PBRG Sub-project Completion Report (PCR)

A. Sub-project Description

1. **Title of the PBRG sub-project:** Formulation of biopesticides to control bakanae disease of rice in field condition
2. **Implementing organization (s):** Bangladesh Rice Research Institute and Islamic University
3. **Name and full address with phone, cell and E-mail of Coordinator, Associate Coordinator, PI/Co-PI (s):**

Coordinators:

A. Dr. Md. Khalequzzaman
Director Research (C.C.)
Bangladesh Rice Research Institute
Cell: 01715752595
zamanmk64@yahoo.co.uk; directorresearchbrri@yahoo.com

B. Dr. Md. Mostafa Kamal (17/10/2019-15/01/2022)
Coordinator for Advance Studies and Research
Cell: 01716729850 & 01521106497
ijmumu@yahoo.com & mkamal15jan1963@gmail.com

Principal Investigators:

A. BRRI Component
Dr. Quazi Shireen Akhter Jahan
Principal Scientific Officer
Plant Pathology Division
Bangladesh Rice Research Institute (BRRI), Gazipur-1701
Mobile.: +8801855873259
Email: shireenbrri@yahoo.com

B. Islamic University, Kushtia Component
A.T.M. Mijanur Rahman
Professor, Dept. of Applied Nutrition & Food Technology
Islamic University, Kushtia
Mobile: +880-1716053597
Email: mijananftiubd@gmail.com

Co-principal investigators:

A. DR. Mozammel Haque **(BRRI component)**
Senior Scientific Officer,
BRRI Regional Station Habiganj, Tel.: +8801741112529
Email: mhaquesoil@yahoo.com

B. Dr. Md. Asad Ud - Daula (**Islamic University component**)
Assoc. Prof., Dept. Of Applied Nutrition and Food Technology
Tel.: 01675527666; 01837638455
Email address: asad.uddaula@googlemail.com

C. Montasir Ahmed (04/04/2021-31/10/2022)
Scientific Officer
Plant Pathology Division, BRRI, Gazipur-1701
Telephone: +8801783755739
Email: ahmed.montasir@ymail.com

4. Sub-project budget (Tk.):

4.1. Total: (in Tk. as approved): 80,21,000/=

4.2. Latest Revised (if any): 102, 44,280/=

5. Duration of the sub-project:

5.1. Start date (based on LoA signed): 17/10/2019

5.2. End date: 15/2/2023

6. Background of the sub-project

Bakanae caused by *Fusarium fujikuroi/F. proliferatum* is an endemic fungal disease in rice and is sporadic distributed in Bangladesh mainly in greater Cumilla, Habiganj and Mymensingh districts. But, the incidence of bakanae is increasing in Bangladesh (Haq *et al.* 2011) and growing more concern to rice growers as yield loss 21-51.53% has been reported in Bangladesh (Hossain *et al*., 2013; Angeles *et al.*, 2006). In the present perspective of Bangladesh, it is essential to minimize yield loss due to diseases for increasing rice production in decreasing land area. Despite the considerable economic impact of bakanae, efficient and effective control methods are scarcity, except the seed treatment with chemical fungicides. The use of a single chemical to control bakanae is not justified as some strains of *F. fujikuroi* have been found to degrade the chemicals when applied singly (Kim *et al.*, 2010). Moreover, the massive and continued use of these chemicals together at lack of controlled and adequate conditions for using them it have generated numerous problems such as new fungal pathogen strains evolved and resistant to fungicides and the increase of waste residues and the toxic effects for humans and animals. Because of the above limitations, more effective and environmentally sound control measures using antagonistic microorganisms and natural plant products commonly known as biopesticide might have an alternative approach to control *F. fujikuroi/F. proliferatum*. *Bacillus* spp. *Pseudomonas* spp. and *Trichoderma* spp. have been found to control many plant pathogens including sheath blight (Bhattacherjee and Dey, 2014; Kumar *et al*., 2012). Currently, *Bacillus* spp. is identified as a successful biopesticide for control bakanae disease (Hossain *et al*., 2016). *Trichoderma* spp. is also identified as a very proactive bio control agent for sheath blight disease management in Bangladesh (Jahan *et al.*, 2016; Kamal and Shahjahan 1995). Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Therefore, it is worth to look for environmentally and toxicologically safe and more effective methods (biopesticide) to control bakanae disease and to replace chemicals gradually with biopesticides which are safe to human, and non-target to other beneficial organisms and cheaper than the chemicals.

In the mean time, 40 biocontrol bacteria, six *Trichoderma* spp., have been identified that can inhibit mycelia growth of bakanae causing pathogen in vitro (61-94%) in NATP-2, CRG project (PI:712). Among the biocontrol agents two biocontrol bacterial isolate (isolate 1 and isolate 4) identified as *Bacillus cereus* and one *Trichoderma* isolate (T2) identified as *T. asperelloides* were tested on seedlings as root dip method and found promising to manage bakanae disease in comparison with control (inoculated). Moreover, four plant product active ingredients (neem seed extraction in ethanol, neem leaf extraction in ethanol, mehogoni seed extraction in ethanol and dodder plant extraction in ethanol) have been identified to inhibit 100% mycelial growth of the bakanae causing pathogen *in vitro* condition.

As the biocontrol agents/active plant products were identified as effective in *in vitro* condition only and could not evaluate in field condition due to short time in CRG project, thus, it is aimed to use plant products to formulate nano particle and formulate biopesticide with the identified effective biocontrol agents against bakanae disease. Moreover, Biocontrol agents were identified at species level. In addition, nano particle was developed from the plant products that were identified in CRG subproject to manage the disease in large scale with lower cost.

In this study, formulation of biopesticides was done using suitable carrier material. Moreover, nano particle of the identified plant products was developed to manage the disease at farmer's field level. Simultaneously, environment friendly and sustainable management package was developed against bakanae disease of rice.

7. Sub-project general objective (s)

Development of environmentally safe biopesticide to control bakanae disease and increase yield of rice.

8. Sub-project specific objectives (component wise)

BRRI component

i) To characterize the identified effective biocontrol fungi/bacteria at species level
ii) Molecular identification of isolated biocontrol agents
iii) To formulate and find out suitable carrier material with prolong shelf life of biopesticides.
iv) To evaluate field efficacy of formulated biopesticiede and nano particle against bakanae disease of rice in field condition.

Islamic University, Kushtia component

i) Formulation of nano particle from identified effective plant product/active ingredients.
ii) In-vitro evaluation of nano particle against bakanae causing *Fusarium* spp.

9. Implementing location (s): Gazipur, Cumilla, Habiganj

10. Methodology

A. BRRI Component

1. Characterization of the bacterial biocontrol agents isolated from bakanae infected field (Activity under objective 1)

Important biochemical tests were performed for the identification and characterization of biocontrol bacterial agents such as gram staining, siderophore production, cellulase production, phosphate solubilization tests etc. as described by Elbeshehy *et al.*(2015).

(a) Gram staining: Gram staining was performed using Potassium hydroxide test (Halebian *et al.*, 1981). In briefly, two drops of a 3% solution of potassium hydroxide were placed on a glass slide with concave wells. A 2-mm loopful of bacterial growth of 48-hr culture on nutrient agar was stirred in a circularotion in the KOH solution. The loop was occasionally raised 1 to 2 cm from the surface of the slide. The KOH solution characteristically became very viscousnd mucoid with gram-negative bacteria. A string of mixture was followed the loop when it was raised. The KOH test was only considered positive if stirring occurred within the first 30 s of mixing the bacteria in the KOH solution. Gram-positive bacteria suspended in the KOH solution generally displayed no action (absence of stringing).

(b) Siderophore production: Siderophore production by Fluorescent *Pseudomonads* or *Pseudomonas* spp/*Bacillus* spp was tested qualitatively using chrome azurol S (CAS) agar as described by Alexander and Zuberer (1991). Selected isolates were grown on King's broth (KB) medium for 48hr in shaker. The medium was amended with 2µmol $L^{-1}Fe^{3+}$ from sterilized $FeSO_4.7H_2O$ for control treatment. After that the grown culture were centrifuged at 10,000 rpm for 10 minutes at 4^0C for remove the cell from medium. One mL of the supernatant was mixed with one mL of the chrome azurol S solution prepared according to Schwyn and Neilands (1987). The mixer color was changed from bluish to reddish brown within 15 min and it indicated the the presence of siderophore production in the medium.

(c) Cellulase production: Cellulase production by biocontrol bacteria was determined on basal medium supplemented with carboxymethyl cellulose (CMC) (10 g/L) (Cattelan *et al.* 1999). Cellulase production was determined by plating the isolates onto M9 MS medium agar amended with 10g cellulose and 1.2g yeast extract/L. The cultures were incubated for 8 days at 28^0C. The clear halos formed around the colonies confirmed as positive for cellulase production.

(d) Indole acetic acid production: Indole acetic acid **(**IAA) production of bacterial isolates was carried out as per the procedure described by Gang *et al.*, (2019) using Salkowski reagent. In briefly, isolates were incubated at 30^0C for overnight. After that 100µl of the young culture were vortexed and incubated again in dark condition at 120 rpm for 30 min. Then mixed 1 ml of culture supernatant with 1 ml of Salkowski reagent and development of pink to reddish color within 30 min confirmed the IAA production.

(e) Cyanide production: Determination of cyanide production in slants of Nutrient agar amended with glycine (4.4 g/L) following Dewihastuti *et al*. (2012). In briefly, each isolate was grown in nutrient agar slant containing glycine (4.4 g/L). A piece of filter paper saturated with 0.5% of picric acid and 2% of Na_2CO_3 solution was placed on the upper portion of the medium in the slant. After incubation of 3-5 days in room temperature change in color of filter paper from yellow to orange-brown indicating the production of cyanide by the isolates.

(f) Phosphate solubilization by *Bacillus* spp: The capacity of selected bacilli strains to solubilize phosphate in form of calcium phosphate was tested qualitatively by plating the bacterial strains in Pikovskaya agar medium {5 g $Ca_3(PO_4)_2$; 0.5 g $(NH4)_2SO_4$; 0.2 g NaCl; 0.1 g $MgSO_4 \cdot 7H_2O$; 0.2 g KCl; 10 g glucose; 0.5 g yeast extract; 20g agar; 0.0025 g $MnSO_4$; 0.0025 g $FeSO_4$; 1000 ml distilled water} by plating the disc soaked in bacterial suspension (Dewihastuti *et al*. 2012). In briefly, each isolate was grown on nutrient agar for 7 days. After that, an agar disc (5mm) from each isolate was placed on petri plate containing Pikovskaya medium and incubated at 28^0C in dark for 2 weeks. Formation of a clear zone around the agar disc indicated the solubilizing activity. Isoaltes producing a clear zone more than 20 mm are considered as high phosphate solubilizing activity.

2. Specify of the biocontrol agents (Activity under objective 2)

A. Molecular identification of fungal biocontrol agents

(a) **Extraction of DNA from *Fungi*:** *Trichoderma* was grown on Potato Dextrose Broth (PDB) medium. After 24-36 h culture was centrifuged at 12000 rpm for 10 min. Supernatant was discarded and centrifuged twice with 500 µl neuclease free water at 12000 rpm for 10 min. After that supernatant was discarded and crushed the pellet with crush ball and CTAB buffer (300 µl). Then add CIP (chloroform isoamyl alcohol phenol) and inverted the solute and stand for 10 min. Then centrifuged for 10 min at 12000 rpm. Approximately 200 µl supernatant was withdrawn and mix with equal volume of isopropyle alcohol and keep for 10 min at room temperature. Then centrifuged again for 10 min at 12000 rpm and discarded the supernatant. After that, 700 µl of cold ethanol (70%) was added and centrifuged for 5 min at 12000 rpm. Then supernatant was discarded and pellet was dried and diluted with TE buffer and stored at -20^0C.

(b) **PCR amplification of purified DNA (*Trichoderma*):** Isolated DNA was amplified using ITS 1/ ITS 4 primers. PCR condition was as follows: initial denaturation at 94^0C for 5 min followed by 35 cycles of denaturation was at 94^0C for 30 sec. Anneleaning temp. was at 53^0C for 30 sec, extension was at 72^0C for 1 min and final extension was at 72^0C for 7 min. *Trichoderma* isolates was identified according to the similarity of ITS region in Blast homology search.

B. Molecular identification of bacterial bicontrol agents

(a) Extraction DNA from bacteria: DNA of bacterial isolates was extracted followed by Cheng & Jiang (2006). One ml cell suspension was centrifuged at 5000 rpm for 10 min. After removing the supernatant, the cells were washed with nuclease free water and centrifuged again. After removing the supernatant, cells were diluted with 1 ml nuclease free water and vortexed for 30 sec and treated with dry heat block at 85^0C for 10 min. After removing from heat block the cells were kept at -20^0C for 10 min. Then

centrifuged again at 12000 rpm for 5 min. Collected 800 µl supernatant and added with 800 µl cold isopropyle alcohol and stand for 10 min. Then centrifuged again at 12000 rpm for 10 min and discarded supernatant. Again, added 500µl (70%) ethanol and centrifuged at 12000 rpm for 5 min and dried the pellet. The pellets were re-suspended in 50µl TE buffer and stored at -20^{0}C for future work.

(b) PCR amplification of purified DNA of bacterial bioagent: To identify the endophytic bacterial isolates, 16S RNA gene sequence analysis was carried out using primer 91E -F GGAATTCAAAKGAATTGACGGGGGC, 13B-RCGGGATCCCAGGCCCGGGAACGTATTCAC. PCR condition was initial denaturation at 94^{0}C for 5 min followed by 35 cycles of denaturation was 94^{0}c for 30 sec, annealing at 55^{0}c for 30 sec, extension was at 72^{0}C for 1 min and final extension was at 72^{0}C for 7 min. Bacterial isolates were identified according to the similarity of 16S RNA genes in Blast homology search.

