Supporting Information

Dual role of copper nanoparticles in Bacterial Leaf Blight infected rice: a therapeutic and metabolic approach.

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1. EXPERIMENTAL SECTION

1.1. Materials. All the chemicals (organic and inorganic) and dyes are purchased from sigma-aldrich and merck millipore. Taichung Native 1 (TN1) seeds are obtained from pure strains from Indian Statistical Institute, Giridih, Jharkhand, India and Rice Research Institute, Chinsurah, Hooghly, West Bengal, India. Pure *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) culture had been obtained from Rice Research Institute, Chinsurah, Hooghly, West Bengal, India.

1.2. Growth of rice seedlings and maintaining *Xoo* culture. TN1 variety of rice was used for the study. TN1 was chosen as it has one of the highest susceptibility towards *Xoo*.[1] The rice was grown in a plant growth chamber under laboratory conditions maintaining a temperature of 30 ˚C and a humidity of 80 %. The seeds were surface sterilized with 4 % NaOCl solution and then grown in sterilized and autoclaved soil in small paper cups. They were grown for 10 days before inoculating them with the bacteria and treating them with CuNP. *Xoo* culture was maintained by constant passaging in 15-20 days at 28 ˚C.

1.3. Interaction with rice plants.

1.3.1. Analysis of surface morphology of rice seedlings treated with CuNP. The surface morphology analysis of the rice seedlings was done with scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDAX). Briefly, the rice plants were grown in triplicates with five surface sterilized rice seeds in each cup for all treatments (control and CuNP treated). The growth conditions were maintained as explained in the previous section for 10 days and the plants were then treated with CuNP (two concentration of CuNP was chosen, 4 mg/L and 5 mg/L, which showed

better *in-vivo* antibacterial activity as will be discussed in the results section). Control was treated with ddH_2O. Each concentration was treated in a set of three cups containing rice seedlings and one set was kept as control. The treated seedlings were kept in a growth chamber under observation for 72 h and were exposed to CuNP treatment every day. The different solutions of CuNP were sprayed to the seedlings by foliar spray using a sprayer. The soil was covered with plastic before spraying to avoid loss of CuNP to the soil. In a single spray, approximately 700 µL of CuNP solution of each concentration was exposed to the seedlings. Control was maintained with ddH_2O. In a span of 72 h, the spray was done 6 times (the number of sprays and the span of exposure of CuNP was standardized before the final experimental setup). Small pieces of treated and control leaves and roots (5 mm) were then cut for the analysis and incubated in FAA fixative for 24 h at 4 ˚C [FAA fixative – 50 % ethanol, 5 % (v/v) acetic acid, 3.7 % (v/v) formaldehyde]. The fixative was then removed and the samples were dipped in 1 % osmium tetroxide solution (v/v) for 24 h at 4 ˚C. The samples were then washed in 25 mM sodium phosphate solution (pH 7.2) three times. The tissues were next dehydrated in 100 % methanol for 15 min followed by dehydration in series of ethanol solutions (30 %, 50 %, 70 %, 95 %, and twice in 100 % ethanol) for 20 min each at room temperature. The samples were finally stored in 70 % ethanol until critical point drying. After drying the samples were analyzed in SEM.[2]

1.3.2. Uptake of CuNP by rice seedlings. The uptake of CuNP by the rice seedlings was studied by TEM analysis. The rice seedlings were grown and treated in the same way as SEM. Small pieces of the treated (same concentration of CuNP was used as in SEM) and control leaves and root samples (2 mm) were cut and fixed in fixative for 6 h

at 4 ˚C. The fixative is a mixture of 2 % paraformaldehyde and 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4).[3] After fixation the tissues were washed 3 times in phosphate buffer each for 1 h at 4 ˚C. The samples were finally stored in a mixture of 4 % paraformaldehyde and phosphate buffer (1:1) until analysis.

1.3.3. Atomic Absorption Spectroscopy (AAS) and zeta potential analysis. AAS analysis was done to quantify the number of copper ions released from CuNP in aqueous solutions. Briefly, 5 mL (5 mg/L) of CuNP and $CuCl_2$ solutions respectively were taken in a dialysis bag (M.W. – 12-14 kDa). The bags were placed in 100 mL buffer having a pH of 6.8 (pH equivalent to leaf cell sap) taken in a 250 mL beaker. The experiment was kept under stirring conditions for 72 h at room temperature. 1 mL of the aqueous solutions, in which bags were placed (CuNP and $CuCl_2$), was taken out at an interval of 12 h (the initial sample was collected after 6 h with a further interval of 12 h).[4] The buffer was replaced every 12 h. AAS analysis of the collected samples was then done. The surface charge of CuNP was measured by zeta potential analysis. For this, the concentration of CuNP was taken the same as in AAS. The particle was well dispersed by sonication before the zeta potential study.[5]

1.3.4. Determine the role of metal transport proteins in intracellular copper transport. Copper is transported inside the cell by metal transporters called chaperones. Once inside the cell, copper reaches to various enzymes like superoxide dismutase (SOD) and cytochrome C oxidase (COX) where it acts as a co-factor. To determine the efficiency of transporters in carrying copper, the increase/decrease in the activity of SOD and COX was determined in treated seedlings. The seedlings were grown and treated as explained in the above sections. After 10 days the seedlings were

treated with 2 mg/L, 3 mg/L, 4 mg/L, and 5 mg/L concentrations of CuNP. Each concentration was treated in a batch of three cups of seedlings (each cup initially containing five surface sterilized seeds) and one batch was kept as control. The treatment was done for 72 h with the seedlings being exposed to CuNP every day as mentioned previously. The solutions were sprayed onto the seedlings using a sprayer. The control was maintained with ddH_2O.

