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Bio Efficacy of Biogenic Silver Nanoparticles against Rice Sheath Blight Causing Pathogen *Rhizoctonia solani* Kuhn

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Silver nano particles were synthesized using two potential isolates of *Trichoderma*, *Psudomonas fluorescens* and one isolate of pathogen *Rhizoctonia solani* cell free culture filtrates incubated at different days 0, 5, 10 and 15. The present investigation revealed that bionano preparations irrespective of the isolate source used, *i.e.*, *Trichoderma*, *P. fluorescens* or *R. solani* were found better in decreasing the sheath blight incidence *in vitro* though variation existed in terms of quantum of disease reduction. Bionano preparation from nutrient broth alone was also found effective in decreasing sheath blight severity compared to bioagents used alone or PDB. When the sensitivity of rice leaves from cv. NLR 34449 was assessed by dipping the leaf segments in 100% concentration of bionano silver (prepared using 170ppm silver nitrate), rice leaves turned yellow from the third day of incubation in detached leaf method *in vitro*.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops grown all over the world with a production of 550 million tonnes. In India, rice is grown over an area of 43.95 million hectares with production of 106.54 million tonnes and 2424 kg per hectare productivity. In Andhra Pradesh, rice is grown over an area of 4.51 million hectares with production and productivity of 13.03 million tonnes and 2891 kg per hectare, respectively (Government of India, Ministry of Agriculture, Department of Agriculture & Cooperation, Directorate of Economics & Statistics 2014). Sheath blight caused by *Rhizoctonia solani* is an important fungal disease of rice. The disease was first recorded from Japan (Miyake 1910). In India, the disease was first reported from Gurudaspur, Punjab (Paracer and Chahal 1963) and later it was reported from Uttar Pradesh (Kohli 1966). Currently, this disease is distributed in almost all the rice growing states.

A modest estimation of losses due to sheath blight disease alone in India has been up to 54.3%. Disease management is currently focused on extensive use of fungicides Such as carbendazim 50% WP, copper oxy chloride

50% WP, hexaconazole 5% SC and mancozeb 75%WP which has created concerns about environmental pollution, pathogen resistance and escalating costs. Although the pathogen is soil borne rice sheath blight develops into a major production limiting disease in an alarmingly short time. In fact, the disease has become the most important rice disease in the southern rice producing areas of the United States over the last 10 years. Yield losses as large as 50% occur in susceptible cultivars when all the leaf sheaths and leaf blades are infected (Roy 1993).

Nanotechnology is an emerging field in the area of interdisciplinary research especially in biological sciences. The advancement of nanotechnology mainly requires the development of reliable and ecofriendly approaches for the synthesis of nanomaterial over a range of biological composition, sizes, shapes and high monodispersity. Nanoparticles possess exceptional physical and chemical properties which lead to rapid commercialization. Nanoparticles are considered as fundamental molecular building blocks for nanotechnology. They are the pre-requisites for preparing many nanostructure materials and devices. Biosynthesis of nanoparticles is an attractive possibility of advancement of green nanotechnology which has potential to find numerous applications in biology - agriculture in particular.

Specific antimicrobial mechanisms of silver are still not completely understood though the toxic effect is postulated to be through inhibiting the expression of proteins associated with ATP production (Yamanaka *et al.,* 2005). Nano silver particles are used for control of various plant pathogens and compared with synthetic fungicides (Min *et al.,* 2009). (Jo *et al.,* 2009) studied the effect of various forms of silver nanoparticles on two plant pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea.*

Since agriculturally important microorganisms are environmental friendly and they are well known for their formation of extracellular enzymes and metabolites in very large amounts, utilizing these bioagents could be an excellent method for production of silver nanoparticles. However, mechanism of silver nano conversion using bioagents and role of silver nano particles in plant disease management with or without microbiological assistance is yet to be worked out.

Keeping the difficulties in use of fungicides and application of biocontrol agents in rice ecosystem, bionano silver preparations could offer a possible solution for the ever threatening sheath blight pathogen *R. solani*.

However, not much research was done on characterization and use of bionano silver preparations for their utility in plant protection in general and control of *R. solani* in particular.

Materials and Methods

Isolation and characterization of pathogen

Sheath blight susceptible variety of rice NLR-34449 (Nellore Mahsuri) was used in present studies. The test pathogen *R. solani* was isolated from sclerotial bodies attached to the diseased portion of rice plants. Antagonistic isolates of bacteria were isolated from healthy rhizosphere soil of rice field at ARS, Nellore. Isolates of *Trichoderma* and *P. fluorescence* available in the Dept. of pl. pathology were used in the present investigation.

Pathogen was isolated from sclerotial bodies by keeping on Petri plate containing sterilized PDA after sterilizing with 70% ethanol followed by three washing in sterile distilled water. Plates were incubated at 28 ± 2^0 C and observed periodically for growth of the fungus. The culture was purified by single

hyphal tip method and maintained on PDA by periodical transfer throughout the present investigation. The pathogen was identified based on its mycelial and sclerotial characters. Young colonies on the media have some shade of brown, right angle branching of mycelium, sclerotial bodies are irregular in shape and large in size (1.5-2 mm dia.), presence of dolipore septum etc., are morphological characteristics of *R. solani*.