3. Find out suitable carrier material and shelf-life study (activity under objective 3)

(a) Multiplication, formulation, carrier material and shelf-life study of fungal bioagents

Trichoderma spp. was grown on PDA plates. At the same time, broken corn seeds were soaked in water for overnight, then sterilized in conical flask and cooled down to room temperature. Broken corn seeds were used as carrier material. After 5 days of incubation, when *Trichoderma* spp. fully covered PDA plates, then the sterilized broken corn seeds inoculated with the fungus. Further incubated the fungus in conical flask for 5-7 days for fully colonized the corn seeds. Then the colonized corn seeds with *Trichoderma* were taken out from the conical flask and dry it on trays in open air condition for 5-7days. After that, dried corn seeds colonized with *Trichoderma* were ground into powder and preserved it in polythelene bag. Shelf-life study of formulated *Trichoderma* based on biopesticide was carried out after one month interval on PDA plates up to 12 months.

(b)*Multiplication, formulation, carrier material and shelf-life study of bacterial bioagents*
The identified potential biocontrol bacterium was formulated as bacterial biopesticide on carrier material in liquid medium. Two types of formulations were prepared using two carrier materials- nutrient broth and water. Formulation-1 was formulated with and without glycerol in combination with starch in nutrient broth (100%) in conical flask and stored in room temparature. Formulation-2 was formulated with and without glycerol in combination with starch in water (86%) and in combination with nutrient broth (12-14%). In bacterial biopesticidal formulation concentration was calculated as bellow:

$$\text{CFU/mL} = \frac{\text{Cololinies formed}}{\text{Dilution x mL plate}}$$

Shelf-life study of formulated bacterial biopesticide was carried out after one month interval on nutrient agar (NA) plates up to 12 months.

(c) *Preparation of Trichocompost using Trichoderma powder based biopesticide*

Trichocompost was prepared with water hyacinth, Cow dung and Trichoderma powder based biopesticide. Trichoderma powder based biopesticide was prepared with *Trichoderma asperelloides* inoculum on broken corn seeds in Plant Pathology Laboratory (Fig 1a). All

materials were mixed and placed in layers in a composting pile in ratio of water hyacinth (60% moisture): Cow dung (30% moisture): *Trichoderma* powder based biopesticide: 3: 1: 0.16 (w/w) (Fig. 1b & c). Urea solution (10%) was used for rapid decomposition. Trichocompost was prepared within 6-8 weeks (Fig. 1d). Nutrient analysis in Trichocompost produced from *Trichoderma* based biopesticide was also analysed with the help of Soil Science Division, BRRI.

Fig.1.Procedure of *Trichoderma* based Trichocompost preparation (a=inoculums preparaion, b=mixing procedure with Trichocompost preparation materials, c=Trichocompost preparation pile, d= prepared Trichocompost).

4. Field efficacy of formulated biopesticides(Activity under objective 4)

(a) Disease protection studies under net house conditions with fungal and bacterial bio-control agents

This experiment was conducted in a net house condition with rice variety BRRI dhan29. The seeds were surface sterilized with 70% ethanol, washed with sterilized distilled water and soaked in sterilized distilled water for 48 hr. The excess water was drained off and sprouted seeds were further soaked in spore suspension (10^6 conidia/ml) of the virulent isolate of bakanae disease for 48 hr. Pre-soaked seeds in water for the healthy control

treatment were soaked further in sterile distilled water for 48 hr before sowing. The seeds were sown in sterilized soil in trays (2 kg soil/tray). After 7 days of planting both inoculated and un-inoculated seedlings were uprooted from the soil and the roots of seedlings were treated with identified biocontrol agents for 30 minutes following root dip method (Fig.2.). Before that, five biocontrol bacterial isolates i.e. *Bacillus cereus* (isolate-1), *Leucobacter aridicollis* (isolate-2), *Serratia* sp. (isolate-3), *Bacillus cereus* (isolate-4) and *Bacillus cereus* (isolate-5) were cultured on nutrient broth medium for 24 hr. At the same time two Trichoderma based biopesticides i.e. T1 (*Trichoderma asperellum*) and T2 (*Trichoderma asperelloides*) were also cultured on broken corn seed. Root dip method was used for inoculation with both fungal and bacterial biocontrol agents. The inoculated and non inoculated seedlings were arranged in a completely randomized design with 3 replications (40 seedlings/replication) in different trays contains sterile soil respectively. For healthy control and disease control roots of seedlings were soaked further in sterile distilled water for 30 minutes also before planting. All trays were placed in a net house at room temperature and watered once daily with a hand sprinkler. The bakanae symptom appeared on treated seedlings were count at 14, 21 and 28 days after treatment and were expressed in % bakanae infection compared with diseased control seedlings.

Fig.2. Seedling root dip method with biocontrol agents

(b)Net house trial with nano particles

Two net house trials were conducted at Plant Pathology division, BRRI. First trial with nano particles synthesized from neem leaf @ 12 mg/L was applied as seed treatment on sprouted seeds as well as spraying on plants. Seed treatment was done after sprouting and on inoculated seeds. Sprouted seeds were inoculated first with *F. fujikuroi* and then treated in synthesized nano particles for 2, 4, 6 and 8 hr as seed treatment and transplanted in trays. For spraying method, inoculated sprouted seeds were transplanted and sprayed with synthesized nano particles after 7 days of transplanting in trays. Along with seed treatment and spraying method, seeds inoculated with *F. fujikuroi* and non-inoculated seeds were transplanted for comparison. Forty seedlings/tray and ten seedlings/rows were transplanted for each treatment. Data was recorded on Percent (%) infected and Percent (%) healthy seedlings after 14 days of nano treatment.

In second trial, $AgNO_3$ (1 mM) was used along with neem leaf mediated silver nano particle to observe the effect of $AgNO_3$ on bakanae disease development. Inoculation method was followed as described before but silver nano particle using neem leaf extract and $AgNO_3$ treatment was lasting for overnight. Fifty seedlings/tray and ten seedlings/rows were transplanted for each treatment. Data was recorded on % infected and % healthy seedlings after 14 days of nano treatment. Plant height and root length was also noted for comparison with inoculated versus neem nano treatment and inoculated versus $AgNO_3$ treatment.

(c) Field trial with formulated biopesticide

Field trial was conducted with the variety BRRI dhan 49 at BRRI R/S, Habiganj farm in T. aman 2020 season. Bakanae incidence was approximate 30-40% in seedbed (Fig. 3). In this trial, bakanae infected and non-infected (symptomless) both type of seedlings was collected from seedbed (Fig. 4). Both infected and non-infected seedlings were treated with biopesticides. There were 4 treatments: T1= Trichocompost produced from *Trichoderma* based biopesticide, T2= *Bacillus cereus* (isolate 1), T3= *Bacillus cereus* (isolate 5) and T4= Control. Trichocompost produced from formulated *Trichoderma* based biopesticide was applied @ 2.0 t/ha during final land preparation. Bacterial antagonists were applied as root dip for 30 minutes before seedlings transplantation. Control plots were sprayed with sterilized water. Each treatment was replicated thrice. Fertilizer management was done according to BRRI recommended dose.

Fig. 3. Bakanae infected seedlings in seedbed at Habiganj in T. Aman season, 2020

Fig 4. Bakanae infected seedlings collected from affected seedbed in T. Aman, 2020.

In Boro 2020-21, BRRI dhan81 was used at Cumilla whereas BRRI dhan28 and BRRI dhan29 were used at Habiganj for field trials. At each location two trials and thus a total of 4 trials were conducted in those locations. Three treatments were used in each trial; T1=Trichocompost was applied in seedbed (0.5t/ha) before seeding, T2= root dip with *Trichoderma* biopesticide formulation @10g/L H_2O was done before transplanting for 30 min + root dip in bacterial biopesticide formulation for 30 min before transplanting, T3= control. The bakanae incidence with visible symptom in seedbed at Habiganj was 30-40% on BRRI dhan28 whereas, no bakanae symptom was observed at Cumilla. Root dip method in *Trichoderma* powder based biopesticide and in bacterial biopesticide is shown in Fig. 5 and Fig.6 at Cumilla and Habiganj region respectively.

Fig.5. Root dip method in *Trichoderma* powder based biopesticide and in bacterial biopesticide in Boro season, 2020-21 at Cumilla on BRRI dhan81.

Fig.6. Root dip method in *Trichoderma* powder based biopesticide and in bacterial biopesticide in Boro season, 2020-21 at Habiganj.

In Gazipur, two more trials were conducted in highly bakanae infected fields 20-25 days after transplanting where bakanae incidence was 60-70% in Boro 2020-21 (Fig.7). Seeds were supplied by Lalteer seeds Ltd. and variety was hybrid Tia. In this trial two treatments were applied. In first trial T1= *Trichoderma* biopesticide based Trichococompost (2.0 t/ha) and T2= No compost (control) was applied whereas, in second trial T1= *Trichoderma* biopesticide based Trichococompost (1.0 t/ha) + bacterial biopesticide (*Serattia* sp.) spray (20 ml/L H_2O) and T2= none treatment (control) was applied.

Fig.7. Bakanae infected seedlings in seedbed at Gazipur in Boro, 2020-21.

In T. Aus 2021 season, three trials were conducted. Two were at Cumilla and one at Habiganj with BRRI dhan48. Four treatments were applied at field condition as follows: T1: Trichocompost (2.0 t/ha) during final land preparation, T2: Trichococompost (2.0 t/ha) + *Trichoderma* powder based biopesticide treated (root dip for 30 min) before transplanting, T3: Trichococompost (2.0 t/ha) + Bacterial biopesticide (root dip for 30 min) before transplanting, T4: Control. At Cumilla no bakanae infection was observed at seedbed as well as in field condition. At Habiganj, bakanae infection was observed 5-10% at seedbed condition (Fig.8).

Fig.8. Bakanae incidence (5-10%) at seedbed condition at Habiganj in T. Aus, 2021.

In T. Aman 2021 two trials were conducted at Cumilla and Habiganj. Four treatments were applied including control. T1= Trichococompost (1.5t/ha) during final land preparation T2= bacterial biopesticide (20 ml/ha) 10-12 days after transplanting, T3= T1 + T2 and T4= control. Variety BRRI dhan49 was used at Habiganj whereas; BRRI dhan32 and BR22 were used at Cumilla. In BRRI dhan32 bakanae disease incidence was higher (20%) compared to BR22 (10%). At Habiganj disease incidence was 10-15% in BRRI dhan49.

Moreover, In T. Aman 2021 another trial was conducted with neem leaf extract mediated silver nano particle at Cumilla to find out the efficacy of neem mediated silver nano particle to manage bakanae disease of rice in field condition. Two treatments were applied including control. T1= root dip in neem leaf extract mediated silver nano particle and T2= control. Root dip method followed for 30 min for nano treatment and BRRI dhan32 was used where bakanae infection was observed approximately 15% incidence at seedbed condition (Figure 9).

Fig.9. Bakanae infection at seedbed of BRRI dhan32 in Cumilla, T. Aman, 2021

In Boro 2021-22, four trials were conducted at Hotapara, Gazipur and three trials were conducted at Cumilla. At Gazipur, trial-1 was conducted with Trichococompost (1.5 t/ha) along with control using variety katarivhog where disease incidence was 20% in seedbed. Trial-2 was carried out with three treatments i.e., T1= neem leaf extract mediated nano particle following root dip method overnight, T2= bacterial bio pesticide (*Serratia* sp.) (20 ml/L) and T3= control using variety BRRI dhan28 where incidence was 25% in seedbed. In Trial-3, three treatments were applied: T1= Trichocompost, T2= dodder stem extract mediated silver nano particle spray on seedbed, T3= control using BRRI dhan92 where incidence was 22% in seedbed. In Trial-3 ten bakanae infected plants/plot of each treatment was marked after treatmennt application and total tiiler/hill and total effcetive tiller/hill was counted at harvest time to find out the effcetiveness of treatments on bakanae disease. On the other hand, Trial-4 was set up based on Trial-3 in Gazipur to get more conclusive results of dodder stem extract mediated siver nano particle treatment on bakanae disease management in field condition. Two treatments were used in Trial-4. In this trial prominent bakanae infected (internode highly elongated) plants were uprooted from the seedbed where BRRI dhan92 were seeded and incidence was 22% in seedbed. Roots of 30 infected plants were soaked in dodder stem extract mediated silver nano particle for overnight and then transplanted in the field (T1). At the same time 30 infected plants were also transplanted in the field without any treatment (T2). Normal cultural practices were done in both treatments including watering and weeding. Fertilizer application was done in the field before transplanting and urea application was done once at recommended dose at 30 days after transplanting.

At Cumilla, three trilas were set up after transplanting where bakanae symptom was visible in farmers' field in Boro 2021-22. At Cumilla, Trial-1 was conducted with formulated biopesticides where, T1= Trichocompost (1.5t/ha), T2= bacterial biopesticide (*Serratia* sp.) (20 ml/L) and T3= control using BRRI dhan48. Disease incidence was 5%

in seedbed condition (Fig 10). At Cumilla, Trial-2 was conducted with formulated nanoparticles along with control where T1= neem leaf extract mediated silver nano particle following root dip method, T2= dodder stem extract mediated silver nano particle following root dip method and T3= control using BRRI dhan86. Disease incidence was 20% in seedbed (Fig.11). In trial-3 at Cumilla four treatments along with control were used where, T1= Trichococompost @1.5t/ha, T2= dodder stem extract mediated silver nano particle following root dip method, T3= bacterial biopesticide (*Serratia* sp.) sparay @20ml/L and T4= control was applied in BRRI dhan86. Disease incidence was 20% in seedbed. At Cumilla, treatments were applied at field during transplanting after symptoms appear at seedbed condition. From all three trials at Cumilla, ten bakanae infected plants/plot and ten non-infected plants/plot of each treatment was marked after treatment application. Total tiiler/hill and total effcetive tiller/hill was counted from both infected versus non-infected plants during harvesting time that were marked to find out the effcetiveness of treatments on bakanae disease management.

