International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 1917-1922

Received: 23-03-2018
Accepted: 25-04-2018

N Chiranjeevi
Department of Plant Pathology, S.V. Agricultural college, Tirupati and Nanotechnology Laboratory, Institute of Frontier Technology, RARS, Acharya N. G. Ranga Agricultural University, Tirupati, Andhra Pradesh, India

P Anil kumar
Department of Plant Pathology, S.V. Agricultural college, Tirupati and Nanotechnology Laboratory, Institute of Frontier Technology, RARS, Acharya N. G. Ranga Agricultural University, Tirupati, Andhra Pradesh, India

R Sarada jayalakshmi
Department of Plant Pathology, S.V. Agricultural college, Tirupati and Nanotechnology Laboratory, Institute of Frontier Technology, RARS, Acharya N. G. Ranga Agricultural University, Tirupati, Andhra Pradesh, India

KV Hari Prasad
Department of Plant Pathology, S.V. Agricultural college, Tirupati and Nanotechnology Laboratory, Institute of Frontier Technology, RARS, Acharya N. G. Ranga Agricultural University, Tirupati, Andhra Pradesh, India

TNVKV Prasad
Department of Plant Pathology, S.V. Agricultural college, Tirupati and Nanotechnology Laboratory, Institute of Frontier Technology, RARS, Acharya N. G. Ranga Agricultural University, Tirupati, Andhra Pradesh, India

Correspondence
N Chiranjeevi
Department of Plant Pathology, S.V. Agricultural college, Tirupati and Nanotechnology Laboratory, Institute of Frontier Technology, RARS, Acharya N. G. Ranga Agricultural University, Tirupati, Andhra Pradesh, India

Testing the effect of silver nanoparticles on the radial growth of *R. Solani*, *Trichoderma* Spp. and *Pseudomonas fluorescens in vitro*

N Chiranjeevi, P Anil kumar, R Sarada jayalakshmi, KV Hari Prasad and TNVKV Prasad

Abstract
In the present investigation, efforts were made to test the effect of nano preparations on the growth of *R. Solani, Trichoderma* Spp. *and Pseudomonas flourescens* using poisoned food technique. nano preparations from *R. solani*, *Trichoderma* isolate ET-1 five day old culture filtrates, *P. fluorescens* isolate PF-2 five day old culture filtrate and nano preparations from PDB were assessed along with pure silver nitrate solution at 50%, 5%, 0.5%, 0.05%, 0.005% concentrations of 170 ppm silver nitrate or silver nano preparation. Observations on radial growth were recorded at on 1, 2, and 3 days after inoculation. Studies using poisoned food technique indicated that silver nitrate preparation (pure or bionano) had fungistatic effect on *R. solani* at lower concentrations (5% and below) as the inhibitory effect was nullified after three days of inoculation. However, at higher concentrations, *i. e.*, at 50% concentration, inhibitory effect continued to exist even after third day. Further, it is also interesting to note that the silver nano preparation from *R. solani* was inhibitory to itself. The fungistatic effect of silver preparations at <5% conc. were also observed on *Trichoderma* ET-1 when assessed using poisoned food technique. Similar to *R. solani*, *Trichoderma* ET-1 was also more sensitive to silver preparations at 50% concentration. The inhibitory effect of silver preparations was found more of bactericidal in nature when the sensitivity of *P. fluorescens* PF-2 was assessed using inhibition zone technique as evidenced by continued inhibition zone even after 4 days of seeding the agar.

Keywords: poisoned food technique, Silver nitrate, Bio nano and inhibition

Introduction
Nanotechnology can offer green and eco-friendly alternatives for plant disease management. Apart from being eco-friendly, fungi and bacteria are used as bio-manufacturing units, which will provide an added benefit in being easy to use, as compared to other microbes. The non-pathogenic nature of some fungal species in combination with the simplicity of production and handling will improve the mass production of silver nanoparticles. Recently, a diverse range of fungi have been screened for their ability to create silver nanoparticles. Mycosynthesis of gold, silver, goldsilver alloy, selenium, tellurium, platinum, palladium, silica, titania, zirconia, quantum dots, usnic acid, magnetite, cadmium telluride and uraninite nanoparticles has also been reported by various researchers. Nanotechnological application in plant pathology is still in the early stages. For example, nanofungicides, nanopesticides and nanoherbicides are being used extensively in agriculture practices [1].

