

E-ISSN: 2278-4136 P-ISSN: 2349-8234 JPP 2019; 8(3): 222-226 Received: 21-03-2019 Accepted: 24-04-2019

CS Azad

Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Amarendra Kumar

Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

G Chand

Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

RD Ranjan

Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Correspondence Amarendra Kumar Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



Evaluation of host defense inducing nanoparticles against *Alternaria tenuissima* (Kunze ex pers.) Wiltshire causing dieback disease of chilli

CS Azad, Amarendra Kumar, G Chand and RD Ranjan

Abstract

Dieback disease, caused by *Alternaria tenuissima*, (Kunze ex pers.) Wiltshire, is one of the most important disease that affecting all the plant parts of chilli. Among sixn nano compounds, all treatments significantly increased the enzymatic activities over control. Silver nanoparticles were found to best followed by Aluminum nano particles as lowest No. of spot/leaves as well as severity was recorded. The accumulation of enzymes i.e. polyphenol oxidase (PPO), peroxidase (PO) and total phenol significantly increased upto 72 hours. Among different nanoparticles highest significantly increase in PPO, PO and total phenol activity at 100 μ gml⁻¹ were recorded in Silver nano particles i.e. 0.039, 0.441 and 10.069 μ mol/min/mg protein respectively. The average No. of spot/leaf (3.11), No. of infected leaves/plant (2.00) and disease severity after 60 DAI (days after inoculation) (8.00%) of dieback disease of chilli were minimum at 100 μ gml⁻¹ as compare to control.

Keywords: Alternaria tenuissima, dieback, nanoparticles, polyphenol oxidase (PPO), peroxidase (PO) and total phenol

Introduction

Chilli (Capsicum annum L.) is considered as one of the most important commercial spice crop. Different varieties are cultivated for varied uses like vegetable, pickles, spice and condiments. It is cultivated over an area of 775 thousand hectares with an annual production of 1492 thousand tonnes and productivity of 1.9 metric tonnes per hectare in India (NHB, 2016). Chilli is affected by 750 pathogens of different origins, reported from different part of the world, but only few are responsible for considerable loss of production and productivity. Among the fungal diseases, dieback, leaf spot and fruit rot is caused by Alternaria spp., damping off caused by *Pythium* spp., *Phytophthora* spp. and other fungi, seedling blight caused by Rhizoctonia spp., wilt caused by Fusarium spp., anthracnose and dieback caused by Colletotrichum capsici are major diseases. Among all these Alternaria spp. are responsible for dieback, leaf spot and fruit rot have been identified as major limiting factor in chilli cultivation is very common problem in fields and greenhouse. Use of host defence nano particles has been considered an alternate and effective approach for the control of pathogenic microbes. Plants can be sensitized for a more rapid and intense mobilization of defence responses leading to enhanced resistance to biotic stresses (Beckers and Conrath, 2007)^[1]. Induction of systemic resistance is associated with gene induction, activation of a wide range of resistance mechanisms and the production of variety of defence compounds. It is a race non-specific and is often effective against a broad spectrum pathogenic agents (Walters and Fountaine, 2009) ^[14, 15]. Thus, study on induction of host defence through nano compounds can be considered as one of the effective sustainable approaches in disease management.

Materials and Methods

Soil was collected from the upper 0-15 cm layer from Vegetable Research Centre (VRC), GBPUA&T Pantnagar and was sterilized by autoclaving at 20 lb psi (121.6°C) for one hour on three consecutive days. The sterilized soil was filled in 1.5 kg capacity plastic pots and kept in glasshouse. Thirty days old seedlings of chilli cultivar Pant C1 grown in portrays under polyhouse was used in this experiment. Pots were watered regularly as and when required to maintain optimum moisture. Six nano compounds viz. Silver Nanoparticle, Aluminium Nanoparticle, Silicon carbide, Silicon dioxide, Titanium dioxide and Zinc oxide were tested at three concentrations (25, 50 and 100 μ gml⁻¹) were applied under glass house these six nano compounds were prepared and applied at three different concentrations through atomizer to the point of runoff. Experiment was laid out in a completely randomized block design with five replications.