Pathogenecity test

Then the pathogenecity of rice sheath bight pathogen was tested using germination test.

In germination test (Agarwal 1994) mycelial suspension of *R. solani* was prepared by using pestle and mortar from 3 day old culture. Ten rice seeds of cv. NLR 34449 were placed on moistened towel paper in a row. Then 2 µl of mycelial suspension was poured on to each seed. Without mycelial suspension on the seeds was taken as a control. The paper towels were then rolled in a proper way and kept in incubator at 28 ± 1°C. After one week, the paper towels were rolled back to unwrap the seeds carefully so that the fragile shoots are not destroyed. Seedlings that have shoots longer than 1½ inch (and at least one strong root) were considered as viable seeds. Observations on no. of seeds germinated, root length (cm), shoot length (cm) were taken, based on which shoot length: root length ratio and vigour index were calculated. The formula for vigour index is given below.

Vigour index = Germination percentage (shoot length + root length)

Testing the efficacy of biosilver nano particles against *R. solani*

Detached leaf technique was used to assess the efficacy of bio nano silver particles on rice sheath blight disease development.

Sheath blight susceptible variety cv NLR34449 (Nellore Mahsuri) was selected for the experiment. Then the seeds were sown in the pots under greenhouse conditions and grown for 45 days. After forty five days after transplantation in pots, the leaves were detached from the plants, they were cut in to 6cm small segments then they were surface sterilized with 75% ethyl alcohol after drying of the leaves they were washed 3-4 times with sterile distilled water and and dipped in different bio nano silver solvents separately for 10 min, and then placed in moist chamber. Moist chamber builded in the petriplates with the help of filter papers and sterilized distilled water. Two days old culture disc (2 mm) of *R. solani* was inoculated on each rice leaf segment. Uninoculated rice leaf segments served as control. The moistened cotton swabs were placed on the both sides of leaf segments. The moist chamber was made wet with sterile distilled water regularly. The entire process was carried out under aseptic conditions (Laminar air flow). Observations were recorded on lesion length.

In order to assess the phytotoxic effect of bio nano silver, detached leaf technique was used. The method followed was similar to the above procedure except that no pathogen was inoculated. The experiment continued till leaves showed senescence in untreated control.

Results and Discussion

Isolation and characterization of pathogen

Rice sheath blight pathogen *Rhizoctonia solani* was isolated from the diseased samples obtained from Agricultural Research Station, Nellore. The disease was characterized with symptoms such as necrotic lesions extending along the veins with brown margin and greyish center. The margins were irregular. Symptoms were seen on leaf sheaths

spreading from base to top extending up to boot leaf sheath and also up to panicles in severe cases. Sclerotial bodies whitish when young but later turned brown when old were observed on the affected portions of rice plant.

The culture obtained on PDA at 28±1^{o}C was light brown colour occupying 9 cm diameter Petriplate in 3 days of incubation. The pathogen produced dark brown, irregular, loose type of sclerotial bodies on PDA. Microscopic examination of the fungal culture revealed broad brown coloured hyphae with branching at right angles.

Proving of Pathogenicity by rolled paper towel method

In the present study pathogenicity of *R. solani* on rice leaves and germinating seedlings was assessed following rolled paper towel method. In this method individual rice seeds of cv. NLR 34449 were placed on rolled paper towel and inoculated with 2µl of *R. solani* mycelial suspension and incubated at 28±1^{O}C.

When observations were collected on per cent germination, root length and shoot length, the data indicated that seeds without *R. solani* inoculation had 90% germination, 13.70 cm of shoot length and 11.9 cm of root length with seedling vigour index of 2302 and shoot length : root length ratio (S:R ratio) of 1.15. In *R. solani* inoculated seeds, germination was 75% (16.7% reduction compared to check), shoot length was 8.35 cm (38.9% reduction), root length was 6.20 cm (48.2% reduction), vigour index was 1089 (52.7% reduction) and S:R ratio of 1.34. Increase in S: R ratio was due to higher reduction in root length than that of shoot length.

(Suryanarayana and Bhombe 1961) and (Oral *et al.*, 2011) were experimented with paper towel method for studying *R. solani* pathogenicity.

In the present investigation, *R. solani* was found to cause quantifiable disease *in vitro* in detached leaf method. However, in rolled paper towel method, only growth of rice seedlings was affected without any external manifestation of disease symptoms such as necrosis, yellowing *etc*. within the test period of ten days. Hence, detached leaf technique was chosen for further investigations on bionano silver. Among the two different inocula assessed, inoculation with *R. solani* mycelial disc was chosen as the method was swift in symptom development compared to sclerotial body inoculation. The results were in accordance with (Sharma and Thrimurthy 2004) who reported maximum sheath blight severity on rice leaf bits in detached leaf technique with seven day old mycelial propagules of *R. solani*.