A Nanotechnology is one of the most fascinating and rapidly advancing sciences and possess potential to revolutionize many disciplines of science, technology, medicine and agriculture. Conversion of macromaterials in to nano size particles (1-100 nm) gives birth to new characteristics and the material behave differently. Nanoparticles may act up on in a way similar to chemical pesticides or the nanomaterials can be used as carrier of active ingredients of pesticides, host defence inducing chemicals, etc. To the target pathogens. Because of ultra small size, nanoparticles may hot/target virus particles and may open a new field of virus control in plants [2].

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.

The world's huge rice agro-ecosystem, designed to feed the ever increasing human population, also provides a habitat for great number of pests and pathogens. Rice diseases can cause significant quality and yield losses. Rice sheath blight (ShB), caused by *Rhizoctonia solani* Kuhn (Teleomorph: Thanatephorus cucumeris (Frank) Donk), is a destructive disease worldwide that causes significant yield loss and quality degradation *Rhizoctonia solani* is a universal soil saprotrophic and facultative plant parasite.. The pathogen has limited movement due to lack of spores and survives in unfavorable conditions by forming sclerotia or dormant mycelia. Sclerotia in soil can survive for 2 years, and are spread during field preparation and flooding the field for irrigation. During permanent flooding the sclerotia may float and move within the field or to bordering fields through continuous flood irrigation. Sclerotia or hyphae attach to the plant, infecting and causing ShB disease, and the pathogen spreads under conditions favorable to disease development. Initial symptoms occur on leaf sheaths near the water line as water-soaked lesions. Secondary infections are caused by hyphae growing upward towards uninfected plant parts, producing additional lesions and sclerotia on leaf sheaths to complete the disease cycle [3].

The disease is alarming due to the intensive cultivation of modern high fertilizer responsive high yielding varieties. Crop with high plant density and close canopy favours disease build up from panicle initiation onwards. Poor weed management practices and increase in frequency of irrigation have aggravated incidence of the disease due to modified micro climatic conditions. Seedlings may be infected in the nursery. Infection starts at the base of plant and death of the seedling is observed. Seedling often observed only in patches. The pathogen has a wide host range and can infect plants belonging to more than 32 plant families and 188 genera [4].

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 [5].

Several reports have been published on the biocontrol of *R. solani*, rice sheath blight pathogen using *Trichoderma* spp. [6, 7] and *Pseudomonas fluorescens* [8, 9]. However, during recent times, *P. fluorescens* is being used widely in rice ecosystem as the agent is a bacterium and can sustain better under high moisture conditions. The advantage of using biocontrol agents like *Trichoderma and P. fluorescence* in managing soil borne plant pathogens are ecofriendly, effective, ease of mass culturing with less cost of production and growth promoting effect. However, commercialization of *Trichoderma* for its utility in field crops could not be achieved successfully. A series of abiotic and biotic parameters has an influence on the biocontrol efficacy of *Trichoderma*. The effects of temperature, water potential, pH, presence of pesticides, metal ions and antagonistic microorganisms in the soil are some important parameters to be considered.

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.

Material and Methods

Poisoned food technique was used to assess the efficacy of bionano silver against selected *Trichoderma* isolates and *R. solani*. Inhibition zone technique was used to assess the efficacy of bio silver nano against *P. fluorescens* [10].

Five different conc. i.e., 50, 5, 0.5, 0.05, and 0.005 of bio nano silver were used in the present investigation by mixing appropriate quantity of bionano silver solution in equivalent mass of double strength PDA.

Such prepared PDA plates were inoculated with 6 mm culture disc of (2 day old) of either *Trichoderma* or *R. solani* for poisoned food technique.

For inhibition zone technique, *P. fluorescens* cell suspension was mixed with PDA just before pouring and the mixture was poured in to the Petri plates.

Plates were incubated at 28±1ºC and observed for mycelia growth in case of fungal cultures and zone of inhibition incase of bacterial cultures. Appropriate controls with only silver nitrate and pathogen check were maintained for comparison.

Results and Discussion

Effect of Silver Nano Particles on The Radial Growth of *R. Solani in vitro*

In the present investigation, efforts were made to test the effect of nano preparations on the growth of *R. solani* using poisoned food technique. nano preparations from *R. solani*, *Trichoderma* isolate ET-1 five day old culture filtrates, *P. fluorescens* isolate PF-2 five day old culture filtrate and nano preparations from PDB were assessed along with pure silver nitrate solution at 50%, 5%, 0.5%, 0.05%, 0.005% concentrations of 170 ppm silver nitrate or silver nano preparation. Observations on radial growth were recorded at on 1, 2, and 3 days after inoculation. The results were analysed using 2 factorial CRD and data was represented in Table 1 and Fig 1.