After 30 days of transplanting plants were inoculated by spraying with spore suspension of 1×10^6 spores/ml by sterilized atomizer of the *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire.

Effect of Nanoparticles on enzymatic activity after post inoculation of *Alternaria tenuissima* on chilli

Top five leaves per treatments were harvested at 0, 24, 48, 72, 96 and 120 hours after inoculation and brought to the laboratory in an ice box for analysis of the enzymes as mentioned below.

Polyphenol oxidase (PPO) activity

PPO activity was determined as per the procedure given by Mayer *et al.* (1965) ^[6]. Leaf samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant was used as the enzyme source. The reaction mixture consisted of 200 μ l of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 μ l of 0.01M catechol was added and the activity was expressed as μ mol/min/mg protein.

Peroxidase (PO) activity

Assay of Peroxidase (PO) activity was carried by Hammerschmidt *et al.* (1982) ^[3] method. Enzymes extract was prepared by homogenizing one gram of leaf samples in 0.1M sodium phosphate buffer (pH 6.0). It was centrifuged at 10,000 rpm for 20 minutes. The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01 M sodium phosphate buffer (pH 6.0) and 0.1 M hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction, which was followed colorimetrically at 480 nm. The boiled enzyme preparation served as blank. Activity was expressed as µmol/min/mg protein.

Total phenolics

Total phenolics content was determined as per methodology given by Swain and Hills (1959). One gram leaves were homogenized in 10 ml of 80 per cent methanol and agitated for 15 minute at 70°C. One milliliter of methanolic extract was added to 5 ml of distilled water and 250 μ l of Folinciocalteu reagent, after this the solution was kept at 25°C. After 3 minutes, 1 ml of a saturated solution of Na₂CO₃ and 1 ml of distilled water were added, and the reaction mixture was incubated for 1 hr at 25°C. The absorbance of the developed color was measured using spectrophotometer at 725 nm. The total soluble phenolic content was calculated by comparison with a standard curve obtained from Folin-Ciocalteu reaction with catechol.

Effect of Nanoparticles on dieback disease of chilli

The effect of nano compounds on No. of spots/leaf and disease severity of dieback disease of chilli was observed. Observations on number of spots/leaf, number of infected leaves/plant were recorded 15 days after inoculation. Disease severity was also recorded 15, 30, 45 and 60 days after inoculation on 0-5 scale suggested by Vishwakarma and Sitaramaiah (1986)^[12, 13] and percent disease index (PDI) was calculated as described by McKiney (1923).

$$PDI = \frac{Sum of all disease ratings}{Total number of plants observed x maximum rating value} \times 100$$

Results and Discussion

Effect of Nanoparticles on Polyphenol oxidase (PPO) activity in chilli

The results of the experiment presented in Table 5 revealed that a significant increased in polyphenol activity was recorded in chilli plants treated with abiotic elicitor as compared to control. An increased in PPO activity was recorded at 24 hour interval and reached its higher value at 72 hours after inoculation of Alternaria tenuissima while after 72 hrs PPO activity decreased significantly in all the treatments and their concentrations. Among three different concentration of nanoparticles application, 100 µgml⁻¹ was found significantly superior over 50 µgml⁻¹ followed by 25 µgml⁻¹ at every 24 hrs interval. The interaction between treatments, concentrations and time interval was found none significant. This trend was observed at all time durations at its peak *i.e.* 72 hours after treatment. At 100 µgml⁻¹ concentration of silicon carbide nano particles the increased PPO activity was (61.11%) which was followed by Titanium dioxide (56.52%), Silver nanoparticles (54.84%), silicon dioxide (53.85%), Aluminium nanoparticles (50.00%) and Zinc oxide (41.67%) respectively higher than initial value (0 hour). Among nanoparticles highest mean increase in PPO activity was recorded in Silver Nanoparticles followed by Aluminium Nanoparticles, Titanium dioxide, Silicon carbide, and Silicon dioxide and Zinc oxide i.e. 0.039, 0.035, 0.029, 0.023, 0.016 and 0.015 µmol/min/mg protein respectively at 100 µgml⁻¹ concentration.