For estimating SOD activity, an enzyme extract was prepared by homogenizing 0.1 g of plant tissue from control and treated samples in 3 mL of 50 mM sodium phosphate buffer (pH 7) containing 1 % (w/v) of polyvinylpyrrolidone. The homogenate was further centrifuged at 3000 rpm for 15 min at 4 ˚C and the supernatant was collected. SOD activity was quantified by measuring its efficiency to inhibit the photochemical reduction of nitroblue tetrazolium salt (NBT). The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8), 26 mM methionine, 20 µM riboflavin, 750 µM NBT, 1 µM EDTA and enzyme solution in the ratio of 2:2:1:1:0.5:2 respectively. The final volume was made to 2 mL by adding phosphate buffer. The reaction mixture was kept under sunlight for 10 min beyond which the absorbance was measured at 560 nm.[6]

For determining COX activity, 0.1 g of plant tissue from both control and treated samples was homogenized in a media containing 0.2 M sucrose and 0.05 M phosphate buffer (pH 7) at 0 ˚C. The resulting homogenate was then centrifuged at 3000 rpm for 15 min at 4 ˚C. The supernatant was again centrifuged for 20 min at 3000 rpm at 4 ˚C. The pellet containing mitochondria was resuspended in the above media and was centrifuged at 3000 rpm for 20 min at 4 ˚C. The pellet was finally suspended in 3 mL of the medium and was stored at 0 ˚C until further use. COX activity was determined in a

reaction mixture containing 90 mM phosphate buffer (pH 7), 30 µg (50 µM) cytochrome C, reduced with sodium hydrosulfite, and 15 µg of mitochondrial protein. The final volume was adjusted to 2 mL with phosphate buffer. The activity of COX was determined from the rate of oxidation of reduced cytochrome C measured at 550 nm.[7] The experiments were repeated twice.

1.4. Oxidative stress produced in rice seedlings on treatment with CuNP.

1.4.1. Proline, phenol, and peroxide assays. Proline and phenol are known as stress indicators in plants.[8, 9] To quantify their amount in CuNP treated rice, the seedlings were grown and treated as explained in the above sections. The concentrations used for treatment were 2 mg/L, 3 mg/L, 4 mg/L and 5 mg/L of CuNP. The control was maintained with ddH_2O. Proline estimation was done following the protocol of Bates.[10] Briefly, 0.1 g of plant tissue from each of the treated samples and control was homogenized with 3 mL sulphosalicylic acid and then centrifuged at 5000 rpm for 30 min at 4 ˚C. The soup was collected in test tubes and the volume of all the tubes was adjusted with sterilized ddH_2O. Further, an equal ratio of glacial acetic acid and ninhydrin was added to them. The tubes were then heated in a boiling water bath for an hour. After cooling to normal temperature the mixture was extracted with 10 mL toluene in a separating funnel with discarding the aqueous layer. The optical density (O.D.) of the organic layer was recorded at 520 nm with toluene as blank. The quantity of proline present in tissues was estimated against a standard curve of known concentrations of proline.

Phenol was estimated following the protocol of Malik and Singh.[11] Briefly, 0.1 g of plant tissue was extracted in 0.3 N HCl (3 mL) in methanol and centrifuged at 10,000 rpm for 20 min at 4 ˚C. The soup was collected and evaporated to dryness. The residue was dissolved in ddH_2O and 1 mL of each solution (treated and control) was taken in test tubes. The volume in each tube was adjusted with ddH_2O. Thereafter, folin's phenol reagent (10 % in water) was added to each tube and shaken well. After allowing standing for 5 min, 35 % sodium carbonate solution was added (in equal ratio to folin reagent) and test tubes were kept for 1 h at room temperature. The absorbance was recorded at 630 nm. The amount of phenol in tissues was estimated against a standard curve with known concentrations of gallic acid.

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) produced in live cells. Its production may increase on exposure to external stress and acts as an important signal of stress.[12] To quantify the amount of peroxide produced in tissues the rice plants were grown and treated the same way as in phenol and proline. The estimation was done following the protocol of Velikova et.al.[13] Briefly, 0.1 g of plant tissues (treated and control) were homogenized in an ice bath with 3 mL (0.1 %) w/v trichloroacetic acid (TCA). The homogenate was then centrifuged at 12,000 rpm for 15 min at 4 ˚C. 1 mL of the supernatant was taken in respective test tubes. To it, 1 mL of potassium phosphate buffer (10 mM, pH 7) and 2 mL KI solution (1 M) were added respectively. The solutions were allowed to stand for 2 min following which the absorbance was read at 390 nm. The amount of peroxide in tissues was estimated against a standard curve with known concentrations of H_2O_2. All the assays were repeated twice in triplicates.