On day-1, of all the silver preparations used, pure silver nitrate should significantly highest inhibition in the growth of *R. solani* (36.67% mean inhibition) over all the concentrations tested followed by PDB nano (32.96% mean inhibition) which differed significantly among themselves and also with *R. solani* nano (31.11%) or ET-1 nano (29.26%). This indicated that pure silver is highly toxic to *R. solani* growth followed by silver nano preparations from PDB. Non significant variation in inhibiting the growth of *R. solani* by *R. solani* N-5d and ET-1 N-5d indicated possible similarities between the two as both of them are fungi.

When mean inhibition in the growth of *R. solani* due to concentrations was assessed over all the preparations, it was found that 50% concentration of 170 ppm pure silver nitrate (62.96% mean inhibition) was significantly more toxic to *R. solani* than the remaining four concentrations. All the other four concentrations gave insignificant difference among them with respect to *R. solani* growth.

When interaction effects were observed, it was found that 50% concentration of any of the test preparations was

significantly more toxic than that of other concentrations. Further, at 50% conc., PDB nano (81.48%) was inhibitorier followed by fungal nano preparations and the least was by pure silver nitrate preparation (46.30%). However, at lower concentrations pure silver nitrate was more toxic than that of other nano preparations.

On day-2, inhibition in the growth of *R. solani* was continued though there was an apparent reduction in the quantum of inhibition compared to the first 24 hours.

Mean comparisons indicated that the inhibition was significantly higher with pure silver nitrate preparation (29.33%) compared to other preparations. Silver nano preparation from either *R. solani*, ET-1 or PDB were on par among themselves and significantly lower than pure silver nitrate preparation in showing inhibitory effect on *R. solani* growth. Among the five different concentrations tested, maximum and significantly higher inhibition observed at 50% concentration (68.81%) compared to other lower concentrations which were on par among themselves.

Interaction effects revealed that irrespective of the type of silver (pure or nano), 50% concentration was more inhibitory than that of lower concentrations. It may be noticed that the effect of nano preparation using PDB which showed maximum inhibition at 50% after 24 hours (day-1) decreased by 48th hour (day-2). Further, overall decreased inhibitory effect signified fungi static effect of silver nitrate (pure or nano) on *R. solani* than fungicidal effect. This may be due to the fact that *R. solani* is capable of converting silver in to silver nano to make it less toxic or nontoxic.

To observe the effect of silver (nano or pure) on *R. solani* mycelia growth *in vitro*, inoculated plates were further incubated up to 72 hours (day-3). Result indicated that there was a further decrease in the quantum of inhibition in the radial growth of *R. solani*. When comparisons were made with mean inhibition percent, the inhibitory effect of pure silver nitrate was highly nullified by the pathogen *R. solani* (9.19% mean inhibition) and was significantly lower than the three test nano preparations which were on par among themselves. Further, it was interesting to note that the inhibitory effect existed only at 50% concentration (67.96% inhibition) while at other concentrations inhibitory effect was zeroed down. The interaction effect also signified the same result, *i.e*., inhibitory effect of individual test silver preparation was effective only at 50% concentration and zeroed down at lower concentrations.

Further, it was to be noticed that the inhibitory effect of 50% concentration of silver preparations on *R. solani* continued to be uniform from 24 hours to 72 hours of incubation while, at lower concentrations, inhibitory effect was nullified over a period of 72 hours.

Thus the result indicated that silver nitrate preparation (pure or nano) had fungi static effect at lower concentrations (5% and below). Initial inhibition was higher with silver nano prepared even from dissimilar organisms. Further, silver nano preparation from *R. solani* was also inhibitory to itself.

Similar results were obtained by [11] who assessed inhibitory effect of Ag NPs at different concentrations. Generally, higher suppression of fungal radial growth was noticed at a concentration of 0.0019 mol/L. The highest inhibition was observed against *R. solani* AG-2-2, *R. solani* AG-6 and *R. solani* AG-5 with 77.77%, 73.60% and 71.10% of linear growth inhibition, respectively, when compared to the control.