Testing the efficacy of silver nano particles (100%) by detached leaf technique

The bioefficacy of synthesized silver nano particles was done using detached leaf technique in decreasing the disease severity due to rice sheath blight pathogen *R. solani*. Six cm cut pieces of rice leaves were dipped in 10 ml of 170 ppm silver bionano solution for 10 minutes and inoculated with 2mm mycelial disc of *R. solani* (48 hours old culture) at the center of each leaf segment placed in the moist chamber. The results obtained were presented in Plates 1 and Fig. 1a and 1b. For ease in expression, nano material synthesized by bioagents with different aged cultures was designated as <isolate> N-<age of culture filtrate>. For example PF-2 N-10d indicating PF-2 nano preparation form 10 day old culture filtrate.

On day-1, in pathogen uninoculated control there was no disease up to four days after inoculation. In pathogen inoculated control, a lesion length of 0.1 cm was observed which was significantly higher compared to treated

leaf bits. In treatments involving ET-1 and RT-4 spore suspension dip (0.05 cm), PF-2 and PF-5 cell suspension dip (0.05 cm) and PF-2 N-10d (0.05 cm) least disease severity was noticed compared with control. In ET-1 N-10d, a lesion length of 0.03 cm was observed. In all other treatments no disease symptoms were observed.

On day-2, pathogen control had the highest lesion length (0.8 cm) which differed significantly with all other treatments. In PDB silver nano alone (0.22 cm), ET-1 N- 5d (0.20 cm), RT-4 N-5d (0.20 cm), *R. solani* N-5d (0.20 cm), PF-5 cell suspension (0.12 cm), PF-2 N-10d (0.1 cm) and PF-2 cell suspension (0.1 cm), ET-1 and RT-4 spore suspension (0.05 cm) disease was observed. In all other treatments no disease was observed.

On day-3, all the treatments showed significantly less disease severity compared to pathogen inoculated control (5.23 cm). Significantly lowest lesion length (0.03 cm) was observed in ET-1 N-5d with the highest disease reduction of 99.5% followed by PF-2 N-15 d with 99.35% disease reduction compared with control (5.23 cm).

When comparisons were made with in different treatment types, variation existed with type of organism used and age of culture filtrate used for nano silver preparation. In case of *Trichoderma* ET-1 isolate *viz*., ET-1 N-5d (0.06 cm), ET-1 N-10d (1.52 cm), ET-1 N-15d (0.78 cm) had significantly lower lesion length compared with ET-1spore suspension (2.17 cm). In case of *Trichoderma* RT-4 also RT-4 spore suspension had the significantly highest lesion length (1.98 cm) compared with RT-4 N-5d (1.18 cm), RT-4 N-10d (1.14 cm), RT-4 N- 15d (0.20 cm).

In case of *P. fluorescens*, PF-2 cell suspension had significantly highest lesion length (2.54 cm) compared with PF-2 nano *viz*., PF-2 N-5d (0.08 cm), PF-2 N-10d (1.33 cm), PF-2 N-15d (0.01 cm). In case of PF-5, cell suspension had significantly higher lesion (3.73 cm) length compared with PF-5 N-5d (0.32 cm), PF-5 N-10d (0.6 cm), PF-5 N-15d (3.50 cm).

In case of pathogen *R. solani*, *R. solani* N-10d and *R. solani* N-15 d had significantly lower lesion length (0.78 cm and 0.66 cm respectively) compared to that with nanomaterial from biocontrol agents. However, *R. solani* N-5d had significantly higher lesion length compared with ET-1 spore suspension, RT-4 spore suspension, PF-2 cell suspension but had significantly lower lesion length than the PF-5 cell suspension.

PDB nano had significantly higher lesion length (2.86 cm) compared with ET-1 spore suspension, RT-4 spore suspension, PF-2 cell suspension but significantly lower lesion length than PF-5 cell suspension (3.73 cm).

NB nano (0.28 cm) had significantly lower lesion length than the biological control agents *viz*., *Trichoderma* (ET-1 and RT-4) spore suspension and *P. fluorescens* (PF-2 and PF-5) cell suspensions.

Carbendazim @0.1% also had significantly lower lesion length (0.96 cm) compared with bio control agents *viz*., *Trichoderma* (ET-1 and RT-4) spore suspension and *P. fluorescens* (PF-2 and PF-5) cell suspensions.

On day-4, least lesion length was observed in ET-1 N-5d (0.03 cm) with the highest disease reduction (99.50%), followed by PF-2 N – 15d with 99.33% disease reduction which were significantly differed with all other treatments. In control leaves, maximum disease (6 cm) was observed (entire leaf segment was diseased) which was significantly higher than that in treated leaf bits. All the remaining treatments also significantly differed except carbendazim (1.06 cm), ET-1 N-10d (1.04

cm), ET-1 N-15d (0.78 cm), *R. solani* N-15d (0.76 cm), *R.solani* N-10d (0.61 cm), PF-5 N-10d (0.60 cm), PF-5 N-5d (0.30 cm) and NB (0.28 cm).