Effect of nanoparticles on Peroxidase (PO) activity in chilli

The peroxidase activity was increased significantly as compared to control in chilli plants treated with abiotic elicitor *Alternaria tenuissima*. An increased in PO activity was recorded at 24 hour and reached its higher value at 72 hours after inoculation of *Alternaria tenuissima* in all the treatments. However after 72 hrs, there has been decrease in polyphenol activity in all the different methods of application. Data pertaining to effect of different nanoparticles on peroxidase activity in chilli presented in Table 2 revealed that all the nanoparticles increased the peroxidase accumulation as compared to control.

Among nanocompounds highest increase in PO activity was recorded in Zinc oxide followed by Silicon dioxide, Aluminium Nanoparticles, Titanium dioxide, Silver Among Nanoparticles and Silicon carbide. three concentration, 100 µgml⁻¹ concentration proved to be the best as compared to 50 µgml⁻¹ and 25 µgml⁻¹. In case of 100 µgml⁻¹ ¹ concentration of nanocompunds Zinc oxide, PO activity was 54.31% followed by Silicon dioxide (54.01%) and Aluminium Nanoparticles (54.01%) respectively higher than initial value after 72 hours after treatment.

Effect of nanoparticles on total phenol accumulation in chilli

The effect of nanoparticles on total phenol accumulation in chilli after 120 hours of inoculation of *Alternaria tenuissima* was recorded at every 24 hours interval. Among three different concentration of nanoparticles 50 μ gml⁻¹ concentration but 100 μ gml⁻¹ concentrations proved to be the best. The data revealed in Table 3 showed that the total phenol accumulation increases upto 72 hours but later on total phenol activity decreases in all the three concentrations.

Among different nanoparticles highest increase in total phenol content was recorded in *Titanium dioxide* followed by Zinc oxide, Silver Nanoparticles, Aluminium Nanoparticles, Silicon dioxide and Silicon carbide. At 100 μ gml⁻¹ concentrations of *Titanium dioxide*, total phenol content was 53.41% higher than initial value (0 hour). In case of Zinc oxide, Silver Nanoparticles, Aluminium Nanoparticles and Silicon dioxide it was 53.38, 53.36, 53.35 and 53.34 per cent, respectively.

Effect of nanoparticles on leaf infection of dieback disease of chilli

The nanoparticles reduced the No. of spot/leaf and No. of infected leaves/plant significantly as compared to control. The results of the experiment presented in Table 4 revealed that among three different concentrations 25 μ gml⁻¹, 50 μ gml⁻¹ and 100 μ gml⁻¹ the highest concentration of nanoparticles showed significantly minimum No. of spot/leaf and No. of infected leaves/plant as compared to other concentrations. The minimum No. of leaf spots/ leaf and No. of infected leaves/plant was recorded 3.11 and 2.00 respectively at 100 μ gml⁻¹ concentration of Silver Nanoparticles which was followed by Aluminium Nanoparticles *i.e.* 4.11 and 2.56 respectively.

Effect of nanoparticles on severity of dieback disease of chilli

The results of the experiment presented in Table 5 revealed that 15, 30, 45 and 60 days after inoculation (DAI), all the nanoparticles reduced the disease severity of *Alternaria tenuissima* as compared to control. Among treatments having various nanoparticles, at 100 μ gml⁻¹ Silver Nanoparticles was found best followed by Aluminium Nanoparticles while least effective was in case of Zinc oxide after control. The percent disease severity was found minimum in Silver Nanoparticles

i.e. 0.00, 0.00, 4.00, 8.00 and 8.00 followed by Aluminium Nanoparticles *i.e.* 4.00, 4.00, 08.00 and 12.00 at 100 μ gml⁻¹ concentration at 15, 30, 45 and 60 DAI respectively.

The fungus causing leaf spot, fruit rot and early dieback of chilli under *tarai* conditions of Uttarakhand was established as *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire which is probably the first report from India so very few works were done with the same crop.