Effect of Silver Nano Particles on the Radial Growth Of *Trichoderma* Et-1 *in Vitro*

In order to assess the effect of silver nitrate and bio silver nano on the growth of bio control agent *Trichoderma* isolate ET-1 *in vitro*, poison food technique was done with three selected bio nano preparations (nano preparations using *R. solani* five day old culture filtrate, ET-1 five day old culture filtrate and PDB) and compared with pure silver nitrate. The data was presented in Table 2 and Fig 2 after 2 factorial CRD analysis.

The result indicated that significant inhibition was noticed in the growth of *Trichoderma* isolate ET-1 with silver nitrate (pure or nano) in comparison to control up to 48 hours of incubation. Prolonged inhibition resulted in nullifying the effect of silver nitrate especially at or below 5% concentration. Observations on day-1 revealed insignificant differences in inhibitory effect of different silver nitrate preparations when mean inhibitory effect was considered over all the concentrations tested. Among the concentrations tested, 50% conc. had highest inhibitory effect (57.81% mean inhibition over all the preparations tested).

Interaction effects revealed that individual silver nitrate preparation had significantly maximum inhibitory effect at 50% conc. Unlike in *R. solani* where a progressive trend was observed with decreased conc. In case of *Trichoderma* ET-1, the inhibitory effect with different conc. showed variation. With pure silver nitrate solution the effect decreased with 50 to 0.5% concentrations and then increased from 0.5 to 0.005% concentrations. With silver nano prepared using *R. solani* culture filtrate, the inhibitory effect decreased with decrease in conc. up to 0.5% but increased with 0.05% conc. and later again decreased with 0.005% conc. With *Trichoderma* and PDB nano preparations inhibitory effect decreased with decrease in conc. It may be noted here that silver nano prepared using *R. solani* was highly toxic to *Trichoderma* ET-1 followed by PDB nano. Unlike in the effect on *R. solani* growth, where in *R. solani* nano and ET-1 nano had similar inhibitory effect, in case of *Trichoderma* ET-1, the fungus was more sensitive to *R. solani* and PDB silver nano preparations compared to its own (ET-1) silver nano preparation. Continued inhibition up to 48 hours (day-2) after incubation revealed that silver nano preparation from *R. solani* and PDB had significantly higher mean inhibition effect on *Trichoderma* ET-1 growth (28% and 27.81% respectively) with insignificant differences between them. With ET-1 silver nano preparation, a mean inhibition of 18.38% was observed which was significantly lower than the inhibition due to *R. solani* or PDB nano and significantly higher than pure silver nitrate preparation. Among five different conc. tested, 50% conc. gave significantly highest mean inhibition over all the silver nitrate preparations tested (54.39%), conc. of 5, 0.05, 0.005% were on par in their inhibitory effect on ET-1 growth. Significantly least inhibition was observed in 0.5% conc. *i.e.,* at higher or lower conc. (on either side of 0.5%) the inhibitory effect was significantly higher. Interaction effect of the two different factors (type of preparation and conc.) revealed that at 50% conc., inhibitory effect of two of the three nano preparations and pure silver nitrate was significantly higher 67.63%, 66.62% and 64.59% respectively with *R. solani* nano, silver nitrate and PDB nano) than that of ET-1 nano (18.61%). This indicated that similar to 24 h, even after 48 h, ET-1 nano had no change in its inhibitory effect on ET-1 growth. However, at lower conc. (less than 50%) the inhibitory effect of silver nano, irrespective of the source of preparation, inhibitory effects

decreased and were all on par. Significantly lower inhibitory effect was observed with pure silver nitrate below 50% conc. tested (5.83, 0.95, 6.84, 8.77, 17.80% respectively with 5, 0.5, 0.05, 0.005% conc.). Silver nitrate was found to be more toxic to ET-1 at 50% conc. (66.62%) than that of other lower concentrations (0.95% to 8.77% inhibition). With *R. solani* nano preparation, highest inhibition in the growth of ET-1 was observed at 50% conc (67.63%) while at all other conc. inhibitory effect was on par (17.60 to 19.56%). Similar effect was noticed with PDB nano with highest inhibitory effect noticed at 50% conc. (64.59%) and all other lower conc. showed significantly lower inhibition compared to 50% conc. which did not differ significantly among them. ET-1 silver nano had similar effect at all the conc. tested with insignificant difference among them (17.60% to 19.56%).