When comparisons were made with in different treatment types, variation continued to exist with type of organism used and age of culture filtrate used for nano silver preparation as observed on day-3. In case of *Trichoderma* ET-1 spore suspension had significantly more lesion length (2.47 cm) compared with ET-1 N-5d (0.03 cm), ET-1 N-10d (1.04 cm), ET-1 N-15d (0.78 cm) respectively. In case of *Trichoderma* RT-4 also spore suspension had significantly more lesion length (2.23 cm) compared with RT-4 nano viz., RT-4 N-5d (1.45 cm), RT-4 N-10d (2.03 cm), RT-4 N- 15 d (0.22 cm).

Isolate PF-2 of *P.flourescens*, cell suspension had significantly more lesion length (2.71 cm) compared with PF-2 nano *viz*., PF-2 N-5d (0.13 cm), PF-2 N-10d (1.52 cm), PF-2 N-15d (0.04 cm).

In case of PF-5, cell suspension had significantly higher lesion length (5.4 cm) compared with PF-5 *viz*., PF-5 N-5d (0.30 cm), PF-5 N-10d (0.61 cm), PF-5 N-15d (5.02 cm).

In case of *R. solani*, *R. solani* N-5d had significantly higher lesion length (3.06 cm) compared with biocontrol agents, but *R. solani* N-10d (0.61 cm) and *R. solani* N 15d (0.76 cm) had lower lesion length compared with both the bio control agents used individually.

PDB nano had significantly higher lesion length (3.35 cm) compared with bio control agents except PF-5 cell suspension (4.71 cm).

NB nano had significantly lower lesion length (0.28 cm) compared with bio control agents spore suspension and cell suspension.

Overall performance of ET-1 N-5d (99.5% disease reduction) and PF-2 N -15d (99.33%disease reduction) was better than control, bio control agents and carbendazim on fourth day.

Thus the present investigation revealed that bionano preparations irrespective of the isolate used, *i.e.*, *Trichoderma*, *P. fluorescens* or *R. solani* were found better in decreasing the sheath blight incidence though variation existed in terms of quantum of disease reduction.

Bionano preparation from nutrient broth alone was also found effective in decreasing sheath blight severity compared to bioagents used alone or PDB. (Papaiah *et al.*, 2014) reported efficacy of silver bionano particles prepared using *Agaricus bisporus* against *R. solani*. (Elgorban *et al.*, 2015) reported inhibitory effect of silver nano on the growth of *R. solani in vitro.*

Sensitivity of rice leaves to silver nano particles (100%) in detached leaf technique

The 6 cm cut leaf pieces were dipped in the 100% (170 ppm) silver nano solution for 10 min and then placed in the moist chamber without inoculation of pathogen. Absolute control without inoculation of pathogen and without silver nano dipping was maintained for comparison.

On day-1 and day-2, there was no change in colour of leaves indicating nonphytotoxic effect on nano treated leaf bits. All the leaf bits looked green as in absolute control. By day-3, all the nano treated leaves were turned in to yellow colour in contrast to absolute control. This result indicated that the silver nano particles showed phytotoxicity on the leaves from third day. On fourth day in all the treatments including control the leaves turned yellow indicating natural senescence.

