over the control at 30, 60 and 90 days of pathogen inoculation, respectively. Thus, it is evident from the current evaluation that the use of green synthesis nanoparticles can be employed to manage tomato wilt in sustainable manner.

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Conflict of Interest. None.

REFERENCES

Ahmad, W. and Kalra, D. (2020). Green synthesis, characterization and anti microbial activities of ZnO nanoparticles using *Euphorbia hirta* leaf extract. *Journal of King Saud University-Science, 32*(4), 2358-2364.

Desai, P., Jha, A., Markande, A. and Patel, J. (2021). Silver nanoparticles as a fungicide against soil-borne *Sclerotium rolfsii*, A case study for wheat plants. *Biobased nanotechnology for green applications*, 513-542.

Giannousi, K., Avramidis, I. and Dendrinou-Samara, C. (2013). Synthesis, characterization and evaluation of copper based nanoparticles as agrochemicals against

Phytophthora infestans. *RSC advances*, *3*(44), 21743-21752.
Joshi, S. M., De Britto, S. and Jogaiah, S. (2021). Myco-engineered selenium nanoparticles elicit resistance against tomato late blight disease by regulating differential expression of cellular, biochemical and defense responsive genes. *J. Biotechnol*., *325,* 196-206.
Karki, N. S., Bisht, T., Pal, M., Pathak, B. C. and Bisht, M. (2023). Exploring the Classification, Properties, and Biological Green Synthesis of Nanoparticles: A Comprehensive Review. *Biological Forum – An International Journal, 15*(5), 1559-1566.
Kumari, S., Kansotiya, A. K., Bharti, N., Yadav, P., & Mali, P. C. (2023). Herbal Nanoparticles to Control Fertility and Regulation: A Review. *International Journal on Emerging Technologies, 14*(1), 01–08.
Lahiani, M. H., Chen, J., Irin, F., Puretzky, A. A., Green, M. J. and Khodakovskaya, M. V. (2015). Interaction of carbon nanohorns with plants: uptake and biological effects. *Carbon, 81*(2), 607-619.
Ramyabharathi, S. A., Meena, B. and Raguchander, T. (2012). Induction of chitinase and β-1, 3-glucanase PR proteins in tomato through liquid formulated *Bacillus subtilis* EPCO 16 against Fusarium wilt. *J. Today's Biol. Sci. Res. Rev*., *1,* 50-60.
Rhaman, M. M., Islam, M. R., Akash, S., Mim, M., Nepovimova, E., Valis, M. and Sharma, R. (2022). Exploring the role of nanomedicines for the therapeutic approach of central nervous system dysfunction: *At a glance. Frontiers in Cell and Developmental Biology, 10*, 989471.
Schiavi, P. G., Altimari, P., Branchi, M., Zanoni, R., Simonetti, G., Navarra, M. A. and Pagnanelli, F. (2021). Selective recovery of cobalt from mixed lithium ion battery wastes using deep eutectic solvent. *Chemical Engineering Journal*, *417*, 129-249.
Synder, W. C. and Hansen, H. N. (1940). The species concept in Fusarium. *Am. J. Bot., 27,* 64-67.
Takehara, Y., Fijikawa, I., Watanabe, A., Yonemura, A., Kosaka, T., Sakane, K. and Ito, S. I. (2023). Molecular Analysis of MgO Nanoparticle-Induced Immunity against Fusarium Wilt in Tomato. *International Journal of Molecular Sciences*, *24*(3), 2932-2941.
Weitang, S., Ligang, Z., Chengzong, Y., Xiaodong, C., Liqun, Z. and Xili, L. (2004). Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. *Crop protection, 23*(3), 120-123.
Worrall, E. A., Hamid, A., Mody, K. T., Mitter, N. and Pappu, H. R. (2018). Nanotechnology for plant disease management. *Agronomy, 8*(12), 285.
Yadav, A. K., Arvind, K. and Pandey, R. (2022). Nanotechnology in food processing industries. *J. Phytonanotech. Pharmaceut. Sci*., *2*(2), 1-8.
Yamanaka, M. Hara, K. and Kudo, J. (2005). Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl Environ Microbiol.*, *71*, 7589–7593.

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