The present study clearly showed increased activity of PO, PPO and Phenol in treated plants as compared to control. These finding are in accordance of Hasanuzzaman and Fujita (2013) who reported that application of silver nano particles enhanced the activity of different antioxidant enzymes like poly phenol oxidase, peroxidase and total phenol. Chandra et al. (2015)^[2] and Zhao (2007)^[17] have reported that exogenous application of chitosan nanoparticles on tea leaves elevated the defense response in the tea plant and observed higher accumulation of PO, PPO, β -,3 glucanase, and PAL in the CNP treated leaves. Park et al. (2006) [9] observed the efficacy of silica-silver nanoparticles in the control of plant pathogenic fungi viz. Botrytis cinerea, Rhizoctonia solani, Colletotrichum gloeosporioides, Magnaporthe grisea and Pythium ultimum. Wani and Shah (2012)^[16] observed release of extracellular enzymes and metabolites by application of Magnesium oxide and Zinc oxide nanoparticles against Alternaria alternata, Fusarium oxysporum, Rhizipus stolonifer and Mucor plumbeus. Imada et al. (2015)^[5] have found that Magnesium oxide nanoparticles induce systemic resistance in tomato plants against Ralstonia solanacearum. Exogenous application of chitosan nanoparticles on tea leaves elevated the defense response in the tea plant and promoted higher accumulation of Peroxidase, Polyphenol oxidase, β -1,3-glucanase, Phenylalanine lyase in the chitosan nanoparticles treated leaves.

		POLYPHENOL OXIDASE (µmol/min/mg protein)																			
		0 Hr			24 Hrs			48 Hrs			72Hrs		96 Hrs			120 Hrs				Mean	
Treatments	25	50	100	25	50	100	25	50	100	25	50	100	25	50	100	25 µgml ⁻¹	50 ugml-1	100 µgml ⁻	25 µaml-1	50 uaml-1	100
	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	25 μgmi	50 µgiii	1	25 μgiii	50 µgnn	µgml ⁻¹
Silver Nanoparticles	0.023	0.026	0.031	0.026	0.030	0.035	0.030	0.035	0.041	0.036	0.041	0.048	0.031	0.036	0.041	0.028	0.033	0.038	0.029	0.034	0.039
Aluminium Nanoparticles	0.020	0.024	0.028	0.023	0.027	0.031	0.027	0.031	0.036	0.032	0.035	0.042	0.027	0.032	0.037	0.025	0.031	0.035	0.026	0.030	0.035
Silicon carbide	0.013	0.016	0.018	0.016	0.018	0.021	0.018	0.021	0.024	0.022	0.025	0.029	0.018	0.022	0.024	0.018	0.020	0.023	0.017	0.021	0.023
Silicon dioxide	0.011	0.012	0.013	0.012	0.013	0.014	0.014	0.015	0.016	0.016	0.017	0.020	0.014	0.015	0.017	0.013	0.014	0.015	0.013	0.014	0.016
Titanium dioxide	0.016	0.021	0.023	0.019	0.023	0.027	0.022	0.026	0.030	0.026	0.032	0.036	0.022	0.027	0.031	0.021	0.025	0.028	0.021	0.026	0.029
Zinc oxide	0.011	0.012	0.012	0.012	0.013	0.013	0.014	0.015	0.015	0.016	0.017	0.017	0.014	0.015	0.016	0.013	0.014	0.014	0.013	0.014	0.015
Control	0.010	0.010	0.010	0.011	0.011	0.011	0.013	0.013	0.013	0.015	0.015	0.015	0.013	0.013	0.013	0.012	0.012	0.012	0.012	0.012	0.012
Mean	0.015	0.017	0.019	0.017	0.019	0.022	0.020	0.022	0.025	0.023	0.026	0.030	0.020	0.023	0.026	0.019	0.021	0.024	0.019	0.021	0.024
CD at 1 %	a=0.0003 b=0.0002 c=0.0003 a x b= 0.0006 b x c=0.0009 c x a =0.0005 a x b x c=0.0011																				
CV											4.5951										