Fig.1a Effect of bionano silver on rice sheath blight development *in vitro*

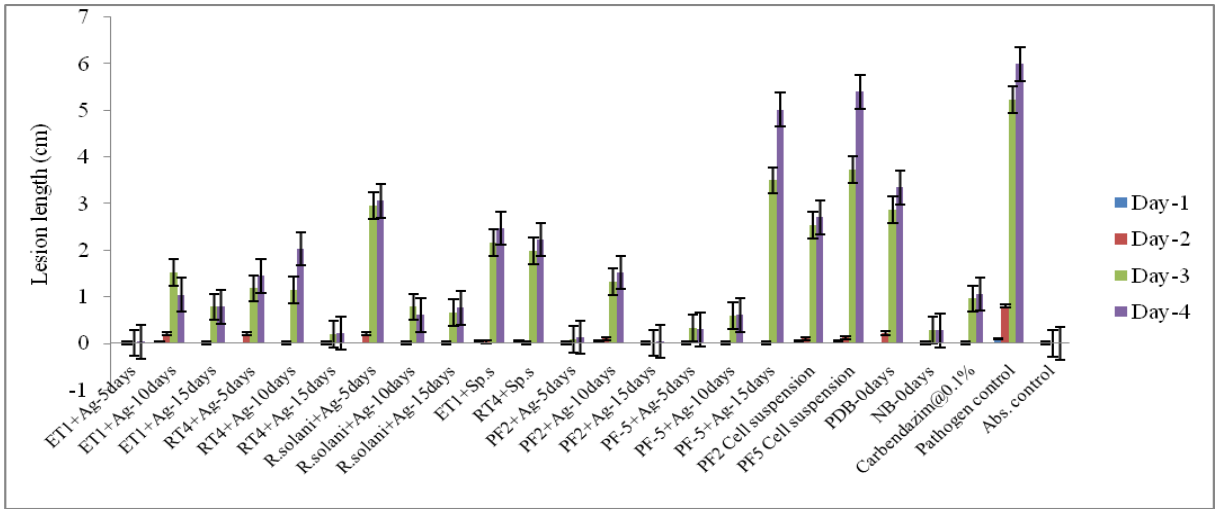

Fig.1b Reduction in rice sheath blight severity due to bionano silver *in vitro*

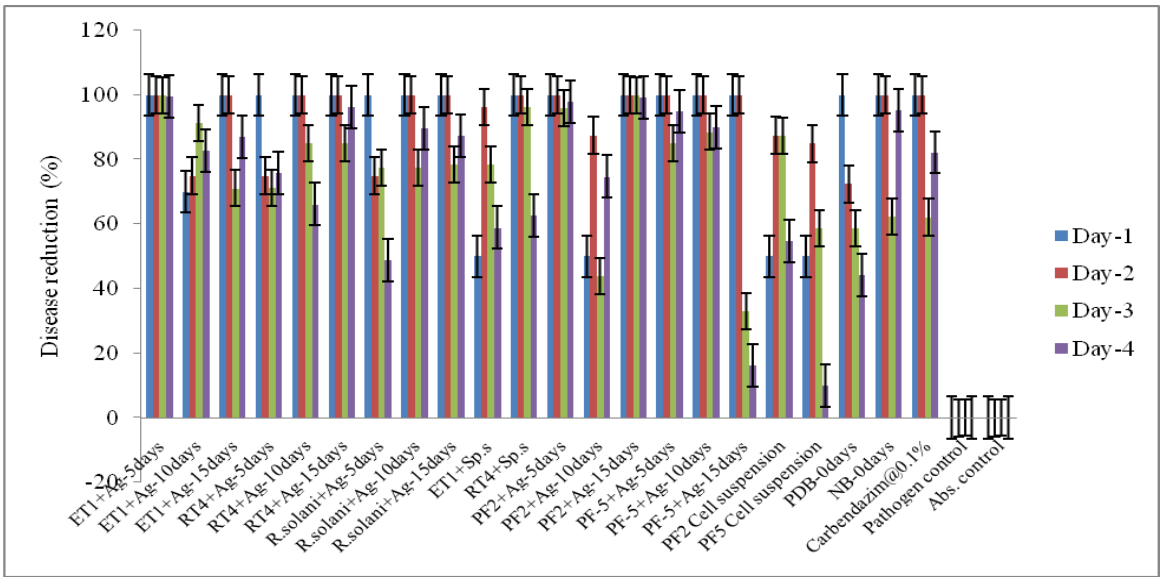

Plate.1 Testing of bioefficacy of biosilver nano particles

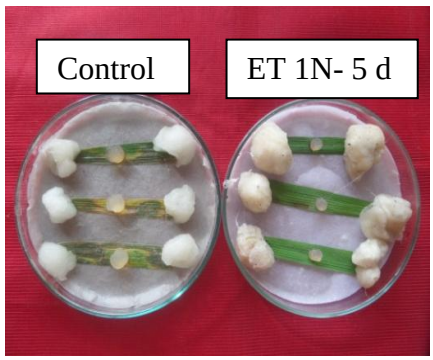

Table.1 Effect of different concentrations of silver bionano particles on rice sheath blight development in detached leaf technique – Lesion length in cm

S. No.	Treatments	Lesion length in cm.											
		Day 1			Day 2			Day 3			Day 4		
		Concentration in %			Concentration in %			Concentration in %			Concentration in %		
		100	50	10	100	50	10	100	50	10	100	50	10
1	ET1 N-5d	0.0 (1.00)^{d}	0.0 (1.00)^{b}	0.0 (1.00)^{e}	0.4^{h}	0.6^{g}	1.5^{g}	2.2^{g}	2.0^{f}	0.5^{h}	4.2^{e}	5.4^{c}	5.6^{b}
2	ET1 N-10d	0.2 (1.09)^{c}	0.0 (1.00)^{b}	0.0 (1.00)^{e}	1.8^{d}	0.1^{h}	2.1^{d}	5.4^{b}	2.8^{e}	4.9^{d}	5.5^{b}	4.5^{b}	6.0^{a}
3	ET1 N-15d	0.0 (1.00)^{d}	0.0 (1.00)^{b}	0.0 (1.00)^{e}	1.1^{f}	0.7^{f}	2.7^{c}	4.8^{d}	3.9^{d}	4.6^{ef}	6.0^{a}	6.0^{a}	6.0^{a}
4	PF-2 N-5d	0.0 (1.00)^{d}	0.0 (1.00)^{b}	0.2 (1.09)^{d}	2.4^{b}	1.7^{c}	1.7^{f}	5.0^{c}	1.7^{g}	5.0^{c}	6.0^{a}	6.0^{a}	6.0^{a}
5	PF-2 N-10d	0.5 (1.22)^{b}	0.0(1.00)^{b}	0.0 (1.00)^{e}	1.3^{e}	0.9^{e}	1.5^{g}	3.2^{f}	6.0^{a}	4.6^{ef}	6.0^{a}	6.0^{a}	5.6^{b}
6	PF-2 N-15d	0.0 (1.00)^{d}	0.0 (1.00)^{b}	0.7 (1.21)^{b}	0.1^{i}	0.1^{h}	4.3^{b}	4.1^{e}	4.2^{c}	5.4^{b}	4.1^{f}	4.2^{d}	6.0^{a}
7	PDB-Nano	0.0 (1.00)^{d}	0.0 (1.00)^{b}	0.0 (1.00)^{e}	2.1^{c}	1.3^{d}	0.1^{h}	3.3^{f}	4.5^{b}	2.1^{g}	5.3^{c}	4.5^{b}	5.6^{b}
8	NB-Nano	0.0 (1.00)^{d}	1.0 (1.41)^{a}	0.6 (1.28)^{bc}	0.5^{g}	2.1^{b}	2.0^{de}	1.9^{h}	6.0^{a}	4.5^{f}	5.0^{d}	6.0^{a}	5.1^{b}
9	Pathogen control	1.0 (1.41)^{a}	1.0 (1.41)^{a}	1.0 (1.41)^{a}	3.9^{a}	4.1^{a}	4.9^{a}	6.0^{a}	6.0^{a}	6.0^{a}	6.0^{a}	6.0^{a}	6.0^{a}
	C.D (P=0.01)	0.03	0.03	0.03	0.07	0.06	0.11	0.14	0.22	0.23	0.16	0.12	0.11
	SEm (±)	0.01	0.01	0.01	0.02	0.02	0.03	0.04	0.074	0.01	0.05	0.04	0.06
	C.V (%)	1.55	1.52	1.70	2.85	3.04	2.63	2.11	3.28	3.35	1.92	1.49	2.35