1.4.2. ROS determination. The ROS produced in response to CuNP treatment in rice seedlings was determined by *in-vivo* ROS and antioxidants estimation assays. The ROS was evaluated using dichloro-dihydro-fluorescein diacetate (DCFH-DA) and antioxidants with 2,2-diphenyl-1-picrylhydrazyl (DPPH). The rice plants were grown and treated in the same way as that of phenol and proline assays. For ROS both quantitative and qualitative estimation was done. Quantitative was done with fluorescence spectroscopy and qualitative was done with confocal microscopy. After the treatment, small pieces of rice leaves of both treated and control (approximately 1 cm) were cut and dipped immediately into DCFH-DA (10 µM) solution. The samples were then vacuum infiltrated for 5 min at 60 kPa pressure and further incubated at room temperature for 10 min in the dark. The samples were washed after incubation three times in autoclaved ddH_2O. For spectroscopic studies, the samples were then homogenized in phosphate buffer (pH 7.4) and subsequently pellet out in a cold centrifuge at 5000 rpm for 20 min at 4 ˚C. The fluorescence of the soup was read at an excitation and emission of 488 nm and 522 nm respectively. Fluorescence of DCFH-DA was taken as the negative control to nullify its fluorescence from the samples. The quantitative analysis was repeated twice in triplicates. For the qualitative assay, after washing the samples were dipped in 20 % glycerol. The samples were then placed on respective slides (treated and control) and a thin coverslip was fixed onto each of them with nail enamel. The samples were next observed under a confocal microscope with excitation and emission at 488 nm and 522 nm wavelengths respectively.[14] The intensity of fluorescence from the samples was analyzed by Image J (n = 3).

For estimation of antioxidants in response to ROS produced in the tissues, 0.1 gm of plant tissue (treated and control) was extracted in 3 mL of chilled methanol. The samples were further centrifuged at 5000 rpm for 20 min at 4 ˚C. 500 µl of the soup was then mixed with 300 µl of DPPH solution (2 mM in methanol) for each sample. The samples were then kept for 30 min in dark at room temperature. Following which absorbance was taken at 517 nm against methanol as blank. DPPH in methanol was taken as the negative control.[15] The experiment was repeated twice in triplicates. Percentage DPPH scavenging by antioxidants was calculated from the formula

$$\text{DPPH Scavenging} = \left(\frac{\text{O.D.}_C - \text{O.D.}_S}{\text{O.D.}_C}\right) * 100 \quad \ldots\ldots. (1)$$

where, O.D._c = O.D. of control and O.D._s = O.D. of treated sample.

1.5. Effect on plant metabolism.

1.5.1. Root length, shoot length, and AAS analysis of copper ions. For measuring the root and shoot length of the rice seedlings, rice was grown and treated in the same way as done for the oxidative stress analysis. Three rice seedlings (each from the replicates of treated and control) were then randomly and carefully plucked. The consecutive root and shoot length was measured using a calibrated scale and expressed in cm. The experiments were repeated thrice in triplicates.[16] For AAS analysis 0.1 gm of shoot and root (from both control and treated seedlings) were weighed. They were then finely surface sterilized with 1 % HCl solution and washed at least 3 times with ddH_2O. The tissues were then kept for drying overnight at 70 ˚C. They were then finely homogenized in cold autoclaved ddH_2O. 1 mL of each homogenized

solution was collected and centrifuged at 5000 r.p.m for 15 min at 4 ˚C. The supernatant was diluted 100 times and subjected to AAS analysis.[17]

To compare the concentration of intracellular copper ions in CuNP treated plants with commercial copper formulations against *Xoo,* Bordeaux mixture (1g/L) was used. The plants were grown, treated and AAS analysis was done as explained above.

1.5.2. Lipid, carbohydrate, and amino acid content. For estimating the lipid, carbohydrate, and amino acid content in the rice seedlings, the rice seeds were grown and treated in the same way as in all other assays. The total lipid content was measured following the protocol of Bligh and Dyer.[18] 0.1 g of fresh plant material was taken in 50 mL centrifuge tubes (control and treated). 2 mL of autoclaved ddH_2O, 2 mL of methanol, and 1 mL of chloroform were added to each of the tubes respectively and was vortexed thoroughly to form an even paste. Further 1 mL of ddH_2O and 1 mL of chloroform were added to the tubes and vortexed again. The tubes were then centrifuged for 10 min at 4500 rpm resulting in the formation of a two-phase extract of the solution. The above layer being of water/methanol and the below layer of chloroform with a protein layer in between. The chloroform was pipette out and the above layer was extracted three more times with chloroform (1 mL). The chloroform layer was separated by centrifugation. The tubes were then subjected to evaporation at 40 ˚C to remove the chloroform. The fraction left in the tubes is the total lipid in the samples and was dried on a stove at 70 ˚C for 24 h. The experiment was repeated twice in triplicates. The lipids are determined gravimetrically by weighing the centrifuge tubes by the following formula.

$$\text{Lipids} = \left(\frac{W_{c+1} - W_c}{W_{sample}}\right) \ldots\ldots (2)$$

where, W_c = weight of empty centrifuge tube.