Fig.10. Bakanae infection (5%) at seedbed of BRRI dhan48 in Cumilla, Boro, 2021-22.

Fig11. Bakanae infection (20%) at seedbed of BRRI dhan86 in Cumilla, Boro, 2021-22.

Islamic University, Kushtia (Collaborating) Component

1. Collection and preparation of neem leaf and *Cuscuta reflexa* stem extracts

Fresh and healthy leaves of *Azadirachta indica* (neem) and fresh and healthy stems of *Cuscuta reflexa* (swarnalata) used in this experiment were collected from the campus of Islamic University, Kushtia, Bangladesh. The surface of the leaves was thoroughly cleaned under running tap water and subsequently with ddH_2O to remove any dirt or adhering contaminants. The cleaned leaves and stems were dried at room temperature, cut into small pieces which were then taken in Erlenmeyer flask followed by adding of 100 ml ddH_2O. The flask was then boiled at 100ºC for 20 min and cooled down at room temperature as depicted in Fig. 12 and Fig.13. The cooled neem leaf extract and dodder stem extract was filtered using Whatman No.1 filter paper. Subsequently, the obtained filtrate was stored in a refrigerator at 4°C for carrying out further experiments.

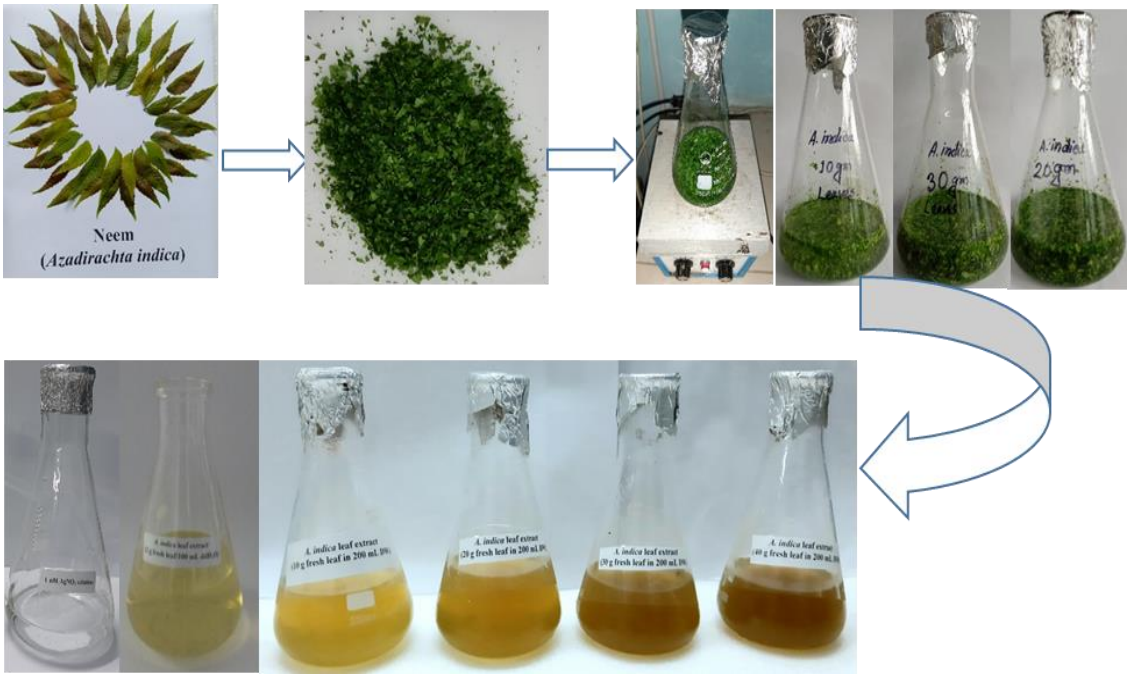


Fig. 12: Preparation of *Azadirachta indica* (neem) leaves extract.

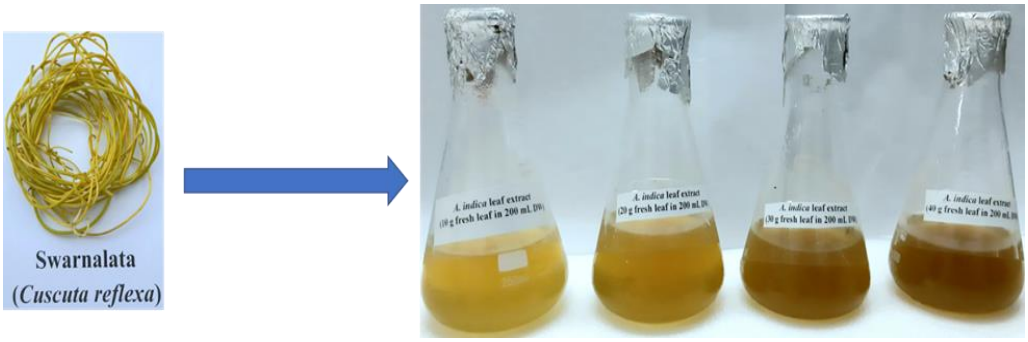


Fig. 13: Preparation of *Cuscuta reflexa* (dodder) stem extract.

2. Biosynthesis of AgNPs

The biosynthesis of AgNPs was carried out by following the procedures of Asimuddin *et al.*, (2020). The AgNPs was biosynthesized by mixing the aqueous neem leaf extract and aqueous dodder stem extract respectively with silver nitrate ($AgNO_3$) solution keeping the final concentration of the mixture always to 1 mM. The mixture was then heated at 85°C in a hot air performance incubator (AP120, Froilabo, France). The color change of the reaction mixture from the initial colorless to yellowish color and finally to red brown color primarily indicated the completion of the reduction of silver ion (Ag+) to silver particle (Ag0) and confirmed the biosynthesis of AgNPs. Subsequently, the formation of AgNPs was further confirmed in aqueous solution using a UV-VIS spectrophotometer (U-2900 UV/VIS Spectrophotometer 200V, HITACHI, Japan) by scanning 3 ml of reaction mixtures in the range between 300 to 700 nm. The absorption spectra for each reaction mixture were recorded as a function of reaction time with a resolution of 1 nm.

3. Optimization of the biosynthesized AgNPs

Optimization process of AgNPs biosynthesis initially started with the formation of nanoparticles from five (5) different amounts of neem leaf extracts and *Cuscuta reflexa* (swarnalata) stem extract such as 2, 5, 10, 15 and 20g. From each of this extract of individual plant parts, 1 ml was mixed with different concentrations of $AgNO_3$ solutions at different ratios of reactants such as 1:4, 1:9, 1:14, and 1:19, (where 1mL was the volume of neem leaf extract/dodder stem extract and the latter was the volume of $AgNO_3$ solution) keeping the final concentration to 1 mM. The mixtures were incubated at 85°C for 1hr followed by measurement of absorption spectrum for each reaction mixture for neem leaf mediated extract whereas, the reaction mixtures were incubated at different temperatures (40, 50, 60, 70, 80, 85, 90, 95, 100, and 110 °C) for different time intervals (30min, 1hr, 2hr, 3hr, 5hr, and 6hr) followed by measurement of absorption spectrum for each reaction mixture. Finally, the optimization of the incubation time of the reaction mixtures (using the optimized amount of leaf extract and dodder stem mediated extract) was carried out based on color development and UV-VIS spectra analysis. By analyzing the obtained UV-VIS spectra of the above mixtures, further experiments were carried out to synthesize AgNPs from the neem leaf and dodder stem amounts of 2, 5, 10, and 15g. The mixtures were incubated at different time intervals in order to investigate the effects of dilution of the reaction mixtures as well as time on AgNPs biosynthesis. Subsequently, UV-VIS spectrum of each of the mixture was carried out in order to confirm the optimum amount of neem leaf/dodder stem, ratio of reactants and incubation time. Finally, the optimization of the incubation time of the reaction mixtures (using the optimized amount of leaf extract/stem extract) was carried out based on color development and UV-VIS spectra analysis. The optimized reactant ratio was further prepared keeping the final volume and concentration to 100 mL and 1mM, respectively. The mixtures were subsequently incubated at different time intervals such as 0, 10, 20, 30, 40, 50, 60, 120, 180, 240, 300, and 360 min, respectively. The color development of each of the reaction mixture at each time interval was monitored and also the UV-VIS spectrum of each of the mixture was carried out in order to confirm the optimum incubation time. The biosynthesized AgNPs in aqueous mixture that exhibited the maximum color development after which no color change observed as well as exhibited the sharpest, narrowest and single SPR band was selected as the optimized AgNPs in this study. The separation and purification of the optimized AgNPs from the reaction mixtures was performed by continuous centrifugation (10000 rpm/min; 20 min; 4°C) with sterile ddH_2O (Acharya *et al.*, 2018). The obtained pellets were repeatedly washed (3-4 times) with ddH_2O water to ensure better separation of the AgNPs from other contaminants. After drying the pellets at 60°C in an oven (AP120, Froilabo, France), the dried AgNPs were kept at 4°C for further characterization.

4. Characterization of bio-AgNPs

The surface morphology, shape and particle size distribution study of the optimized AgNPs were characterized using Field Emission Scanning Electron Microscope (FESEM) equipped with EDX FESEM (JSM-7610F, JEOL Ltd. Japan). In addition, the elemental distributions of the AgNPs were investigated using EDX spectra collected from EDX detector (JSM-7610F, JEOL Ltd. Japan) operating at acceleration voltage of 15 KV. The average particle size of the produced AgNPs was determined by using a particle size analyzer (ZEN3600 Zetasizer, Malvern, U.K.). Furthermore, transmission electron microscope (TEM) image was taken to determine the morphology, size and

shape of the AgNPs. TEM measurements were performed by auto focus, microtrace, autodrive, live FFT display, API (auto pre-irradiation), 120 kV accelerated voltage, multiple lens configurations, including a standard lens for unsurpassed high contrast and a class-leading UHR lens for high resolution. The TEM grid was prepared by placing a drop of the bio-reduced diluted solution on a carbon-coated copper grid and later drying it under a lamp. Besides, the crystalline nature of the AgNPs was determined employing XRD through the XRD patterns of the powder AgNPs sample by an X-ray diffractometer (PHILIPS X'Pert Pro, The Nnetherland) using Cu Kα radiation (λ = 1.54 Å), tube voltage of 33 kV, and tube current of 45 mA. The intensities were measured at 2-theta values from 10 to 90° at a continuous scan rate of 10°/min.

5. Stability test of AgNPs

Evaluation of the stability of the optimized AgNPs was carried out based on SPR (λ_{max} and peak width). AgNPs synthesized using neem leaf extract was preserved at room temperature for 1.5 years and the absorbance was measured whereas, stability of AgNPs synthesized using dodder stem extract is in progress.

6. *In vitro* test of nano particles derived from plant products for controlling bakanae disease

Nano particles synthesized using neem leaf extract and dodder stem extract were sprayed on plants inoculated with bakanae causing pathogen. At Islamic University, silver nano particles were synthesized using neem leaf extract @ 12 mg/L and dodder stem extract @ 8 mg/L and were sprayed on inoculated plants to control bakanae disease. Three different isolates were isolated from bakanae infected plants from Habiganj (40), Cumilla (58) and Gazipur (65) and were used for inoculation for causing infection in plants. Effectiveness of the synthesized silver nano particles were evaluated 21 days after inoculation. Control plants were sprayed with distilled water instead of inoculation with pathogen. Each treatment was replicated thrice following CRD.

7. Data collection

Data was recorded as percent disease incidence on artificially inoculated plants (average 40 plants/tray) at 14, 21 and 28 days after treatment application in net house condition for identifying biocontrol agents against bakanae disease. In net house condition, % infected plants in biopesticide treated trays as well as for nano treated trays were recorded in comparison with control (inoculated) plants. In field trials formulated biopesticides as well as synthesized silver nano particles were applied in natural infected fields or in uninfected seedbeds before infection. After harvesting yield components (number of filled and unfilled grains per plants, grain yield and 1000 grain weight) were recorded in field condition.

8. Statistical analyses

Results obtained from lab test as well as from field test were analyzed statistically.

11. Results and discussion

BRRI Component

1. Characterization of the bacterial biocontrol agents isolated from bakanae infected field (Activity under objective 1)

Gram staining, HCN producing test, IAA producing test, Siderophore production test, phosphate solubilizing test and and cellulase activity results for biocontrol bacteria characterization are presented in in Table 1 and in Fig.14.

Table 1. Characterization of bacterial biocontrol agents on different test parameters

Isolates	Gram staining	IAA test	HCN test	Siderophore production test	Phosphate solubilization test	Cellulase activity test
1.	+	-	++	-	+	+
2.	-	-	+	+	+	-
3.	-	-	+	++	+++	-
4.	-	++	+	+	+	+
5.	-	+	+	+	+	-
6.	+	-	+	+	-	+
7.	+	-	+	-	-	-
8.	+	-	++	+	-	-
9.	+	-	+	-	-	+
10.	-	-	+	+	-	++
11.	-	-	++	+	-	-
12.	-	-	++	-	++	-
13.	-	-	+	+	-	++
14.	-	-	++	+	+	+++
15.	-	-	+	-	+	+

All identified bacterial isolates have shown biocontrol activity against bakanae in *invitro* condition. Among the idntified biocontrol bacterial isolate 4 showed positive reactions of all biochemical tests except gram positive test as shown in Table 1.