Note: Values in the parenthesis are squire root transformed values.
The figures with similar alphabet do not differ significantly.

Phytotoxic effect of nano particles on rice root cells was observed by (Harajyothi and Ahmed 2011; Seif *et al.*, 2011; Salama 2012; Aghajan *et al.*, 2013; Mirajani *et al.*, 2013 and Mazumdar 2014) reported that higher concentrations of nanoparticles were deleterious to plant growth causing phytotoxicity.

Testing of effect of different concentrations (100, 50, 10%) of bio-silver nano on rice sheath blight pathogen in detached leaf technique

In order to avoid Phytotoxic effect on rice leaves, decreased concentrations of silver nano preparations were tested on rice leaf pieces dipped in three different concentrations of nano *viz*., 100%, 50%, 10%, the pathogen was inoculated at the center of each leaf segment and incubated at 28±1^{o}C. The results were represented in Table 1.

On day-1, with 100% silver bio-nano, in most of the treatments there was no disease. Only in the treatments involving ET-1 N-10d (0.2 cm), PF-2 N-10d (0.5 cm) and control (1 cm) lesion length was observed. These treatments significantly differed with all other treatments.

On day-1, in 50% silver nano, in most of the treatments there was no disease.

Only in control and NB-nano lesion length of 1 cm was observed. Both were insignificant with each other but significantly differed with all other treatments.

On day-1, with 10% nano, PF-2 N-15d (0.7cm lesion length), NB-nano (0.6cm lesion length) and PF-2 N-5d (0.2cm) though showed disease development, it was significantly lower than the pathogen inoculated check (1.0cm).

On day-2, with 100% nano, lesion length was significantly lower than pathogen check (3.9cm). Least lesion length (0.1 cm) was observed in PF-2 N-15d with a disease

reduction of 97.43% compared with control which had maximum lesion length (3.9 cm) followed by ET-1 N-5d (89.74% disease reduction) and NB nano (87.17% disease reduction).

On day-2, with 50% nano, least lesion (0.1 cm) was observed in ET-1 N-10d and PF-2 N-15d with the highest disease reduction (97.56%) compared with control (4.1 cm lesion length) followed by ET-1 N-5d (0.6 cm lesion length) with disease reduction (85.36%). All the treatments significantly differed with one another.

On day-2, with 10% nano, least lesion length (0.1 cm) was observed in PDB nano with a disease reduction (97.95%) which significantly differed with all other treatments compared with control ((4.9 cm) followed by PF-2 N-10d and ET-1N-5d (1.5 cm).

On day-3, with 100% silver nano, least lesion length (1.9 cm) was observed in NB nano with disease reduction per cent of 68.33% compared with control (6 cm) followed by ET-1 N-5d (2.2 cm with 66.33 per cent disease reduction). All the treatments significantly differed with one another except PDB nano (3.3 cm lesion length) and PF-2 N 10d (3.2 cm lesion length).

On day-3, with 50% nano, minimum lesion length (1.7 cm) was observed in PF-2 N-5d followed by ET-1 N-5d (2.0 cm) compared with control (6 cm) which significantly differed with one another and also with other treatments.

On day-3, with 10% nano, least lesion length of 0.5 cm was observed in ET-1 N-5d with a disease reduction of 91.66% compared with control (6 cm) followed by PDB-nano (lesion length 2.1 cm with a disease reduction of 65%) which significantly differed with one another and with other treatments.

On day-4, with 100% nano, least lesion length (4.1 cm) was observed in PF-2 N-15d with a disease reduction of 31.66% followed by ET-1 N-5d (lesion length of 4.2 cm equivalent to a disease reduction of 30%) compared with control (6 cm lesion length). These were on par with one another and significantly lower than the other treatments including control.