Prolonged incubation up to 72 h resulted in overall decreased inhibition due to silver nitrate preparations (pure or nano). When mean inhibitory per cent was noticed over all the conc. tested, PDB nano was more toxic (14.37%) followed by *R. solani* nano (13.48%), pure silver nitrate (11.57%) and ET-1 nano (7.48%) which differed individually with all other treatments. Mean inhibitory effect over all the silver preparations was highest at 50% conc. (58.63%). Similar to inhibitory effect on *R. solani*, *Trichoderma* ET-1 also had only fungi static effect below 50% conc. as indicated by complete growth of ET-1 in the 9.0 cm dia. Petriplate resulting in 0.0% inhibition at and below 5% conc. Interaction effect also revealed that PDB nano (71.85%), *R. solani* nano (67.41%), silver nitrate (57.85%), and ET-1 nano (37.7%) had inhibitory effect only at 50% conc., while in all others the inhibitory effect was nullified with 72 h of incubation.

Effect of Silver Nano Particles on the Growth of *P. Fluorescens* Pf-2 *in Vitro*

In order to assess the inhibitory effect of silver nitrate and its nano preparations on the growth of *P. fluorescens* PF-2, inhibition zone technique was conducted by seeding PF-2 cells in nutrient agar and placing 4mm filter paper discs impregnated with either silver nitrate or its nano preparations from 5day old culture filtrates. Unlike in case of fungal cultures where in PDB nano was used, in this case silver nano preparation from nutrient broth (alone) was used along with *R. solani* nano, ET-1 nano, and PF-2 nano. Data obtained were analysed in CRD and presented in Table 3, Plates 3a to n.

Inhibitory effect on PF-2 was obtained only at 50% conc. of the preparations while at all other four lower conc. (5 to 0.005%) inhibitory effect was not noticed with the PF-2 forming bacterial lawn all through the plates. At 50% conc., highest inhibition zone of 1.82 cm dia. was observed with *R. solani* nano followed by NB nano (1.76 cm), PF-2 nano (1.62 cm), ET-1 nano (1.44 cm), and pure silver nitrate (1.32 cm). Unlike in *R. solani* and *Trichoderma* ET-1, the inhibitory effect continued even up to four days of incubation indicating more of bactericidal effect rather than bacteriostatic effect.

Summery and Conclusion

Studies using poisoned food technique indicated that silver nitrate preparation (pure or bionano) had fungi static effect on *R. solani* at lower concentrations (5% and below) as the inhibitory effect was nullified after three days of inoculation. However, at higher concentrations, *i. e.*, at 50% concentration, inhibitory effect continued to exist even after third day. Further, it is also interesting to note that the silver nano preparation from *R. solani* was inhibitory to itself. The fungi static effect of silver preparations at <5% conc. were also observed on *Trichoderma* ET-1 when assessed using poisoned food technique. Similar to *R. solani*, *Trichoderma* ET-1 was also more sensitive to silver preparations at 50% concentration. The inhibitory effect of silver preparations was found more of bactericidal in nature when the sensitivity of *P. fluorescens* PF-2 was assessed using inhibition zone technique as evidenced by continued inhibition zone even after 4 days of seeding the agar.