Gram-negative bacteria have ability to mobilize insoluble phosphate very efficiently (Zhu *et al*., 2011).

IAA is the most important phytohormone that directly promotes the growth of plants and microbes. Root growth and root length can be increased by endophytic bacteria with IAA-

producing ability, resulting in a greater root surface area, which allows the plant to acquire more nutrients from the soil (Souza *et al*., 2015). Among the isolates 4 & 14 showed positive reaction against HCN, siderophore production, phosphate solubilizing test and cellulase activity test. As bakanae causing pathogen *Fusarium fujikuroi*/*Fusarium proliferatum* has ability to produce gibberelic acid and IAA is the precursor of gibberelic acid, therefore, isolate 3 was choose to use as biocontrol agent to produce formulation of biopesticide against bakanae disease.

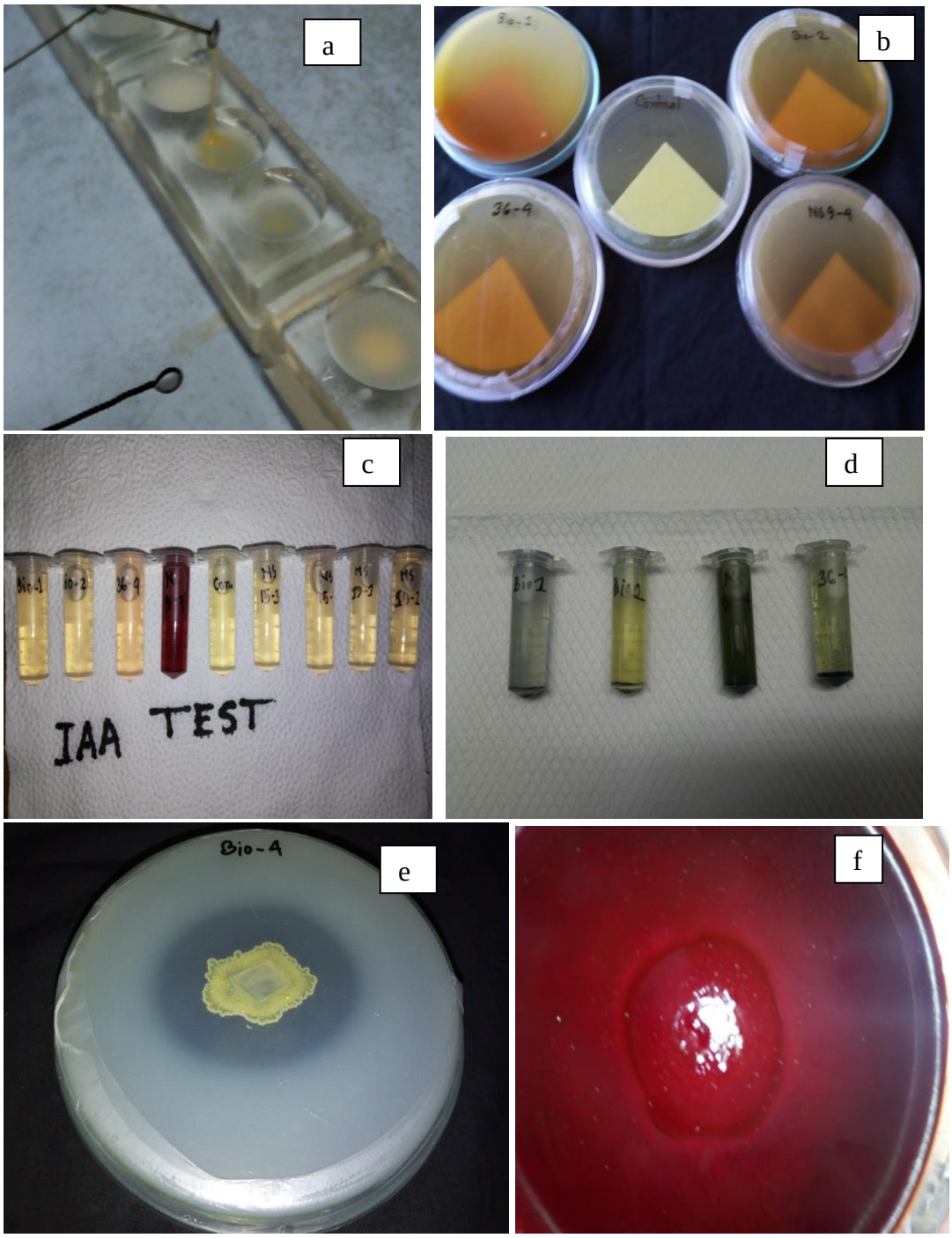


Fig.14. Characterization of biocontrol bacterial agents on different test parameters (a= Gram staining test, b= HCN producing test, c= IAA production test, d= Siderophore production test, e= Phosphate solubilizing test, f= Cellulase activity test).

HCN is a well-known broad spectrum volatile microbial secondary metabolite that is effectively toxic to plant pathogens and can play a key role in disease suppression of various crops (Gupta and Sinha, 2020). Moreover, HCN plays a significant role in biological control of plant pathogens through the limitations of fungal growth contributing to their antagonism (Ahamad *et al*., 2008; Rezzonico *et al*., 2007; Siddiqui *et al*., 2006).

The production of siderophores by microorganisms is beneficial to plants, because it can inhibit the growth of plant pathogens (Sharma & Johri, 2003). Many experiments suggest that production of siderophores when iron is limited is responsible for the antagonism of the phytopathogen and very effective for root growth (Sujatha and Ammani, 2013).

Phosphate solulibilizing bacteria release phosphate from these immobile insoluble forms (Kumar and Ram, 2014). The ability to synthesize fungal cell wall-lysing enzymes such as protease, cellulase or hydrogen cyanide (HCN) and has ability to suppress the growth of fungal pathogen (Modi and Jacob, 2017). The presences of genes such as pqq and gdh which are coding for phosphatase activity in *Serratia marcescens* have already reported (Mohamed *et al*., 2018). In addition to phosphate solubilization, *Serratia* sp. has an antagonistic activity against plant pathogens (Chakraborty *et al*., 2010).

Cellulase has plant growth promoting traits. Apart from plant growth, cellulase is one of them to limit invasion of pathogen to the plants (Panchal, 2021). *Serratia marscens* found to produce maximum yield of cellulases (Sethi *et al*., 2013).

Besides they produce chitinases and siderophores which help to limit fungal growth. Induced systemic resistance is another important mechanism involved in biological control of root pathogens by *Serratia* species (Saha *et al*. 2012). Direct antifungal effect may be based on antibiosis (production of prodigiosin and pyrrolnitrin) and production of lytic enzymes i.e., chitinases and β-1,3glucanases (Kalbe *et al*., 1996).

2. Specify of the biocontrol agents (Activity under objective 2)

Molecular identification of fungal and bacterial biocontrol

PCR amplification of three *Trichoderma* and 15 bacterial isolates were done (Fig.15.). Sequence analysis was done by Macrogen Inc. (Singapore) and similarities were found following NCBI blast analysis that is presented in Table 2.

Table 2. Identification of biocontrol agents following molecular test and sequencing

Isolate	Bio-control agent	Identification	Similarity (%)
1	Bacteria	*Bacillus cereus*	100
2	Bacteria	*Leucobacter aridicollis*	99.77
3	Bacteria	*Serratia sp.*	98.01
4	Bacteria	*Bacillus cereus*	99.56
5	Bacteria	*Bacillus cereus*	99.78
6	Bacteria	*Bacillus cereus*	99
7	Bacteria	*Bacillus cereus*	99
8	Bacteria	*Leucobacter cromireducens*	99
9	Bacteria	*Bacillus thuringiensis*	9999
10	Bacteria	*Brevundimonas diminuta*	99
11	Bacteria	*Brevibacillus brevis*	99
12	Bacteria	*Acinetobacter venetianus*	95.5
13	Bacteria	*Bacillus thuringiensis*	99.78
14	Bacteria	*Bacillus paramycoides*	99.06
15	Bacteria	*Bacillus wiedmannii*	99
16	Fungus	*Trichoderma asperellum* (T1)	99.49
17	Fungus	*Trichoderma asperelloides* (T2)	99.49
18	Fungus	*Trichoderma asperelloides* (T3)	99.66

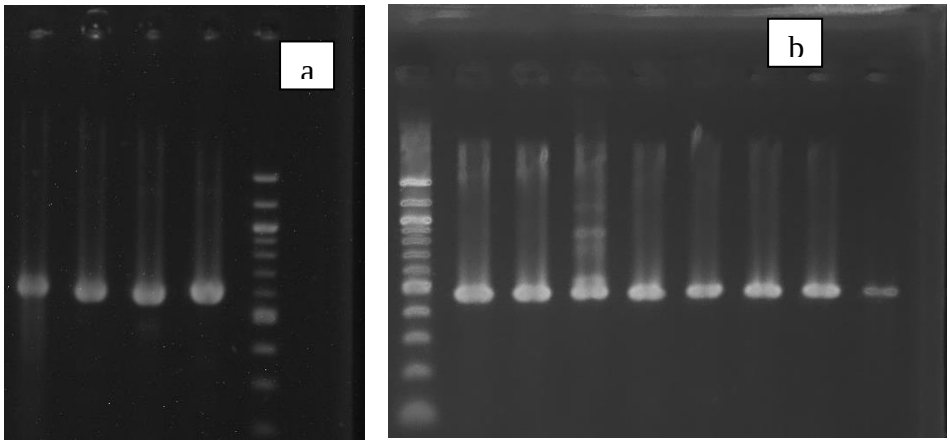


Fig.15. PCR amplification of biocontrol agents (a= *Trichoderma* isolates, b= bacterial isolates).

3. Find out suitable carrier material and shelf-life study (activity under objective 3)

(a) Multiplication, formulation, carrier material and shelf-life study of fungal bioagents

Trichoderma based fungal biopesticide was prepared from both identified *Trichoderma asperellum* (T1) and *Trichoderma asperelloides* (T2) species. Biopesticide with *Trichoderma* was formulated with corn seed and packed in powder form in polythelene bag and stored in room temparature (Fig.16.). It was observed that *Trichoderma* sp. can survive up to 12 months or more in corn formulation (Fig.17.).

Fig 16. Formulated *Trichoderma* based biopesticide

Fig.17. Revive *Trichoderma* biocontrol agent from formulation with corn seeds on PDA medium after 12 months

Other scientists were also stated that solid-state fermentation (SSF) is an effective method for the mass production of fungal biopesticides since it provides micropropagules with higher conidia content (Lewis, 1991; Jeyarajan, 2006).

(b) Multiplication, formulation, carrier material and shelf-life study of bacterial bioagents

It was observed that bacterial biopesticide prepared with *Serratia* sp. can survive up to 12 months or more in both formulations (Fig.18 and 19). In both formulations, bacterial concentration was @ 3.0 x 10^7 CFU/mL. Other researchers were also got the most bacterial concentration of 7×10^4 cfu/ml after fourth month of talc based bio-formulation with bacteria (Ei *et al*., 2017).

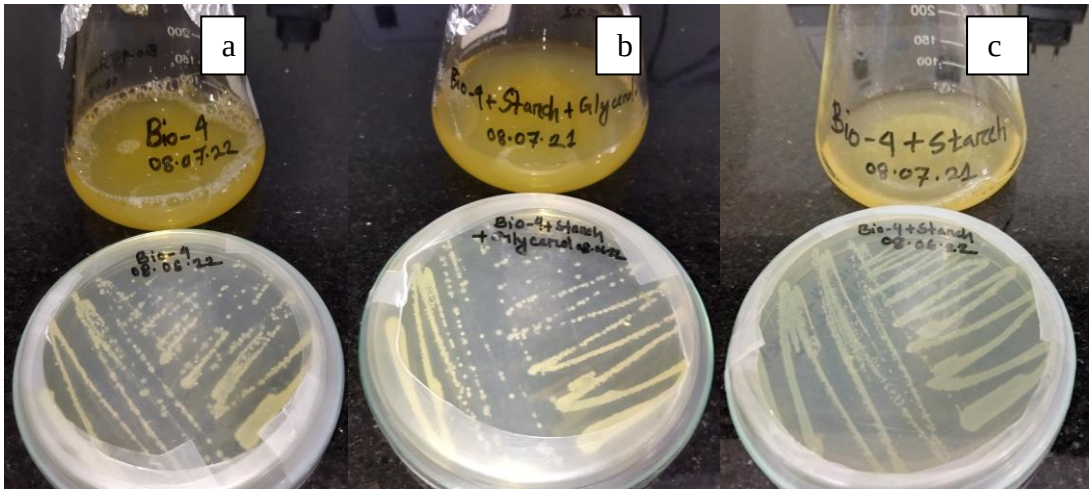


Fig.18. Shelf-life study test of bacterial biopesticide formulation-1 with and without glycerol after12 month

(a=nutrient broth, b= with glycerol, c= without glycerol).