On day-4, with 50% nano, least lesion length was observed in PF-2 N-15d (4.2 cm) with the highest disease reduction (30%) compared with control (6 cm lesion length) followed by ET-1 N-10d and PDB nano (4.5 cm lesion length with a disease reduction of 25%). These were on par with one another and significantly lower than the others.

On day-4, with 10% nano, minimum lesion length (5.1 cm) was observed in NB nano with the disease reduction of 15% compared with control (6cm lesion length) followed by ET-1 N–5d, PF-2 N-10d, PDB nano with a lesion length of 5.6 cm and 6.6% disease reduction. All the treatments were on par with one another. On fourth day overall better performance was observed in 100% and 50% nano than that with 10% nano.

(Sharon *et al.,* 2010; Kabir *et al.,* 2011; Rao and Savithramma 2011) reported variation in the efficacy of silver bionano concentrations against disease development in different crops and diseases.

Sensitivity of rice leaves to different concentrations (100, 50, 10%) of silver nano particles in detached leaf technique

The 6 cm cut pieces of rice leaves were dipped in the different concentrations of nano, *i.e*., 100%, 50%, 10% for 10 min. Then they were placed in the moist chamber. Without inoculation of pathogen and without silver nano dipping was maintained for comparison as absolute check.

On day-1 and Day-2, there was no colour change in the leaf bits. On third day all the 100% nano dipped leaves were turned in to yellow colour. However, 50% and 10% nano solution dipped leaves and control leaves remained green on third day also. On day-4, all the leaf bits turned yellow including absolute control that indicated natural senescence. Thus, decreased nano concentration at or below 50% found to be non-phytotoxic. (Razaq *et al.,* 2016) reported phytotoxic effect of silver nano on wheat at and above 100ppm concentration.

Testing of effect of silver nano particles on rice sheath blight in normal and boot leaves in detached leaf technique

In order assess the effect of bio silver nano preparations from *Trichoderma* and *Pseudomonas fluorescens* isolates, rice leaves (normal or boot leaves) were dipped in 10% of the nano preparation for 10 min and assessed for sheath blight development using detached leaf technique.

After 24 hours of inoculation, mean lesion length over both the types of leaves due to *R. solani* infection on rice leaf bits was maximum in pathogen inoculated untreated leaf bits (1.76cm) which was significantly higher than any other treatment indicating control of disease development due to nano preparations. Further ET-1N-5d and PF-5 N-5d had minimum most lesion length (0.05 cm) which differed significantly with PF-2N-5d (0.1 cm) and RT-4 N-5d (0.45). Mean lesion length over all the nano preparations was maximum in boot leaf (0.50cm) compared to normal leaf (0.46cm). When interaction effects were analysed lesion length was significantly higher on boot leaf compared to that on normal leaf in all the treatments. However, lesion length in untreated leaves was significantly higher (2.30 cm) than that on boot leaf (1.21 cm). When individual treatments were compared in normal and boot leaves separately, on normal leaves, lesion length was significantly lower in treated leaves compared to control. On boot leaves, PF-5 N-5d and ET-1N-5d (0.10 cm) showed significantly lower disease followed by other two treatments.

Forty eight hours of incubation resulted in a mean lesion length of 5.63 cm over both the types of leaves in control which was significantly higher than any other treatments. The lesion length in treated leaves increased compared to day-1 (24 hours after inoculation). Among different treatments tested PF-5 N-5d and ET-1 N-5d could not sustain their effect in decreasing disease when compared to their effect on day-1. RT-4 N-5d (2.60 cm) and PF-2 N-5d (3.25 cm) showed significantly lower mean lesion length. Similar to day-1 observation, on day-2 also boot leaf was found more susceptible (4.39 cm) showing significantly higher lesion length compared to lesion length on normal leaf (3.17 cm). When interaction effects were studied, in treatments involving ET-1 N-5d and RT-4 N-5d lesion length was higher in normal leaf (3.44 cm and 3.03 cm respectively) than that on boot leaf (3.17 cm and 2.17 cm respectively). In PF-2 N-5d and PF-5 N-5d boot leaf had higher disease (5.3 and 5.63 cm respectively) compared to Normal leaf (1.2 and 2.60 cm) respectively. In control, lesion length was on par in boot (5.67 cm) and normal leaves (5.61 cm). When effect of individual treatments on normal leaf was analysed, PF-2 N-5d had significantly lower disease (1.2 cm) compared to all other treatments and control. It may be noted here that unlike in day-1 observation, on day-2, PF-5 N-5d and ET-1N-5d could not sustain their effect when compared with PF-2 N-5d and RT-4 N-5d on boot leaf, however, both PF-2 N-5d and PF-5 N-5d were not successful in decreasing the lesion length while RT-4 N-5d and ET-1 N-5d could show better effect

than that of others though lesion length was higher than that on normal leaf.