W_{c+1} = weight of tube with lipids.

W_{sample} = weight of the sample.

Total carbohydrate estimation was done following the protocol of Krishnaraj et.al.[19] 0.1 g of rice seedlings (control and treated) was ground in 3 mL of chilled phosphate buffer (50 mM). They were then centrifuged at 10,000 rpm for 10 min at 4 ˚C. 1 mL of the supernatant was collected from all the samples in separate test tubes and diluted to 2 mL with extraction buffer. 1 mL phenol (5 %, w/v) and 5 mL concentrated H_2SO_4 were then rapidly added to the tubes and mixed thoroughly. The tubes were further incubated for 10 min at 37 ˚C. The absorbance was measured at 490 nm. Blank was the reagent excluding the plant samples. The experiment was repeated twice in triplicates. The total amount of carbohydrates in the samples was estimated using a standard curve of known concentrations of D-glucose.

Total amino acid content was measured following the protocol of Lee and Takahashi.[20] 0.1 g of rice seedlings (control and treated) was extracted in 3 mL chilled ethanol (80 % solution) and centrifuged at 8000 rpm for 15 min at 4 ˚C. The supernatant was collected and evaporated to dryness to remove the alcohol. 500 µL of autoclaved ddH_2O was added to the samples and gently vortexed to mix. 100 µL of 4 M acetate buffer (pH 5.1) and 300 µL of ninhydrin reagent were then added to the samples. The mixture was vortexed thoroughly and heated at 100 ˚C for 15 min followed by cooling to room temperature. 500 µL of the sample solution was transferred into fresh 2 mL eppendorf tubes and diluted with an equal volume of 50 % ethanol. After a quick vortex, the absorbance was measured at 570 nm. The experiment was repeated twice in triplicates.

The total amount of amino acid in the samples was estimated using a standard curve of known concentrations of l-leucine.

1.5.3. Photosynthetic pigments, photosystem 2, and plastocyanin analysis. The rice seeds were grown and treated in the same way as in all other assays. The chlorophyll and carotenoid content were measured following the formula of Arnon and Davis respectively.[21, 22] 0.1 g of the fresh leaves of both control and treated seedlings were collected and homogenized in 3 mL of chilled acetone (80 % solution). The samples were thoroughly ground to extract the maximum of the pigments. They were then centrifuged at 10,000 rpm for 10 min at 4 ˚C. The absorbance of the soup was taken. The experiment was repeated twice in triplicates. The amount of photosynthetic pigments in the samples was calculated as follows.

$$\text{Chl a} = [12.7\ (O.D._{663}) - 2.69\ (O.D._{645})] * [\frac{V}{1000}] * W \quad \ldots\ldots (3)$$

$$\text{Chl b} = [22.9\ (O.D._{645}) - 4.68\ (O.D._{663})] * [\frac{V}{1000}] * W \quad \ldots\ldots (4)$$

$$\text{Carotenoids} = [\frac{A_{car}}{E_{m\ 100\%}}] * 100;\ A_{car} = [(O.D._{480}) + 0.114\ (O.D._{663}) - 0.638\ (O.D._{645})] \ldots (5)$$

where, V = volume of the sample.

W = weight of the fresh tissue taken for extraction.

$E_{m100\%}$ = 2500.

For photosystem 2 (PS2) analysis first, the thylakoids were isolated from seedlings (control and treated) in SPC buffer (0.5 M sucrose, 0.5 M potassium phosphate, and 0.3 M sodium citrate, pH 7.0). 0.1 g of fresh leaves from the samples were thoroughly homogenized in 3 mL of chilled SPC buffer. The samples were then centrifuged at 5000 rpm for 15 min at 4 ˚C. The supernatant was again centrifuged at 5000 rpm for 15 min at 4 ˚C and the pellet containing the thylakoids was collected. The pellets were then

kept for drying until the buffer got evaporated. 200 µg of pellet from each sample was taken and dissolved in 2 mL of SPC buffer and thoroughly mixed. 200 µL of the thylakoid suspension was mixed with 1 mL of standard assay medium (50 mM Hepes (pH 7.5), 0.3 M sucrose, 10 mM NaCl, 2 mM $MgCl_2$, 20 µM 2,6-dichlorophenolindophenol (DCPIP)). The initial absorbance of the solution was measured at 610 nm. After 10 min of irradiance under bright sunlight, the absorbance was again measured at 610 nm. The difference in absorbance gives the amount of DCPIP reduced as a measure of PS2 activity. The experiment was repeated twice in triplicates.[23]