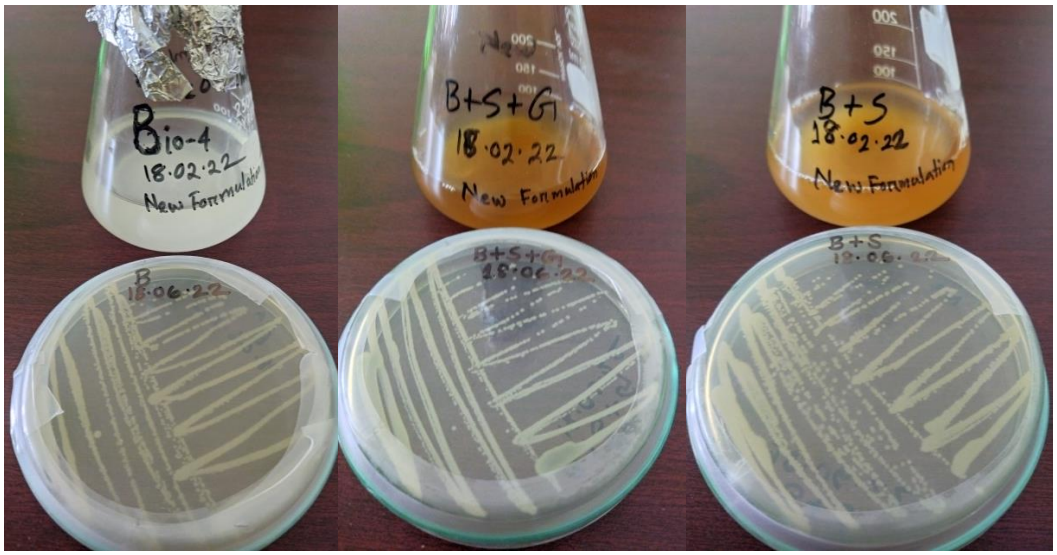


Fig.19. Shelf-life study test of bacterial biopesticide formulation-2 with and without glycerol after 12 month

(a= nutrient broth (14%) in water, b= nutrient broth (12%) in water with glycerol, c= nutrient broth (13%) in water without glycerol).

Formulation is an important factor for biopestide use at field level towards sustaining its activity. Liquid biopesticide formulations are emerging as the way forward for cost effective, eco-friendly and sustainable agriculture (Rao *et al*., 2015). On the other hand, liquid formulation of *Bacillus subtilis* WG6-14 was found effective to control different seedling diseases including bakanae (Miyaki *et al*., 2012).

(c) Preparation of *Trichoderma* biopesticide based Trichocompost

Trichoderma was revived from prepared Trichocompost (Fig. 20).

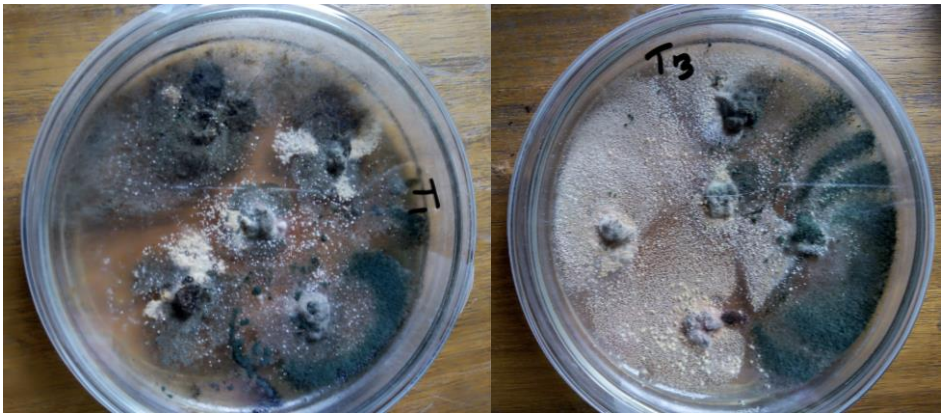


Fig.20. Revive *Trichoderma* from prepared Trichocompost).

High nutrient value was obtained from Trichocompost produced from Tricoderma based biopesticide and are presented in Table 3.

It was observed that after application of Trichocompost soil organic matter inceased by 0.1% and thus soil health can be improved in deficit areas in accordance with disease management. From the nutrient analysis it was observed that soil physical characteristics was improved by increasing organic carbon which also helps microbial activities and thereby increases soil aeration and water holding capacity. Trichocompost, therefore, have an additional benefit as a fertilizer substitute to enhance fertility and productivity. Matin *et al.* (2019) also reported that Trichocompost has an advance benefit to increase soil organic matter.

Table 3. Nutrient analysis of Trichocompost produced from *Trichoderma* based biopesticide and soil organic matter when applied @ 2.0 t/ha in field.

Elements	Content (%)	Kg/ha
N	0.5	10
P	0.44	8.8
K	3.59	71.6
S	0.244	4.9
Zn	0.031	0.62
Organic carbon in soil after Trichocompost application	2.5	2.4

(Nutrient analysis was done with the help of Soil Science Division, BRRI)

4. Field efficacy of formulated biopesticides (Activity under objective 4)

(a) Disease protection studies under net house conditions with fungal and bacterial biocontrol agents

Biopesticide produced from two *Trichoderma* spp. and five biocontrol bacteria were evaluated following root dip method and bakanae infection was recorded. *Trichoderma asperelloides* + *Serratia* sp. performed best followed by *Bacillus cereus*, *Leucobacter aridicollis* and *Bacillus cereus* (Table 4 & Fig. 21). A number of experiments using botanicals were conducted to manage bakanae disease by other researchers. For example, garlic clove extract was found promising to control bakanae disease in Bangladesh (Riazuddin *et al*., 2013). Soaking seeds in the extract of lemon grass at 1:1dilution for 6 hr was also found to control bakanae pathogen successfully (Rahman *et al*., 2014). The leaf extract of *Lawsonia inermis* showed maximum inhibition (60.65%) followed by roots of *Asparagus racemosus* (50.59 %) to control bakanae pathogen (Yasmin *et al*., 2008).

Table 4. Bakanae disease infection (%) with different biocontrol agents in net house conditions

Treatment*	Infected Plant (%)
Trichoderma asperellum	19.6
Trichoderma asperelloides	10
Bacillus cereus (isolate-1)	16.1
Leucobacter aridicollis (isolate-2)	5.4
Serattia sp. (isolate-3)	7.0
Trichoderma asperelloides+*Serattia* sp.	5.2
Bacillus cereus (isolate-4)	10.7
Bacillus cereus (isolate-5)	3.6
Diseased control	87.5
Healthy control	0.0

Fig 21. Effectiveness of biopesticide for control bakanae disease in net house condition.

It was also evaluated that *Trichoderma asperelloides* and *Serratia* sp. both biocontrol agents can coexist at the same time in field condition and are presented in Fig. 22. KNB422 is a fungal isolate was found effective to control bakanae disease, when applied to seeds (Miyaki *et al*., 2012). Moreover, three most prominent *Trichoderma* isolates viz., DPNST-4, DPNST-8 and DPNST-29 were selected for controlling *Fusarium oxysporum* f.sp. *lycoperscisi*, causal agent of Tomato *Fusarium* wilt (Kumar *et al*., 2012). Gangwar and Sinha (2010) screened 52 isolates of *Trichoderma* spp. obtained from soil, rice rhizosphere and rice leaves for their biocontrol ability against *Xoo*. A range of *Bacillus* species had been isolated from the rhizosphere that has biological control activity against rice fungal pathogens (Gnanamanickam, 2009).

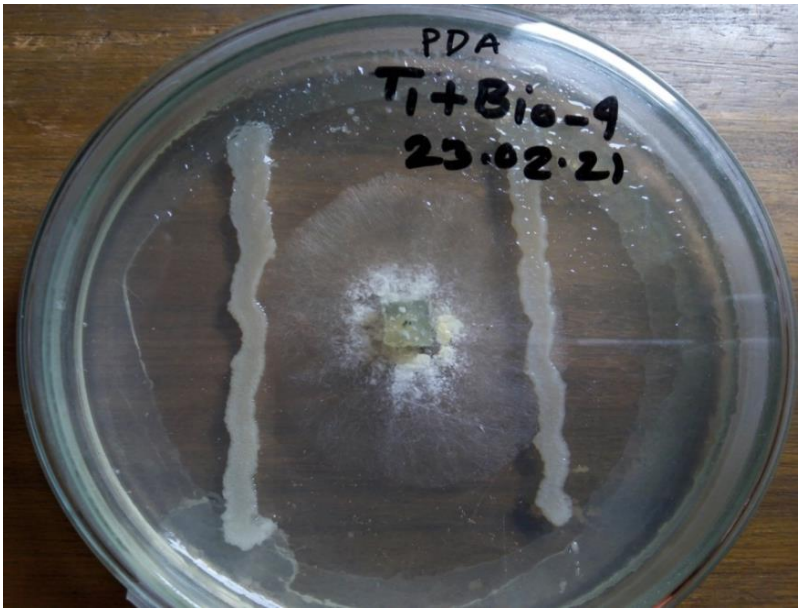


Fig 22. Coexistence test of *Trichoderma asperelloides* and *Serratia* sp.

(b) Net house trial with nano particle

In first net house trial it was observed that neem leaf extract-mediated AgNPs showed a very strong antifungal activity against *Fusarium fujikuroi in vitro* condition (Fig.23).

Fig.23. Results of neem leaf mediated Ag-nano particles on infected plants (a=Healthy non inoculated control plants, b=Inoculated infected plant, c= Inoculated infected plants root dipped with neem mediated Ag nano particle, d= Inoculated infected plants sprayed with neem mediated Ag nano particle)

It was also observed that plants infected with *F. fujikuroi* escaped bakanae infection when sprouted seeds were treated with neem leaf mediated Ag-nano particle for 4-8 hr as seed treatment (Table 5 & Fig.24.). Spraying with nano from neem leaf also promising compared to diseased control plants (Fig.24).

Table 5. Effect of neem leaf mediated silver nano treatment on infected seeds/plants.

Treatment	Seed treatment with neem leaf mediated nano partcle				Spraying with neem leaf mediated nano partcle	Diseased control (Inoculated with *F. fujikuroi*	Healthy Control (Non-inoculated)
	2hr	4hr	6hr	8hr			
% Infected plants	30	0	0	0	12.5	93.3	0
% Healthy plants	70	100	100	100	92.3	6.7	100

*40 seedlings/tray were planted

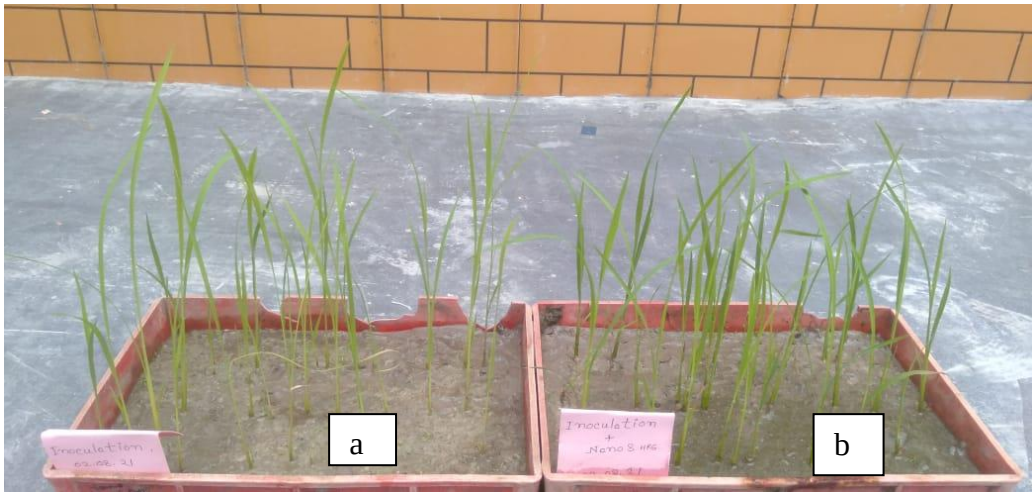


Fig.24.Effect of nano particles on inoculated seedlings after 14 days of nano treatment (a= plants Infected with *F. fujikuroi*, b= plants inoculated with *F. fujikuroi* treated with nano particles for 8 hour).

In second net house trial no bakanae symptom (0%) was observed when neem leaf mediated silver nano particle used and the result was similar to the healthy control (Fig.25). On the other hand, $AgNO_3$ treated plants showed a significant number of elongated plants as like as bakanae symtom (17.80%) and disesed control plants had highest infection (83.75%) (Fig.26). This result suggested that $AgNO_3$ has no role to rescue from bakanae disease symptom development. Moreover, highest plant height was increased in diseased control (8.2%) followed by $AgNO_3$ (1mM) (5.2%) treated seeds. Plant height was somewhat shorter (-2.4%) in silver nano (neem leaf extract mediated) treated plants compared with healthy control plants (Fig.27). This plant height shortness was found in the trays also (Fig.28). The plant height shortness in neem leaf mediated silver nano particle compared to healthy control plants might be due to slower rate of physiological activity occurred after application of neem leaf mediated silver nano particle.

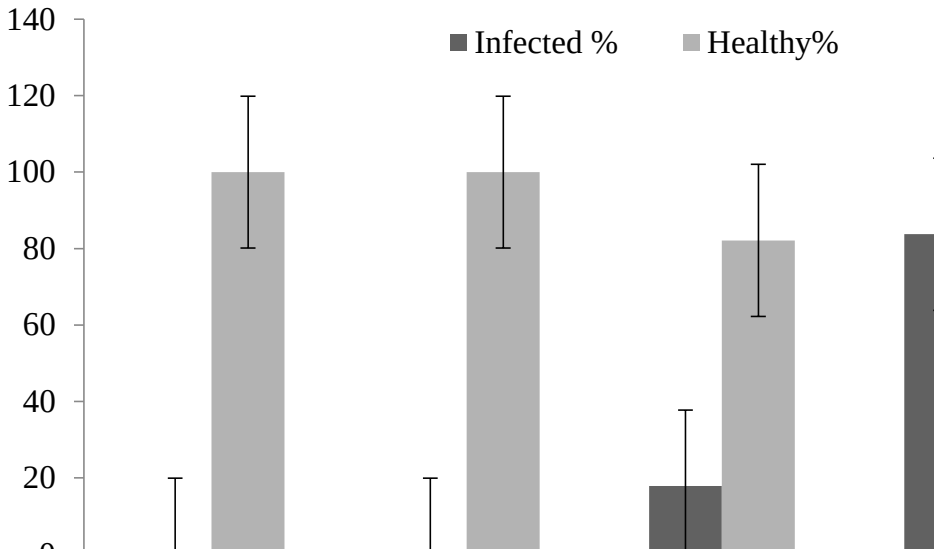


Fig.25. Bakanae incidence (%) observed in different treatments.