Prolonged incubation up to 72 hrs revealed maximum possible mean lesion length in control leaves (6.0 cm) which was on par with PF-2 N-5d (5.97 cm) and ET-1 N -5d (5.83 cm) with insignificant differences among them. Mean lesion length increased from day-2 to day-3 (72 hours of incubation). Least lesion length was observed in PF-5 N-5d (4.17 cm) followed by RT-4 N-5d (5.02 cm). Mean lesion length over all the treatments was higher in boot leaf (5.54 cm) compared to normal leaves (5.25 cm). As similar to the observations made earlier when interaction effect was analysed, except in PF-5 N-5d (2.6 cm on normal leaf and 5.73 cm on boot leaf), in all other treatments disease was more in normal leaf than in boot leaf indicating the increased sensitivity of normal leaf up on prolonged incubation. When individual treatments were assessed on normal leaves, PF-5 N-5d (2.6 cm) showed significantly lower lesion length that differed significantly with all others followed by RT-4 (5.73 cm). All others were on par with control. On boot leaf significantly lowest lesion length was observed with RT-4 N-5d (4.3cm) while others were on par with control.

With 96 hours of incubation, mean lesion length over both the types of leaves tested, except PF-5 N-5d (5.2 cm), all other treatments showed lesion length on par with control (6 cm). This indicated that the effect of nano was much reduced up on incubation indicating temporary effect of nano on the necrotrophic pathogen *R. solani*. Though lesions on boot leaves (5.9 cm) and normal leaves (5.69 cm) differed significantly the variation could not be considered in positive sense as the size of leaf bits placed was only 6 cm. Interaction effects indicated both normal and boot leaf showed equal amount of disease (nearer to 6 cm) except in PF-5 N-5d which showed 4.43 cm lesion length. Among the individual treatments only PF-5 N-5d (4.43 cm) showed same effect in sustaining its inhibitory effect on lesion development in normal leaf while on boot leaf none of the treatments could sustain their inhibitory effect.

Testing of Sensitivity of rice normal and boot leaves towards silver nano particles

The 6 cm cut leaf pieces of boot leaf and normal leaf were dipped in the 10% nano solution for 10 min. Then they were placed in the moist chamber without pathogen inoculation. Absolute control without inoculation of pathogen and without silver nano was maintained for comparison.

In day-1 and day-2, there was no colour change observed in both boot leaf and normal leaf pieces including control. On third day, RT-4 N-5d, PF-2 N-5d, PF-5 N-5d dipped boot leaves turned in to yellow. In ET-1 N-5d and control, boot leaf pieces remained green. In normal leaf on third day also no colour change was observed. On day-4, both the boot and normal leaves turned into yellow including control indicating natural senescence. Thus variation existed in phytotoxic effect depending up on the source of nano preparation and type of leaf tested. Phytotoxicity effect was quicker in boot leaf than that in normal leaf.

Summary

Rice sheath blight disease was characterized by necrotic lesions extending along the leaf sheaths from bottom to top with irregular brown margin and grayish center spreading like the ornamentations of scales of a snake. Symptoms were seen on leaf sheaths spreading from base to top extending up to boot leaf sheath and also up to panicles in severe cases. Sclerotial bodies whitish when

young but later turned brown were observed on the affected portions of rice plant.

From the disease affected rice plants of Agricultural Research Station, Nellore, Andhra Pradesh, the pathogenic fungus was isolated and identified as *Rhizoctonia solani* based on the cultural characters such as brownish broad septate mycelium, right angled branching and loose dark brown irregular sclerotial bodies.

In Pathogenicity When two µl of *R. solani* broth culture was added to rice seeds of cv. NLR 34449 and incubated in moistened rolled towel papers, germination was reduced by 16.7%, shoot length was reduced by 38.9%, root length was reduced by 48.2% and vigour index was reduced by 52.7% compared to pathogen uninoculated rice seeds. However, no necrosis was observed in the leaves of germinated seeds.

The present investigation revealed that bionano preparations irrespective of the isolate source used, *i.e.*, *Trichoderma*, *P. fluorescens* or *R. solani* were found better in decreasing the sheath blight incidence *in vitro* though variation existed in terms of quantum of disease reduction. Bionano preparation from nutrient broth alone was also found effective in decreasing sheath blight severity compared to bioagents used alone or PDB.

When the sensitivity of rice leaves from cv. NLR 34449 was assessed by dipping the leaf segments in 100% concentration of bionano silver (prepared using 170ppm silver nitrate), rice leaves turned yellow from the third day of incubation in detached leaf method *in vitro*.

When three different concentrations of bio-nano silver were assessed up to four days for their effect on rice sheath blight development *in vitro* in detached leaf method, 100% and 50% concentrations of bionano silver were found to have satisfactory reduction in disease development compared to 10%, though all the treatments were found better compared to untreated-inoculated leaves up to two days after inoculation. However, pathogen uninoculated but nano treated rice leaf segments were found sensitive (turning yellow) to 100% concentration of bionano silver but not with 50% or 10%.

Experiment with normal and boot leaves of rice cv. NLR 34449 indicated that boot leaves were more susceptible to *R. solani* sheath blight than the normal leaves and the effect of bionano in decreasing the sheath blight severity was significant in normal leaves than that on boot leaves.

Variation existed in phytotoxic effect depending up on the source of nano preparation and type of leaf tested. Phytotoxicity effect was quicker in boot leaf than that in normal leaf.

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