Table 1: Effect of Nanoparticles on Polyphenol oxidase (PPO) activity in chilli

a = Treatments b =Concentrationc = Hours

Table 2: Effect of Nanoparticles on Peroxidase (PO) activity in chilli

									Per	oxidase (µ	umol/min/	/mg prote	in)									
Treatments	0 Hr				24 Hrs			48 Hrs			72Hrs			96 Hrs			120 Hrs			Mean		
	25	50	100	25	50	100	25	50	100	25	50	100	25	50	100	25	50	100	25	50	100	
	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	
Silver Nanoparticles	0.254	0.297	0.349	0.287	0.335	0.395	0.330	0.386	0.454	0.389	0.455	0.535	0.342	0.400	0.472	0.318	0.372	0.438	0.320	0.374	0.441	
Aluminium Nanoparticles	0.230	0.279	0.303	0.261	0.315	0.343	0.300	0.363	0.395	0.354	0.428	0.466	0.311	0.377	0.410	0.290	0.351	0.381	0.291	0.352	0.383	
Silicon carbide	0.154	0.176	0.202	0.175	0.198	0.228	0.201	0.228	0.262	0.237	0.270	0.309	0.209	0.238	0.272	0.194	0.221	0.253	0.195	0.222	0.254	
Silicon dioxide	0.078	0.124	0.137	0.088	0.140	0.155	0.101	0.162	0.178	0.120	0.191	0.211	0.105	0.167	0.185	0.098	0.156	0.172	0.098	0.157	0.173	
Titanium dioxide	0.186	0.220	0.253	0.211	0.248	0.286	0.243	0.286	0.330	0.286	0.337	0.389	0.252	0.297	0.342	0.234	0.276	0.318	0.235	0.277	0.320	
Zinc oxide	0.068	0.106	0.116	0.076	0.120	0.132	0.088	0.137	0.152	0.104	0.162	0.179	0.092	0.142	0.157	0.086	0.132	0.146	0.086	0.133	0.147	
Control	0.067	0.067	0.067	0.075	0.075	0.075	0.087	0.087	0.087	0.103	0.103	0.103	0.091	0.091	0.091	0.084	0.084	0.084	0.084	0.084	0.084	
Mean	0.148	0.181	0.204	0.167	0.205	0.231	0.193	0.236	0.265	0.228	0.278	0.313	0.200	0.244	0.276	0.186	0.227	0.256	0.187	0.229	0.257	
CD at 1 %		a=0.002 b=0.001 c=0.002 a x b= 0.003 b x c=0.005 c x a =0.003 a x b x c=0.009																				
CV											2.634											

a = Treatments b =Concentration c = Hours

Table 3: Effect of Nanoparticles on total phenol accumulation in chilli

	Total Phenols (mg/gm of fresh leaf)																				
Treatments	0 Hr				24 Hrs			48 Hrs			72Hrs			96 Hrs			120 Hrs				ean
	25	50	100	25	50	100	25	50	100	25	50	10	25	50	100	25	50	100	25	50	100
	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹
Silver Nanoparticles	5.836	6.829	8.029	6.595	7.717	9.073	7.584	8.875	10.434	8.950	10.472	12.313	7.786	9.111	10.711	7.163	8.382	9.854	7.319	8.564	10.069
Aluminium Nanoparticles	5.302	6.414	6.976	5.991	7.248	7.883	6.890	8.335	9.066	8.130	9.835	10.698	7.074	8.557	9.307	6.507	7.872	8.564	6.649	8.044	8.749
Silicon carbide	3.569	4.043	4.651	4.033	4.569	5.256	4.638	5.255	6.044	5.473	6.201	7.132	4.762	5.395	6.205	4.381	4.963	5.708	4.476	5.071	5.832
Silicon dioxide	1.807	2.858	3.155	2.042	3.230	3.565	2.348	3.714	4.100	2.771	4.382	4.838	2.410	3.813	4.209	2.217	3.508	3.872	2.266	3.584	3.956
Titanium dioxide	4.296	5.065	5.834	4.854	5.724	6.595	5.582	6.582	7.585	6.586	7.767	8.950	5.731	6.757	7.786	5.272	6.217	7.163	5.387	6.352	7.319
Zinc oxide	1.536	2.429	2.681	1.732	2.745	3.030	1.996	3.157	3.485	2.355	3.725	4.112	2.049	3.241	3.577	1.885	2.982	3.291	1.925	3.046	3.363
Control	0.755	0.755	0.755	0.854	0.854	0.854	0.982	0.982	0.982	1.159	1.159	1.159	1.008	1.008	1.008	0.927	0.927	0.927	0.948	0.948	0.948
Mean	3.300	4.056	4.583	3.729	4.584	5.179	4.289	5.271	5.956	5.061	6.220	7.029	4.403	5.412	6.115	4.050	4.979	5.626	4.139	5.087	5.748
CD at 1 %		a=0.056 b=0.036 c=0.052 a x b= 0.097 b x c=0.138 c x a =0.090 a x b x c=0.239																			
CV											2.978										