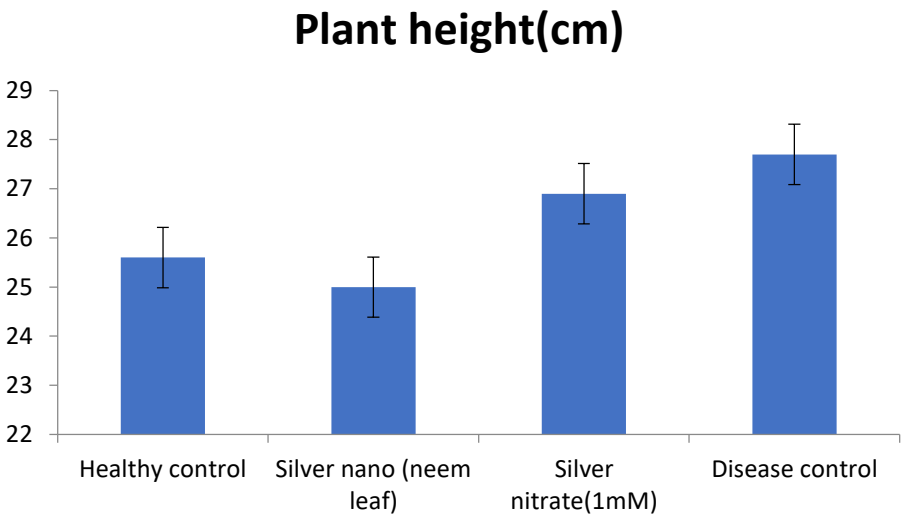


Fig.26. Plant height (cm) in different treatments.

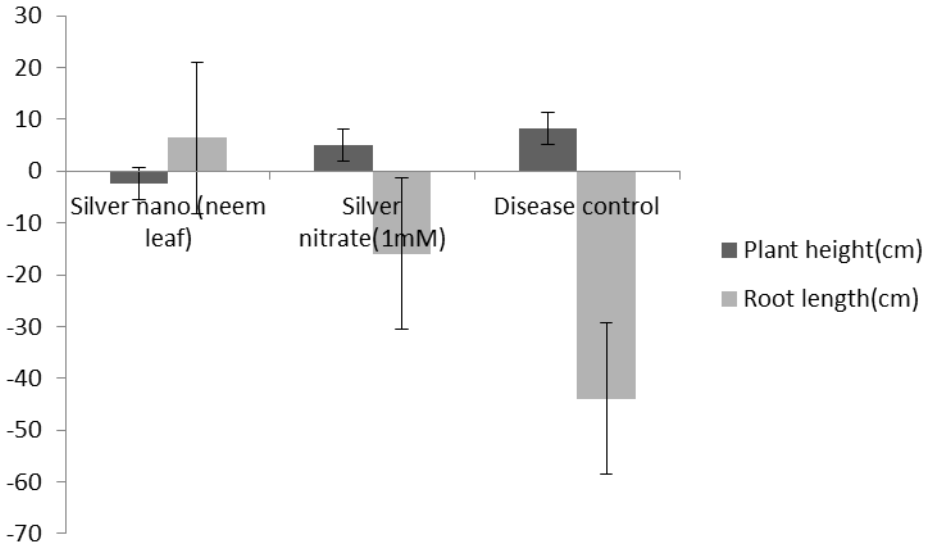


Fig.27. Effect of treatments on plant height and root length increase or decrease in comparison with non inoculated healthy control plants.

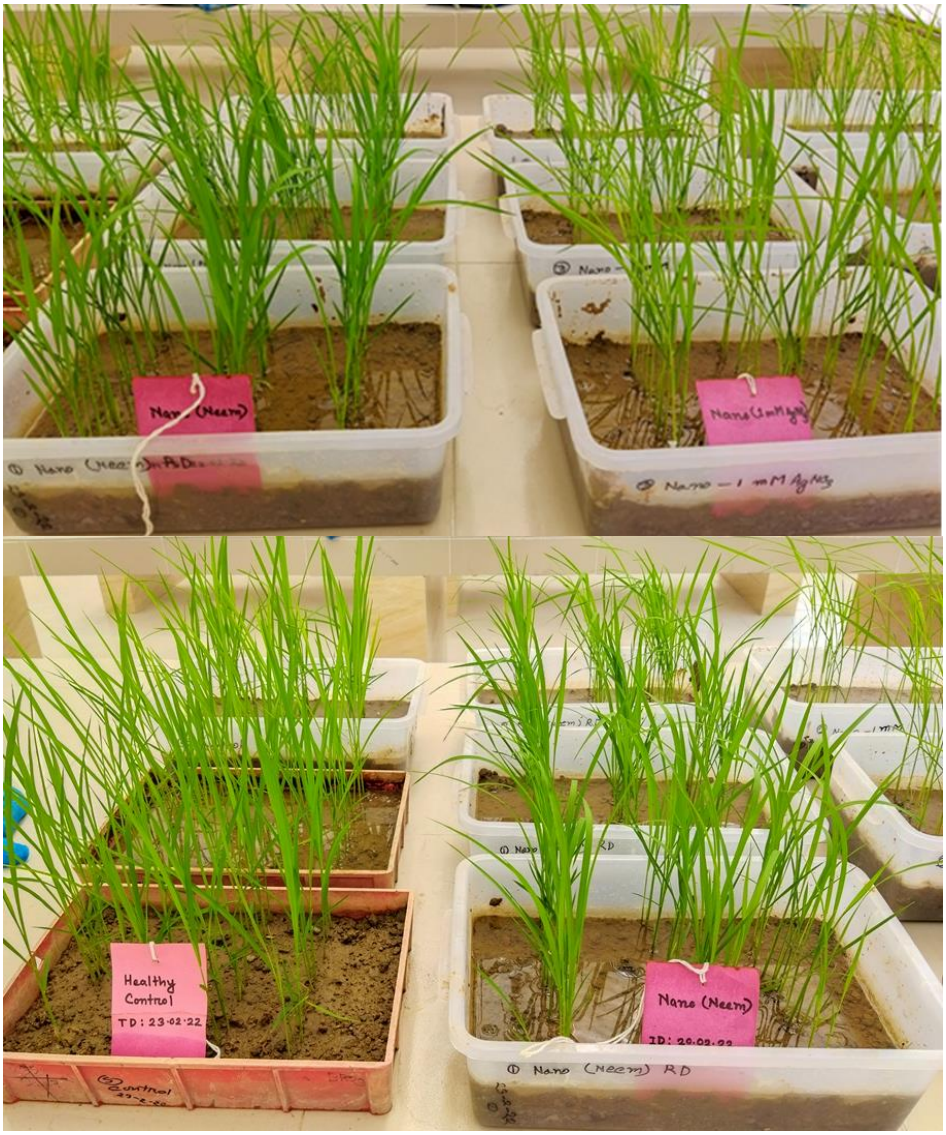


Fig.28. Plant height observation on different treatnments applied after 14 days

In case of root length, it was observed that root length somewhat increased (6.4%) in silver nano (neem leaf extract mediated) treated plants compared to healthy control plants (Fig.29). Other researchers also reported that AgNPs and $AgNO_3$ has effect on increase the root length (Ejaz *et al*., 2018). Lowest root length was observed in diseased control plants (44% decreased) followed by $AgNO_3$ (16% decreased) treated seeds (Fig.27).

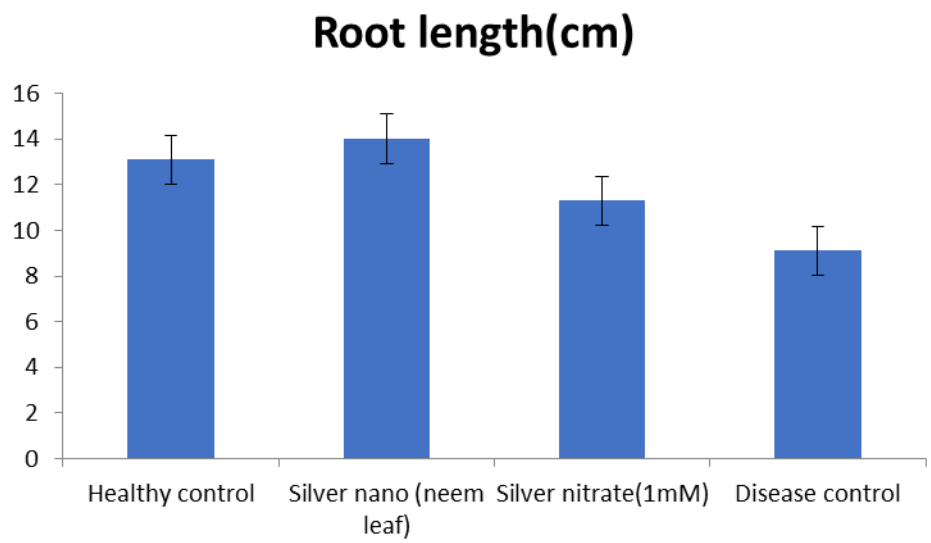


Fig.29. Effect of treatments on root length (cm).

(c) Field trial with formulated biopesticide

At Habiganj trial in T. Aman 2020, it was observed that infected seedlings survived 25-40%, whereas healthy seedlings survived 98-100% after 30 days of transplanting (Table 6 & Fig.30).

Table 6. Survival status of seedlings after 30 days of transplanting in T. aman 2020 at Habiganj

Treatments	**Survival of seedlings**			
	Infected seedlings		Healthy seedlings	
	Number of seedlings survived	Survival seedlings (%)	Number of seedlings survived	Survival seedlings (%)
T1(Trichocompost)	6.3	40	15.7	98
T2 (*Bacillus cereus* -isolate -1)	4.7	29	16.0	100
T3 (*Bacillus cereus* (isolate 5)	4.0	25	16.0	100
T4 (Control)	6.3	40	16.0	100

Fig.30. Survival seedlings after 30 days of transplanting in T. Aman, 2020 at Habiganj.

For all treatments it was found that total tiller (TT) and effective tiller increased in infected plants compared to healthy plants (Fig.31). Higher effective tillers (31-43%) increased where bacterial biopesticide (T2 and T3) were applied compared to *Trichoderma* based biopesticide (T1) and control (T4) treatments (Fig. 32). In all treatments plant height was found increased in healthy seedlings compared to infected seedlings due to bakanae symptom showing infected tiller was died and the plant height was estimated for new tillers in infected bakanae plants which was much lower compared to healthy plants.

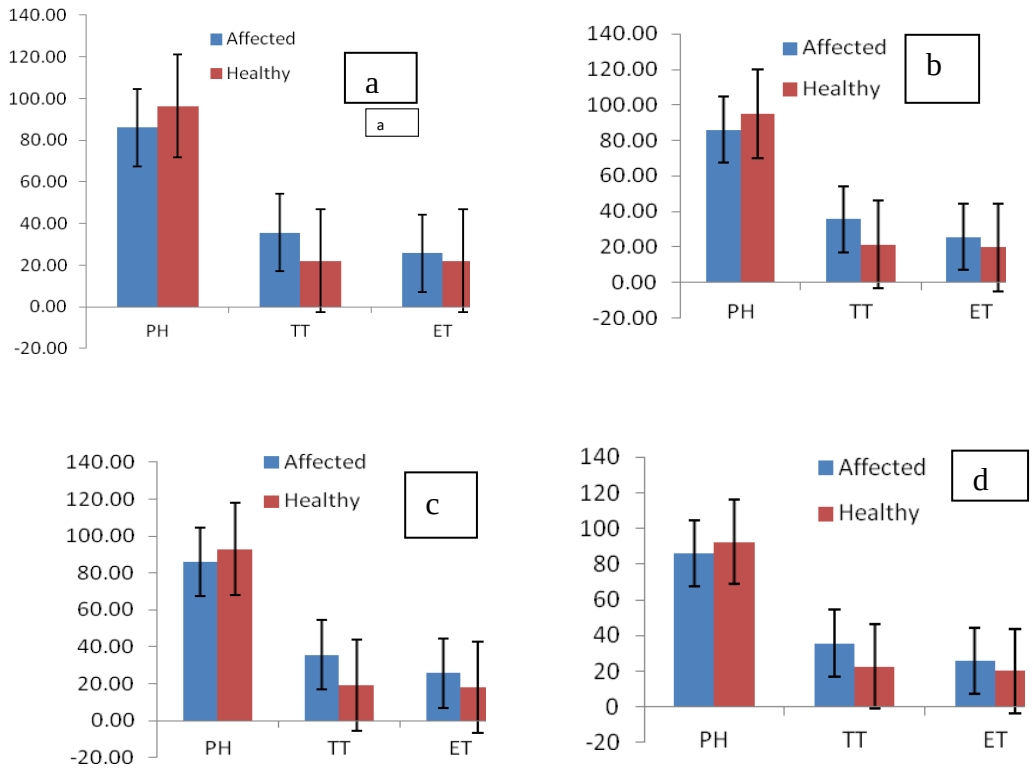


Fig.31. Effectiveness of different treatments on everage plant height/plant (PH), total tiller/plant (TT) and on effective tiller/plant (ET) in T. Aman 2020 at Habiganj.
(a= Trichocompost, b= *Bacillus cereus*, c= *Bacillus cereus* and d=control).