For plastocyanin estimation, 0.1 g of fresh leaves from both control and treated samples were homogenized in 3 mL of chilled 0.2 M Tris-HCl buffer (pH 7.5). The homogenate was then collected and 2 % Triton X-100 was added and stirred for 3 h. The samples were then centrifuged at 10,000 rpm for 15 min at 4 ˚C. Following centrifugation, the supernatant was first purified with 25 % ammonium sulfate solution. The samples were again centrifuged as before and further purified with 55 % ammonium sulfate solution. The samples were further centrifuged and the supernatant was used for the determination of plastocyanin by measuring the absorbance at 597 nm (absorbance wavelength for a 1-copper chromatophore of plastocyanin). The experiment was repeated twice in triplicates.[24]

1.5.4. Hill reaction in the chloroplast. For studying the effect of CuNP on photosynthesis, the effect of the NPs on the light-driven electron transfer cycle or the hill reaction was inquired using the protocol of Vishniac.[25] For this the rice seeds were grown and treated the same way as in previous experiments. 0.1 gm of leaves (control

and treated) were extracted in 3 mL of chilled sucrose phosphate buffer (0.4 M sucrose in 0.05 M phosphate buffer, pH 6.2) and centrifuged at 3000 rpm for 15 min at 4 ˚C. The supernatant was collected and further centrifuged at 5000 rpm for 15 min at 4 ˚C. The pellet obtained contained the chloroplasts. 2 mL of sucrose phosphate buffer was added to the pellets and gently vortexed to evenly mix the solution. 1 mL of chloroplast suspension was then taken in each respective test tubes and to them, 2 mL of buffer and 1 mL of 0.03 % DCPIP were added. The initial absorbance of the solutions was immediately measured at 610 nm. The solutions were then kept for 10 min saturation under sunlight. The absorbance was then again measured at 610 nm. The difference in absorbance gives the amount of DCPIP reduced as a measure of Hill activity. The experiment was repeated twice in triplicates.

1.5.5. Photophosphorylation. The effect of CuNP on photophosphorylation (the phosphorylation of ADP to ATP in the presence of light) cycle of photosynthesis was next evaluated. Fresh leaves of the seedlings of both control and treated (0.1 gm) were collected and finely homogenized in chilled chloroplast isolation buffer (3 mL, pH 7.8) containing mannitol (330 mM), HEPES (30 mM), EDTA (2 mM), $MgCl_2$ (3 mM) and BSA (0.1 % w/v). The obtained slurry was next centrifuged at 5000 rpm for 15 min at 4 ˚C. The supernatant was collected and again centrifuged at 5000 rpm for 15 min at 4 ˚C. The chloroplast (pellet) was washed in autoclaved ddH_2O twice and resuspended in an extraction buffer for tracking the photosynthetic pathway. For estimation of ferricyanide reduction the extraction buffer used consisted of chloroplasts (µg/mL), sorbitol (86 mM), tricine (50 mM, pH 8.1), NaCl (50 mM), $MgCl_2$ (5 mM), K_2HPO_4 (2 mM), ADP (2 mM) and ferricyanide (1 mM). The total volume of chloroplast-buffer solution was made up to

2 mL. Immediately after 2 min of saturation irradiation of the solutions, TCA (2 %) was added. Chloroplasts were settled down by centrifugation at 5000 rpm for 15 min at 4 ˚C. The absorbance of the supernatant was measured at 420 nm. Negative control was taken as a solution of chloroplast-buffer kept in dark. The chloroplast-buffer solution for estimation of NADP reduction consists of the same components except for the addition of purified spinach ferredoxin (3 µM) and 0.66 mM NaDP instead of ferricyanide. After 2 min of saturation irradiance, the reaction was centrifuged. The absorbance of the supernatant was determined at 340 nm. Similar to ferricyanide reduction negative control was the buffer solution kept in dark. The experiment was repeated twice in triplicates.[26]

1.6. Effect on the metabolism of infected seedlings. Effect on pigment (chlorophyll and carotenoid) content, photosynthetic rate and photophosphorylation of CuNP treated *Xoo* infected seedlings were studied. The control was taken as infected seedlings without CuNP treatment. The seedlings were grown, infected, and treated in the same way as explained before. The assays were done following the protocol for measuring pigment content, hill reaction, and photophosphorylation as described in previous sections. The experiment was repeated twice in triplicates.

1.7. Toxicity study on rice seedlings with a higher concentration of CuNP. To study the toxicity of higher concentrations of CuNP on rice seedlings, seedlings were grown and treated with 5 mg/L, 10 mg/L, 50 mg/L, 100 mg/L, and 200 mg/L of CuNP solution in the same way as mentioned in the above experiments. The growths of the treated seedlings were then compared with that of control (no NP treatment). The experiment was repeated thrice.