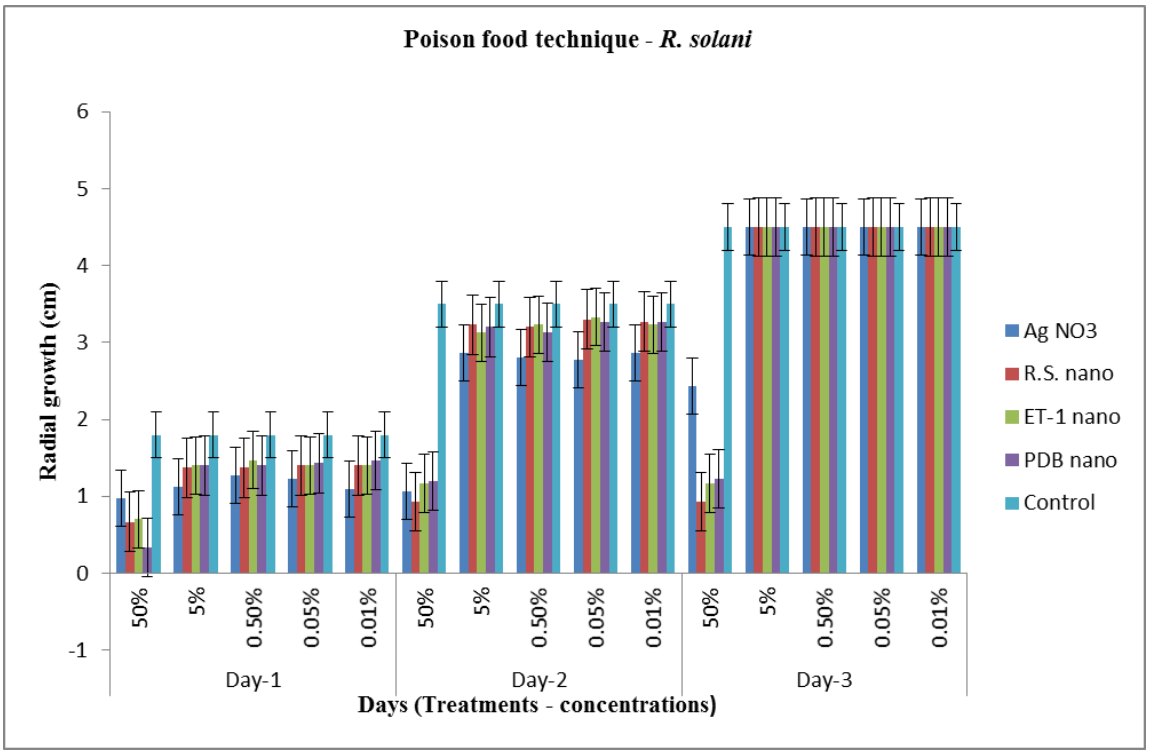


Fig 1: Effect of silver nano particles on radial growth (cm) of *R. solani in vitro*