From this trial it was also established that more effective tillers were produced from infected plants compared to healthy plants in all treatments. It was also found that higher effective tillers were produced when treated with bacterial biopesticides (T3) and (T2) (Fig.32). Although some new effective tillers were produced in T4 (control plots) compared to T1 (Trichocompost) but yielded with empty grains. Yield data was not taken due to rat infestation in plots. Effective tiller/hill was found almost similar from healthy plants in all treatments. There was no effect of treatments on healthy plants in terms of effective tiller production (Fig 32).

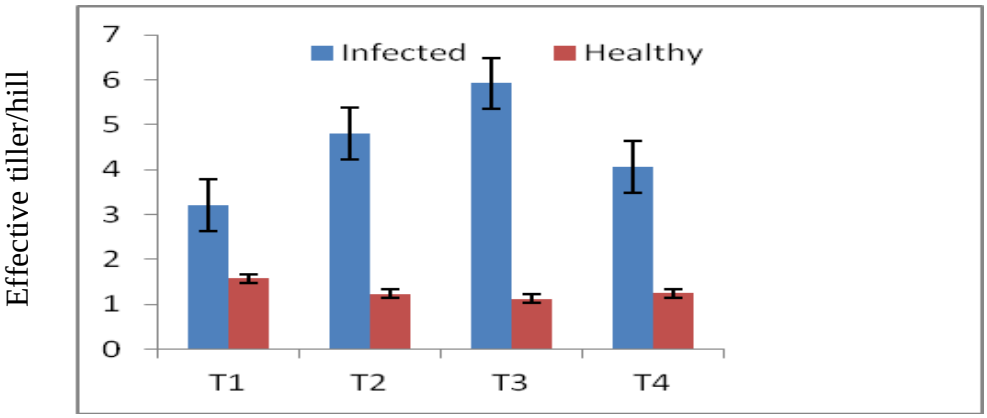


Fig.32. Effective tiller/hill in infceted plants compared to healthy plants in different treatmnents application in T. Aman 2020 at Habiganj.
(T1= Trichocompost, T2= *Bacillus cereus* (Bacterial biopesticide), T3= *Bacillus cereus* (Bacterial biopesticide) and T4=control).

In Boro, 2020-21 season at Cumilla there was no bakanae infection in seedbed condition as well as in field condition. Although there was no significant differences among the treatments at Cumilla but yield increased in treatment T1 (6.07 t/ha in locationa a and 7.1 t/ha in location b) when Trichocompost was applied in seedbed @ 0.5t/ha before seeding at both locations and in T2 (6.9 t/ha) when root was dipped in *Trichoderma* powder formulation @10g/L H_2O before transplanting for 30 min + root dip in bacterial formulation @20 ml/L H_2O for 30 min before transplanting at location-b compared to T3 (control) (Fig.33 a and 21b). This yield increase was due to increase % effective tiller in T1 (3-20% in both locations) and in T2 (10-13% in location-b) over T3 (control) (Fig.34).

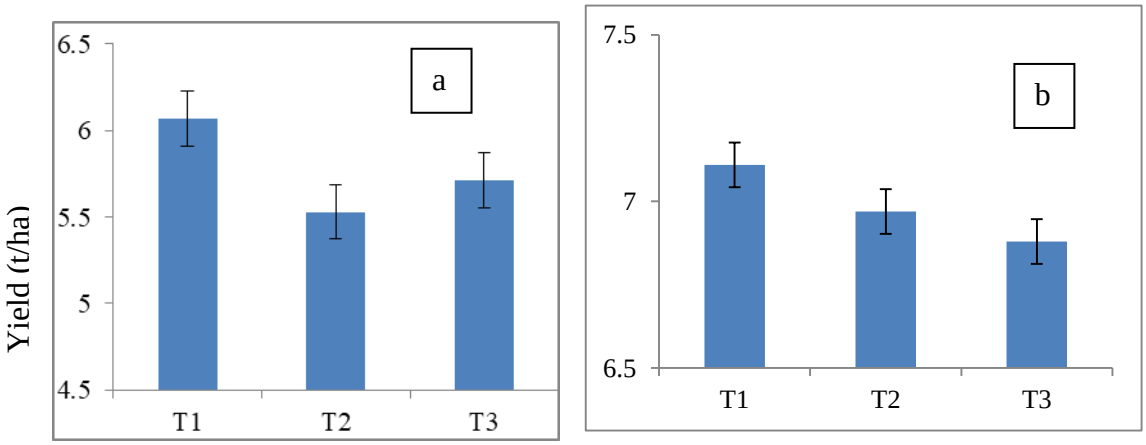


Fig. 33.Yield performance in different treatments applied for bakanae management at Cumilla regions (a=location 1, b=location 2) on BRRI dhan81 in Boro 2020-21.
**(T1 =Trichocompost was applied in seedbed @ 0.5t/ha before seeding) at both locations and in T2=root dip with *Trichoderma* powder formulation @10g/L H_2O was done before transplanting for 30 min + root dip in bacterial formulation @20 ml/L for 30 min before transplanting, T3= control).

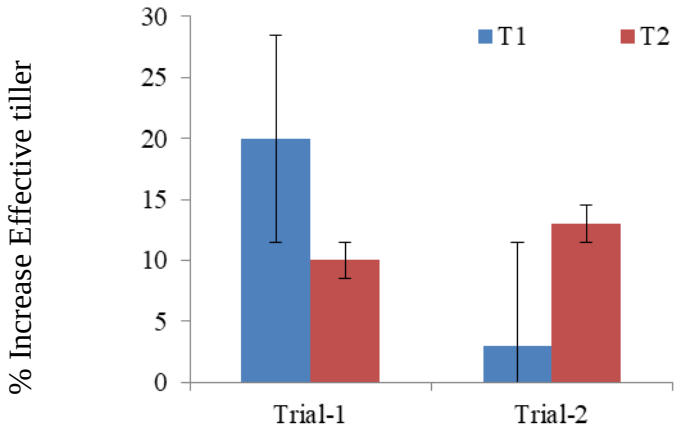


Fig.34. Effective tiller (%) increase compared to control at Cumilla on BRRI dhan81 in Boro 2020-21.**(T1 =Trichocompost was applied in seedbed @ 0.5t/ha before seeding) at both locations and in T2=root dip was done in *Trichoderma* powder formulation @10g/L H_2O before transplanting for 30 min + root dip was done in bacterial formulation @20 ml/L H_2O for 30 min before transplanting).

In Boro 2020-21, at Habiganj trials it was observed that yield increased in T1 at both locations (5.16 t/ha in location-a on BRRI dhan28 and 4.7 t/ha on BRRI dhan29) whenTrichocompost was applied in seedbed @ 0.5t/ha before seeding and in T2 (5.4 t/ha in locatio-a and 4.6 t/ha in location-b) when root was dipped in *Trichoderma* powder formulation @10g/L H_2O before transplanting for 30 min + root dip in bacterial formulation @20 ml/L H_2O for 30 min before transplanting in both locations compared to control treament T3 (5.03 t/ha in location-a and 4.41 t/ha in location-b) (Fig.35). This yield increase was due to increase grain weight/panicle in T1 (37g) and T2 (39g) compared to T3 (32g) (Fig.36). In location-a yield performance was better in T2 compared to T1 on BRRI dhan28 whereas, in location-b both T1 and T2 performed better compared to T3 on BRRI dhan29. From this trial it was observed that when root was dipped in *Trichoderma* powder formulation @10g/L H_2O + root dip in bacterial formulation @20 ml/L H_2O for 30 min before transplanting is effective to manage bakanae and thus suppot to increase yield (0.4 t/ha) in field condition.

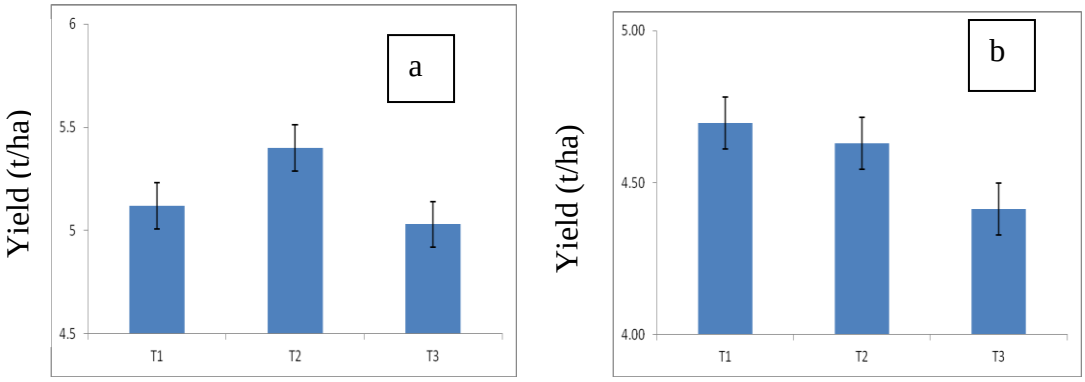


Fig.35. Yield performance in different treatments applied for bakanae management at Habiganj regions (a=location on BRRI dhan28, b=location on BRRI dhan29) in Boro 2020-21. **(T1 =Trichocompost was applied in seedbed @ 0.5t/ha before seeding) at both locations and in T2=root dip with *Trichoderma* powder formulation @10g/L H_2O was done before transplanting for 30 min + root dip in bacterial formulation @20 ml/L H_2O for 30 min before transplanting, T3= control).

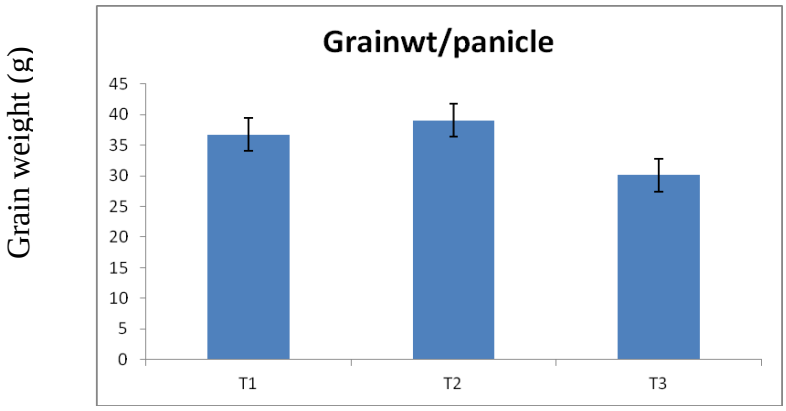


Fig.36. Grain weight/panicle in different treatment in location-a on BRRI dhan28 at Habiganj in Boro 2020-21. **(T1 =Trichocompost was applied in seedbed @ 0.5t/ha before seeding) at both locations and in T2=root dip with *Trichoderma* powder formulation @10g/L H_2O was done before transplanting for 30 min + root dip @20 ml/L H_2O in bacterial formulation for 30 min before transplanting, T3= control).

In first trial (location-a) at Gazipur, there was no significant variation observed for flag leaf length (FLL) in T1= Trichocompost and T2= no compost (control) treatments but significant variation was observed for flag leaf width (FLW) and panicle length (PL). Increase of FLW (14.52%) and PL (11%) due to application of Trichocompost in bakanae infected field is shown in Fig. 37.

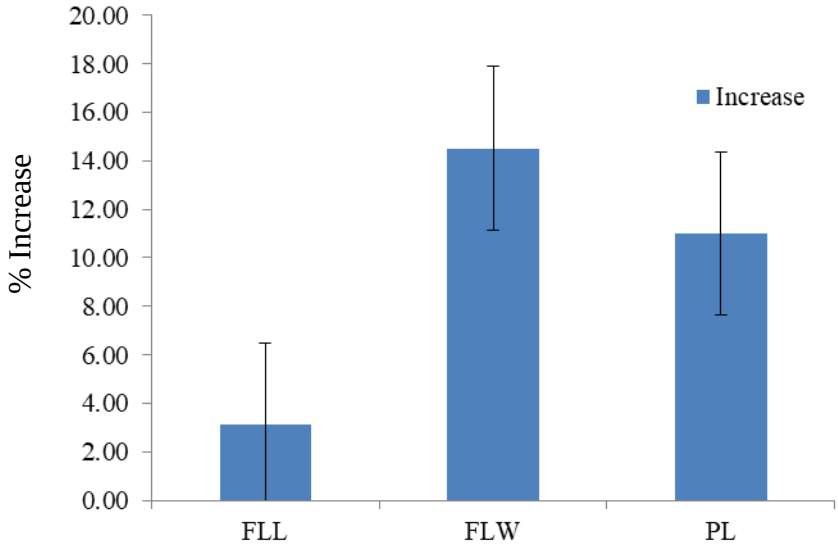


Fig. 37. Increase (%) flag leaf length, flag leaf width and panicle length after Trichocompost application compared to no Trichocompost (control) application in bakanae infected field at Gazipur in Boro 2020-21.

Grain/panicle (no) and 1000 grain wt. (g) was also increased and unfilled grain/panicle (no) was decreased in Trichocompost treated plots compared to no Trichocompost treated plots (Fig.38).