2. Figures.

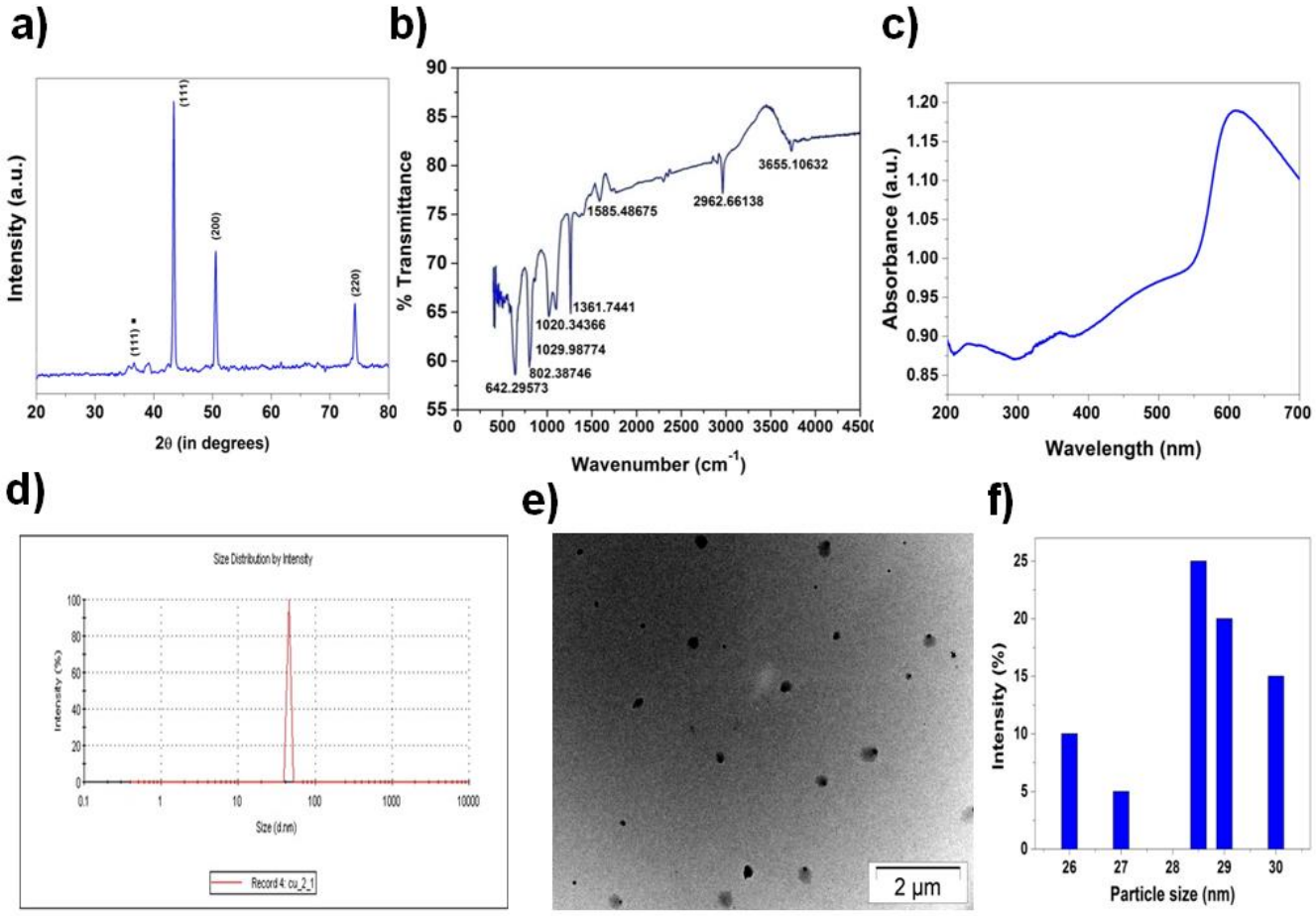

Figure S1. Characterization of CuNP. a) XRD diffractogram of CuNP. b) FTIR spectrum of CuNP. c) UV-Vis absorbance spectrum of CuNP. d) DLS distribution graph of CuNP. e) TEM micrograph of CuNP (Scale bar – 2 µm). f) size-distribution frequency of CuNP as calculated from TEM.