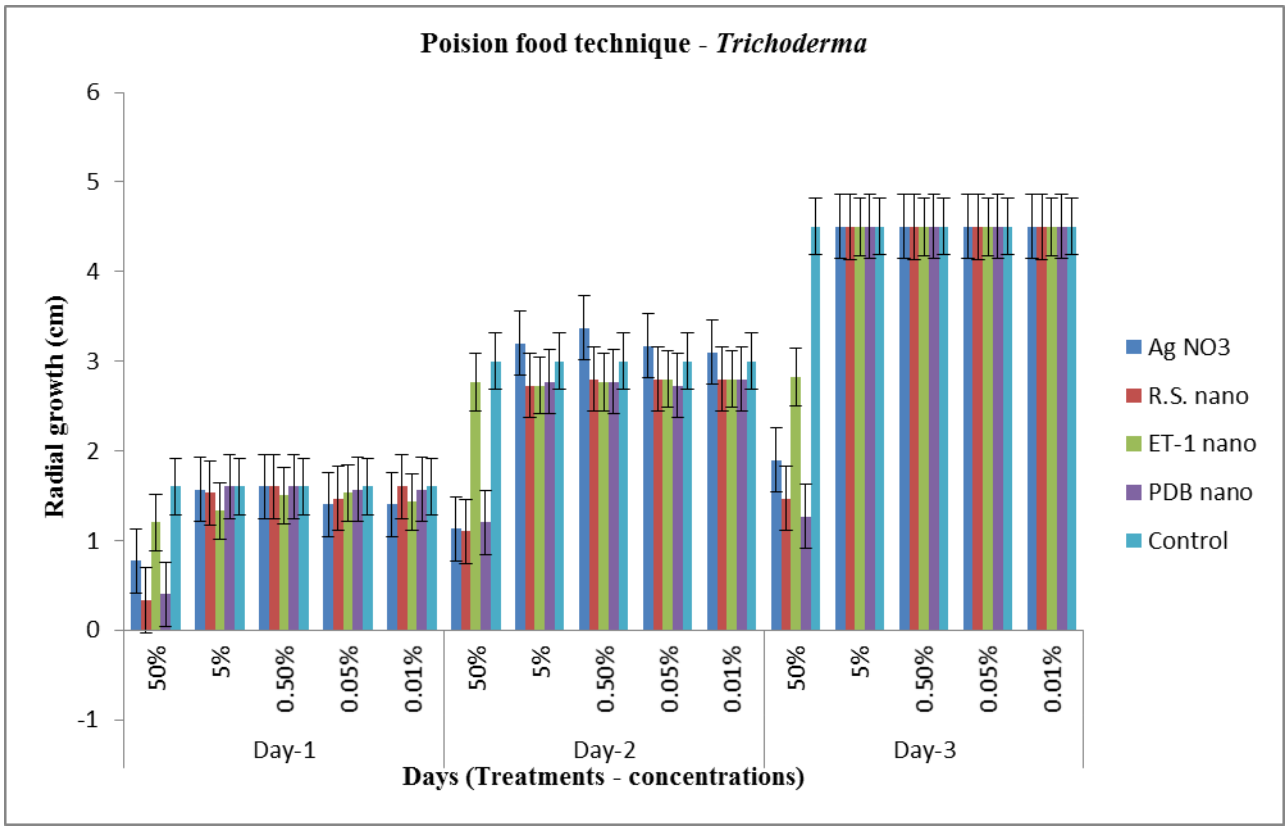
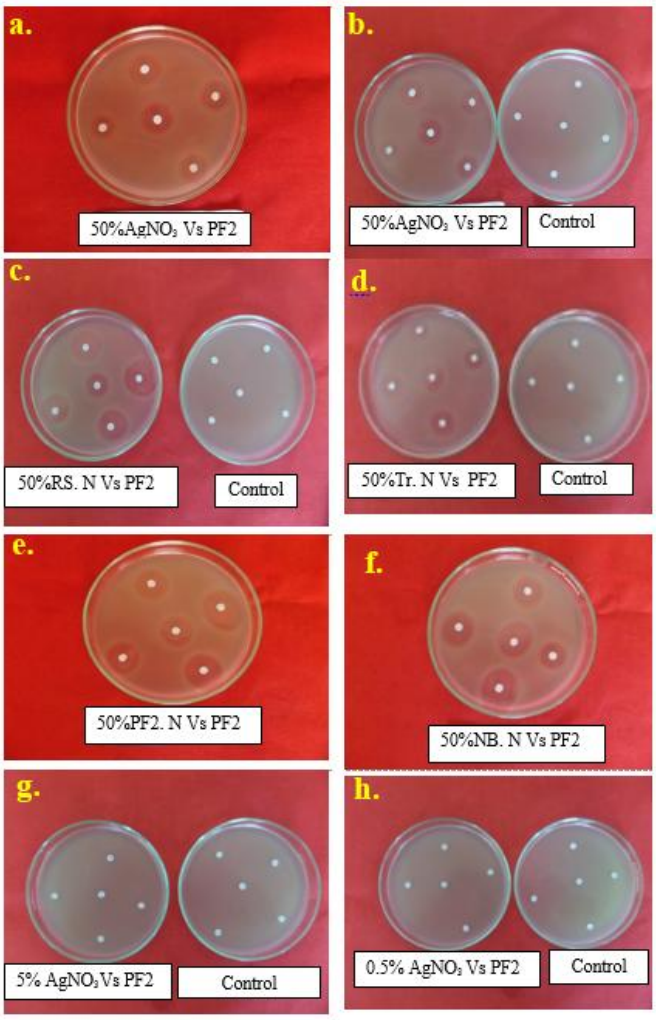
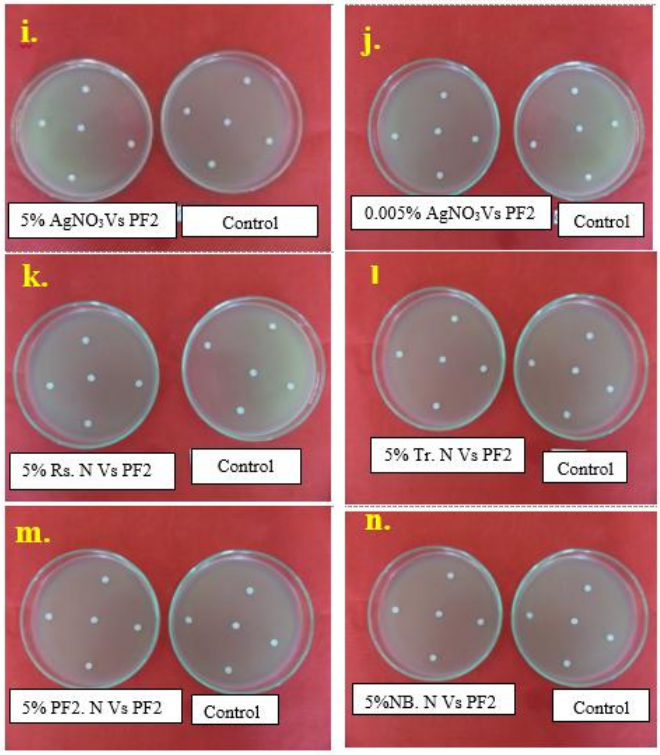