a = Treatments b =Concentration c = Hours

Table 4: Effect of Nano	particles on leaf infection	of chilli by Alt	ternaria tenuissima (Kunze ex Pers.) Wiltshire.
		2	· · · · · · · · · · · · · · · · · · ·	,

Treatments	N	No. of spots/le	eaf	No. of infected leaves/plant					
Treatments	25 μgml ⁻¹	50 µgml ⁻¹	100 µgml ⁻¹	25 μgml ⁻¹	50 µgml ⁻¹	100 µgml ⁻¹			
Silver Nanoparticles	5.56	4.89	3.11	3.56	3.00	2.00			
Aluminium Nanoparticles	6.33	5.67	4.11	4.00	3.67	2.56			
Silicon carbide	9.22	8.56	6.22	6.00	5.56	4.00			
Silicon dioxide	10.89	10.11	7.67	7.11	6.56	4.89			
Titanium dioxide	8.00	7.33	5.33	5.11	4.67	3.33			
Zinc oxide	12.22	10.67	8.44	8.00	6.89	5.44			
Control	14.11	14.11	14.11	9.22	9.22	9.22			
CD at 1 %	a=0.6	55 b = 0.42 ax	b=1.13	a=0.43 b= 0.28 axb=0.75					
CV		10.95		11.31					

Table 5: Effect of Nanoparticles on severity of Alternaria tenuissima (Kunze ex Pers.) Wiltshire.

	PD	I at 15 D.	AI*	PDI at	15 DAI*	30 DAI	PDI at 15 D	AI*45 DAI	PDI at 15 DAI*60 DAI			
Treatments	25	50	100	25	50	100	25	50	100	25	50	100
	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹	µgml ⁻¹
Silver Nanoparticles	5.33	4.00	0.00	9.33	4.00	0.00	13.33	8.00	4.00	17.33	12.00	8.00
Aluminium Nanoparticles	9.33	4.00	4.00	13.33	8.00	4.00	17.33	12.00	8.00	21.33	16.00	12.00
Silicon carbide	9.33	8.00	8.00	17.33	12.00	12.00	25.33	20.00	16.00	33.33	28.00	24.00
Silicon dioxide	13.33	12.00	8.00	21.33	16.00	12.00	29.33	24.00	20.00	36.00	32.00	28.00
Titanium dioxide	9.33	8.00	4.00	17.33	12.00	8.00	21.33	16.00	12.00	29.33	24.00	20.00
Zinc oxide	17.33	16.00	12.00	25.33	20.00	12.00	33.33	28.00	24.00	41.33	36.00	32.00
Control	21.33	21.33	21.33	29.33	29.33	29.33	36.00	36.00	36.00	60.00	60.00	60.00
CD at 1 %	a= 1.43	b=0.94 a	xb=2.49	a=1.43 b=0.94 axb=2.49			a=1.17 b=0.	a=1.17 b=0.76 axb=2.03				
CV		14.70			10.18		5.	4.10				

DAI* = Days after inoculation a= Treatments b= Concentrations

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