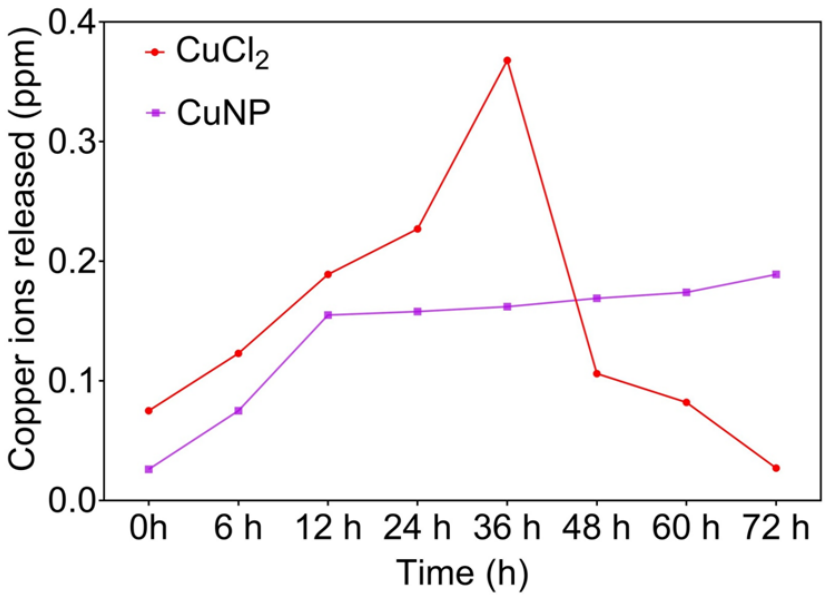

Figure S2. AAS analysis of release of copper ions from $CuCl_2$ and CuNP for 72 h.

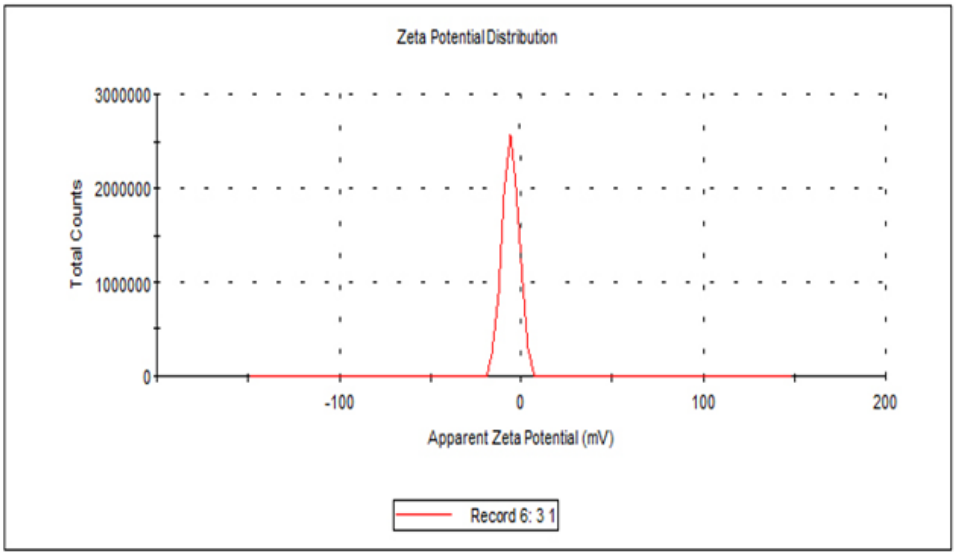

Figure S3. Zeta Potential analysis of the surface charge of CuNP.

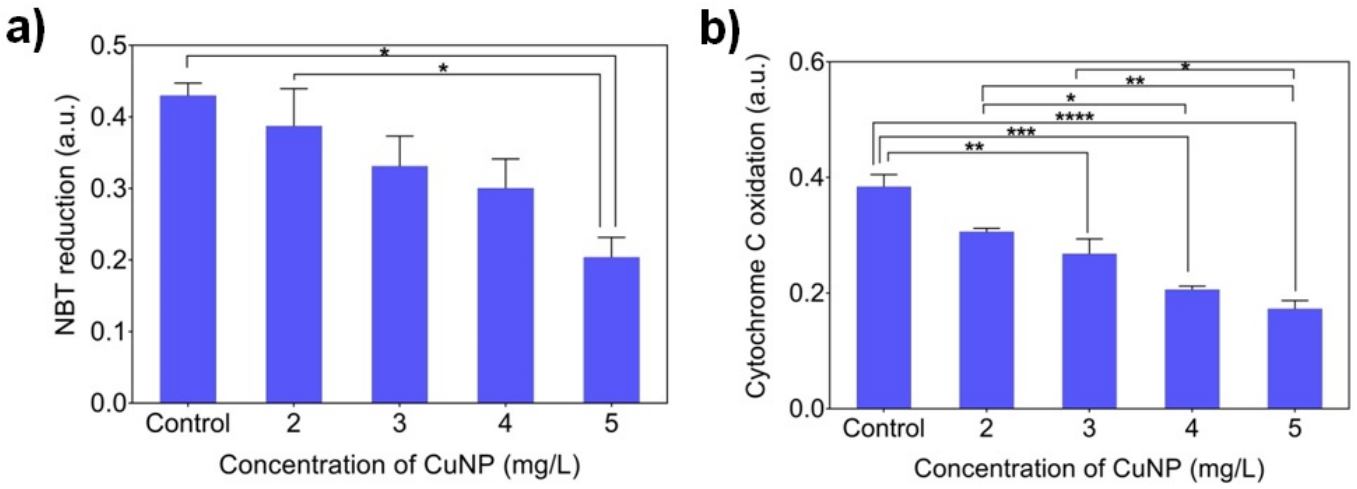

Figure S4. Estimation of the activity of SOD and COX. a) Increase in SOD activity in treated seedlings as compared to control estimated from the inhibition of the photochemical reduction of NBT to form formazan in the presence of SOD (F = 5.142). b) Increase in COX activity in treated seedlings as compared to control estimated by the increase in oxidation of reduced Cytochrome C (F = 24.76). The data are representative of two separate experiments.*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$, ****$p < 0.0001$. The error bars represent the standard error of the mean.