Fig 2: Effect of silver nano particles on radial growth (cm) of *Trichoderma in vitro*



Plates 1a to h: Effect of silver nano particles on the growth of *P. flourescens* PF-2 *in vitro*



Plates 1 i to n: Effect of silver nano particles on the growth of *P. flourescens* PF-2 *in vitro*

Table 1: Effect of silver bionano particles on radial growth of *R. solani in vitro* in poison food method – Day 3

Treatments	Inhibition per cent					
	50% Conc.	5% Conc	0.5% Conc	0.05% Conc	0.005% Conc	Mean
Silver nitrate	45.93 (42.65)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	9.19 (8.53)
R solani N-5d	79.26 (63.78)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	15.85 (12.80)
ET-1 N-5d	74.07 (59.40)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.81 (11.87)
PDB nano	72.59 (58.42)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.52 (11.68)
Mean	67.96 (56.05)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	
	C.D (P=0.01)	SEm (±)	CV (%)			
Nano solution	1.60	0.57	7.8			
Concentrations	1.80	0.64	7.1			
Interactions	3.60	1.30	10.10			

Values in the parenthesis are arc sine transformed values

Table 2: Effect of silver bionano particles on radial growth of *Trichoderma* ET1 *in vitro* in poison food method – Day 3

Treatments	Inhibition per cent					
	50% Conc.	5% Conc	0.5% Conc	0.05% Conc	0.005% Conc	Mean
Silver nitrate	57.85 (49.50)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	11.57 (9.9)
R solani N-5d	67.41 (55.20)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	13.48 (11.03)
ET-1 N-5d	37.41 (37.70)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	7.48 (7.54)
PDB nano	71.85 (58.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.37 (11.60)
Mean	58.63 (50.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	
	C.D (P=0.01)	SEm (±)	CV (%)			
Nano solution	0.20	0.07	2.6			
Concentrations	0.22	0.08	2.8			
Interactions	0.44	0.16	3.9			

Values in the parenthesis are arc sine transformed values

Table 3: Effect of silver bionano particles on the growth of *Pseudomonas fluorescens* isolate PF-2 *in vitro* in inhibition zone technique.

S. No.	Treatments	Inhibition zone (cm)				
		50% Conc.	5% Conc.	0.5% Conc.	0.05% Conc.	0.005% Conc.
1	*R. solani* N-5d	1.82 (1.68)	0.00	0.00	0.00	0.00
2	ET1 N-5d	1.44 (1.56)	0.00	0.00	0.00	0.00
3	PF2 N-5d	1.62 (1.62)	0.00	0.00	0.00	0.00
4	NB nano	1.76 (1.66)	0.00	0.00	0.00	0.00
5	Silver nitrate	1.32 (1.52)	0.00	0.00	0.00	0.00
6	Control	0.00 (1.00)	0.00	0.00	0.00	0.00
	C.D	0.04				
	SE (m)	0.01				
	C.V	1.99				

Values in the parenthesis are arc sine transformed values

References

1. Alghuthaymia MA, Almoammarb H, Raic ME, Galievd S, Abd-Elsalame KA. Biotechnol and Biotechnol equip, 2015; 29(2):221-236.
2. Khan MR, Rizvi TF. J Plant. Pathol. 2014; 13(3):214-231.
3. Anees M, Edel-Hermann V, Steinberg C. Soil. Biol. Biochem. 2010; 42:1661-1672.
4. Gangopadhay S, Chakrabarti NK, J Plant. Pathol. 1982; 61:451-460.
5. Roy AK. Indian. Phytopath. 1993; 46:197-205.
6. Das BC, Khiruzzman ASM, Bora LC. J Mycol. Pl. Pathol. 1998; 28(1):45-47.
7. Bhagavathi R. Crop prot. 2005; 42(4):290-293.
8. Vasantha DT, Malar VR, Sakthivel N, Gnanamanickam, S.S., Plant. Soil. 1989; 119:325-330.
9. Tiwari PK, Trimurthy VS. J Mycol. Pl. Pathol. 37(2):231-234.
10. Nene YL, Thapliyal PN. Fungicides in plant disease control, Oxford and IBH publishing company, New Delhi: Academic, 1993.
11. Elgorban AM, Samawaty AEME, Yassin MA, Sayed SR, Adil SF, Elhindi KM *et al*. Biotechnology. Biotechnology. Equip. 2015; 30:56-62