Figure S5. Shoots and root length of seedlings treated with different concentrations of CuNP (2 mg/L, 3 mg/L, 4 mg/L, and 5 mg/L) as compared to control (not treated with CuNP).

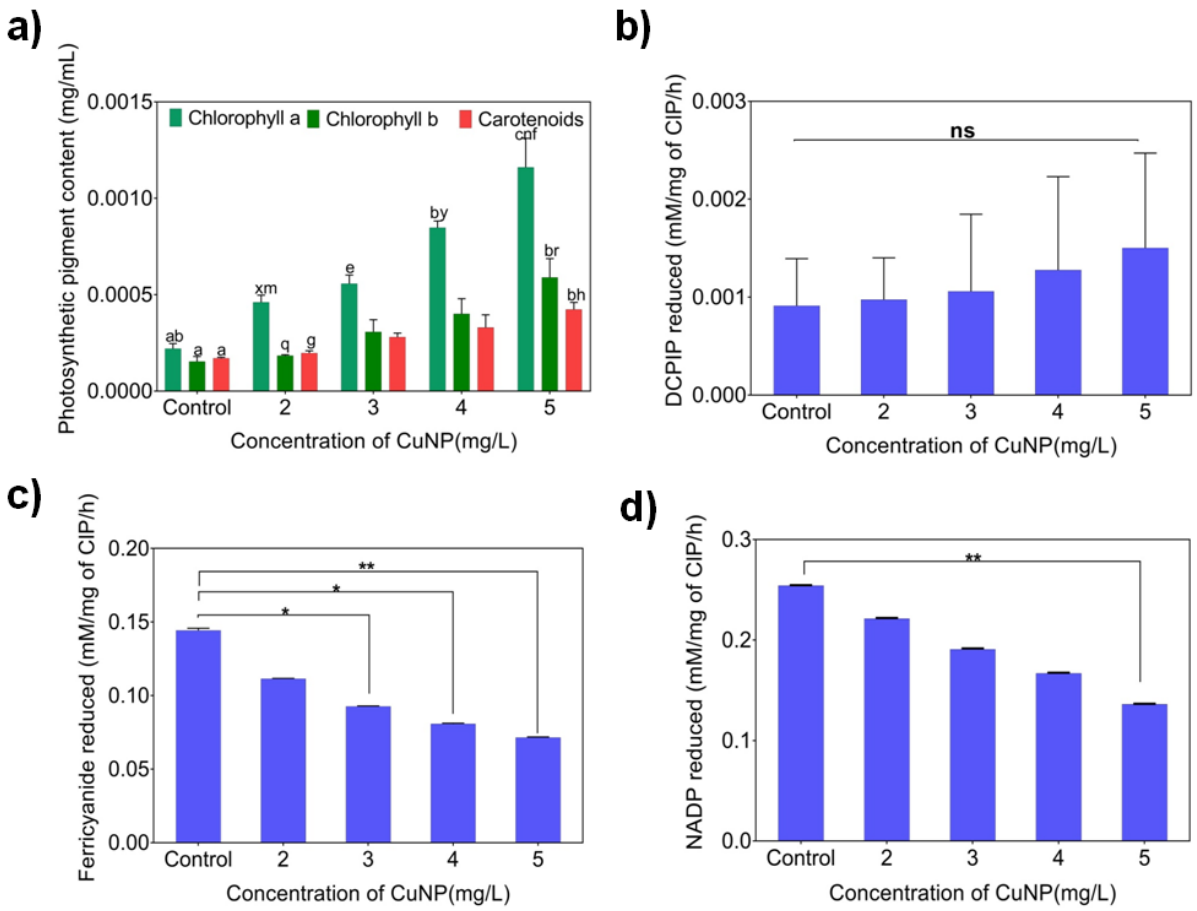

Figure S6. Effect of CuNP on the photosynthetic rate of bacteria infected rice. a) Photosynthetic pigment content (Chlorophyll a (F=23.32), chlorophyll b (F=7.644), and carotenoids (F=8.366)) of treated seedlings as compared to control. Consecutive letters represent significantly different values at $p<0.05$. b) Hill activity or overall photosynthetic rate of treated seedlings as compared to control (F=2.299). c-d) Photophosphorylation rate of rice on CuNP treatment. c) ATP production from ADP (F=7.547) and d) NADPH production from $NADP^+$ (F=5.957) in treated seedlings as compared to control. The data are representative of two separate experiments.*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$, ****$p < 0.0001$. The error bars represent the standard error of the mean.

Figure S7. Toxicity of higher concentrations of CuNP as compared to control. The seedlings start drying up and drooping with the increase in concentration of CuNP from 10 mg/L to 200 mg/L as compared to control and 5 mg/L concentration.

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