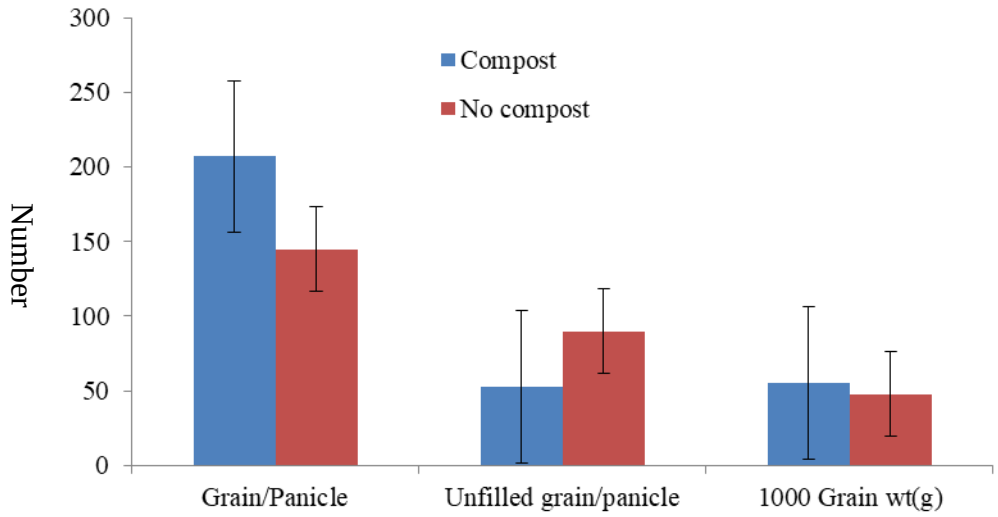


Fig.38. Different yield contributing characters status in Trichocompost versus no Trichocompost application in field condition in Boro, 2020-21 at Gazipur.

Moreover effective tillers/hill (12.2) were increased in Trichocompost treated plots compared to no Trichocompost treated plot (9.4) and thus contributed higher yield (8.1 t/ha) in Trichocompost treated plots compared to no Trichocompost treated plot (3.59 t/ha) (Fig.39).

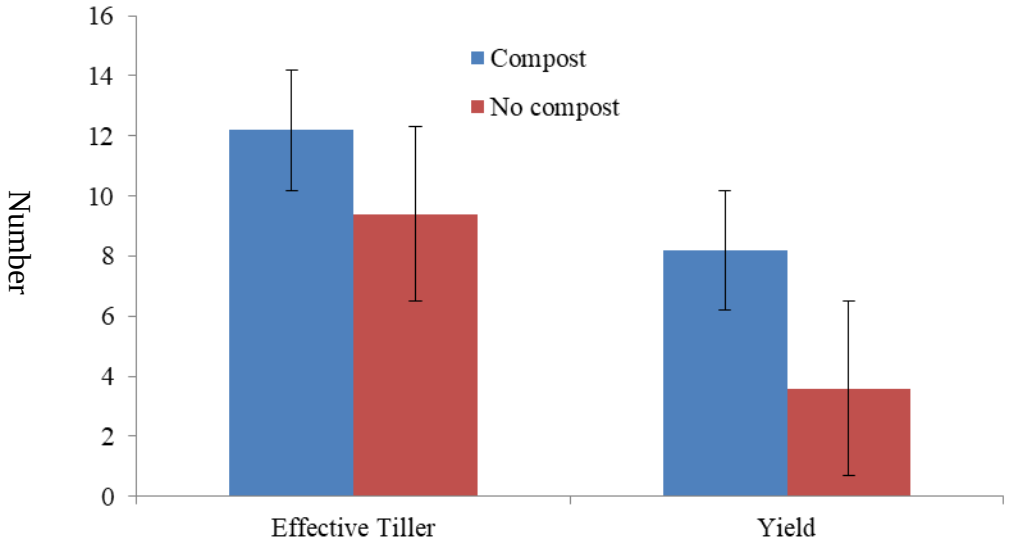


Fig.39. Effect of Trichocompost on effective tiller/hill and yield (t/ha) after Trichocompost application compared to no Trichocompost (control) application in bakanae infected field in Boro 2020-21, at Gazipur.

In second trial (location-b) at Gazipur it was observed that FLL (14.3%), FLW (7.9%) and PL (3.8%) also increased but not significantly when treated with bacterial biopesticide *Serratia* sp. compared to control (Fig.40).

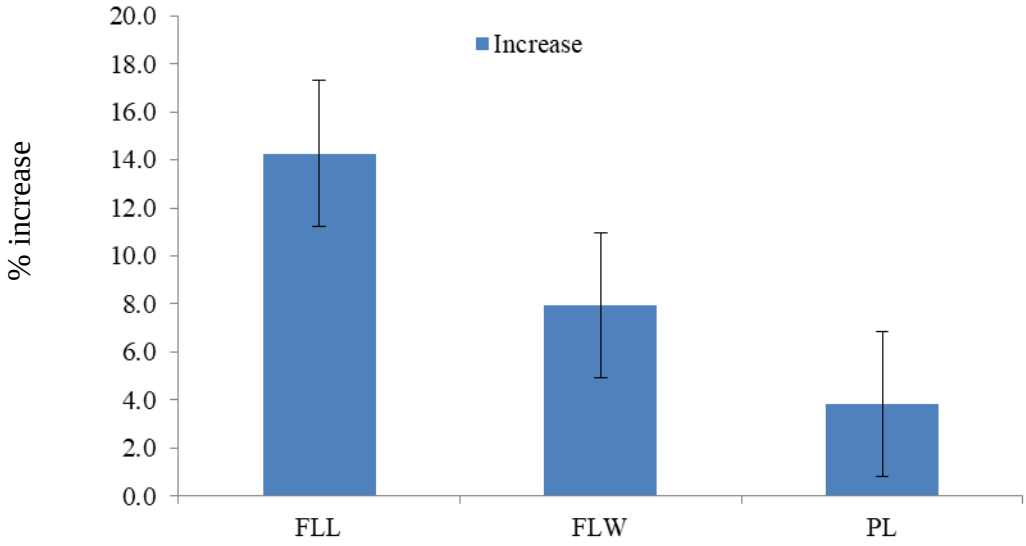


Fig.40. Increase over control (%) in flag leaf length, flag leaf width and panicle length after bacterial biopesticide (*Serratia* sp.) application compared to no Trichocompost (control) application in bakanae infected field at Gazipur in Boro, 2020-21.

Grain/panicle (no) and 1000 grain wt.(g) was also increased and unfilled grain/panicle (no) was decreased in bacterial biopesticide treated plots compared to control plot (Fig.41).

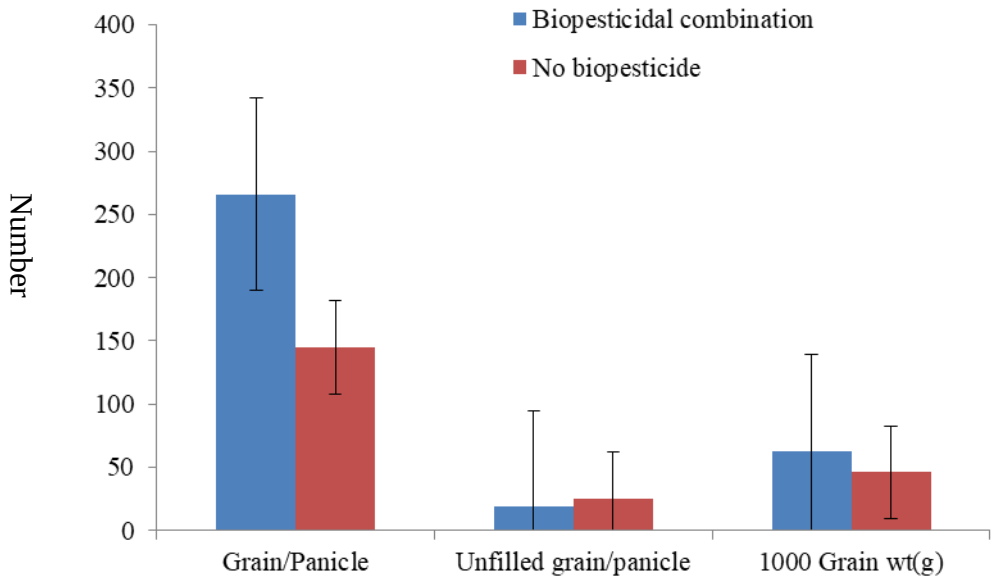


Fig.41. Different yield contributing characters status in *Serratia* sp. bicontrol bacteria versus no bicontrol bacteria application in field condition in Boro, 2020-21 at Gazipur.

Moreover effective tillers were increased and thus contributed higher yield (7.75 t/ha) in bacterial biopesticide treated plots compared to control plot (6.13 t/ha) (Fig.42). In trials at Gazipur it was observed yield may increase 2.0-4.5 t/ha (Fig.43) when formulated

biopesticides were applied compared to control plots in highly bakanae infected (60-70%) plants in field condition.

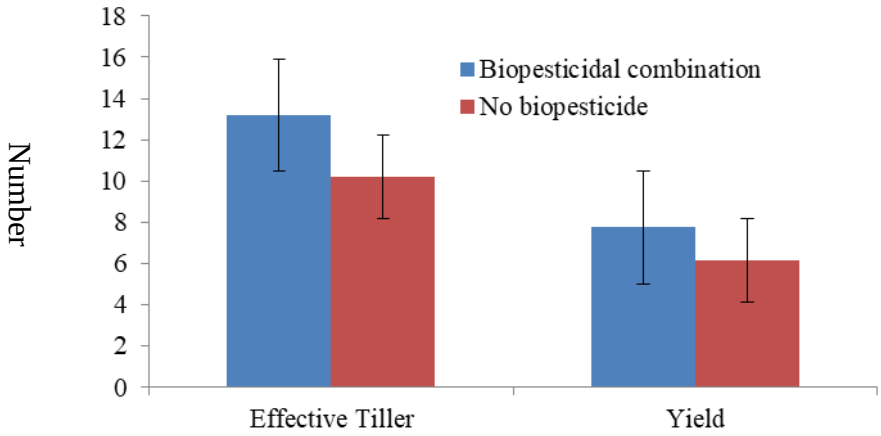


Fig. 42. Effect on yield (t/ha) and effective tillers after bacterial biopesticide (*Serratia* sp.) application compared to no bacterial biopesticide (control) application in field, Boro 2020-21 at Gazipur.

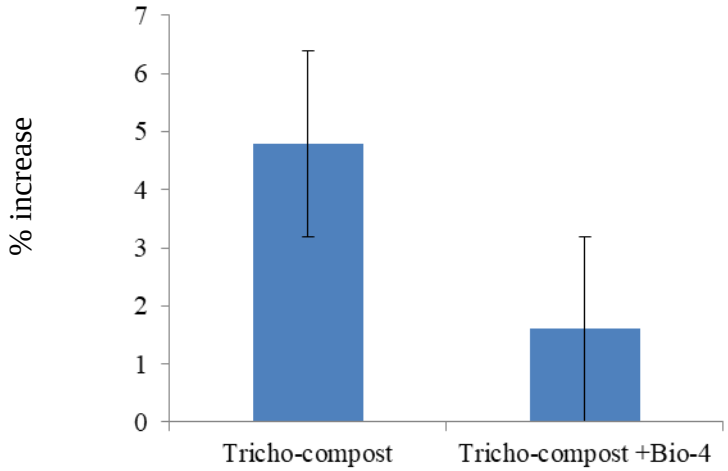


Fig.43. Yield increase (%) at Gazipur trial with formulated biopesticides compared to control plot in Boro 2020-21 at Gazipur.

In T. Aus disease incidence was not recorded at Habiganj after treatment application where disease incidence was 5-10% at initial stage before treatment application. It was observed that yield increased in different treatments compared to control in both Habiganj and in Cumilla (Fig 44 and Fig. 45). More yield increase was observed at Habiganj (1.1- 1.4t/ha) where bakanae infection was observed at initial stage at seedbed (Fig. 46). At Cumilla yield increase was found highest in T2 (0.8-0.9 t/ha) followed by T3 (0.7-0.6 t/ha) and least increase was in T1 (0.2-0.6t/ha) (Fig 45). Higher yield increase in Habiganj was due to more grain weight, and more filled grain /panicle compared to control treatment (Fig. 47). At both locations it was observed that grain weight/panicle increased in treated plots compared to control plot due to treatment effect on bakanae disease management. From this trial it is found that treatmts have more effcet on bakanae infected plants rather than non infected plants by increasing effective grain weight/panicle, increasing filled grain/panicle and decreasing empty grain/panicle in infected plants in Habiganj trial (Fig 47).

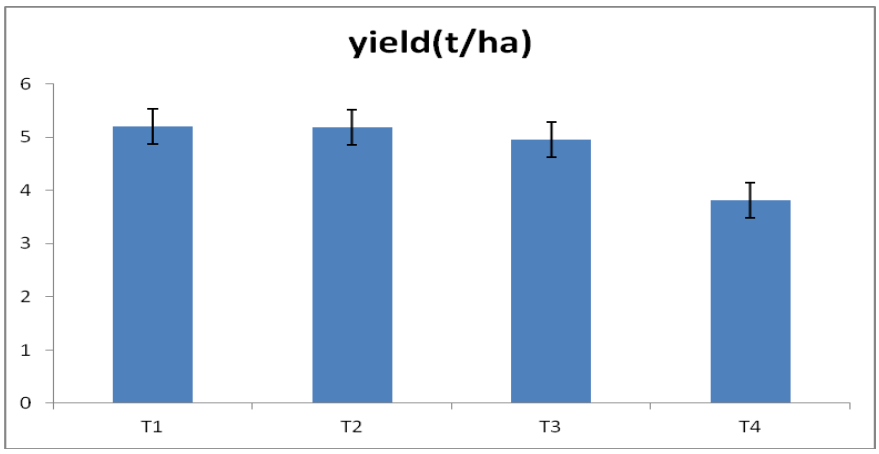


Fig.44. Comparison of yield in different treatments at Habiganj in T. Aus, 2021

**(T1: Trichocompost @2.0 t/ha, T2: T1 +*Trichoderma* treated root dip for 30 min, T3: T1+Bacterial biopesticide root dip for 30 min, T4: control)

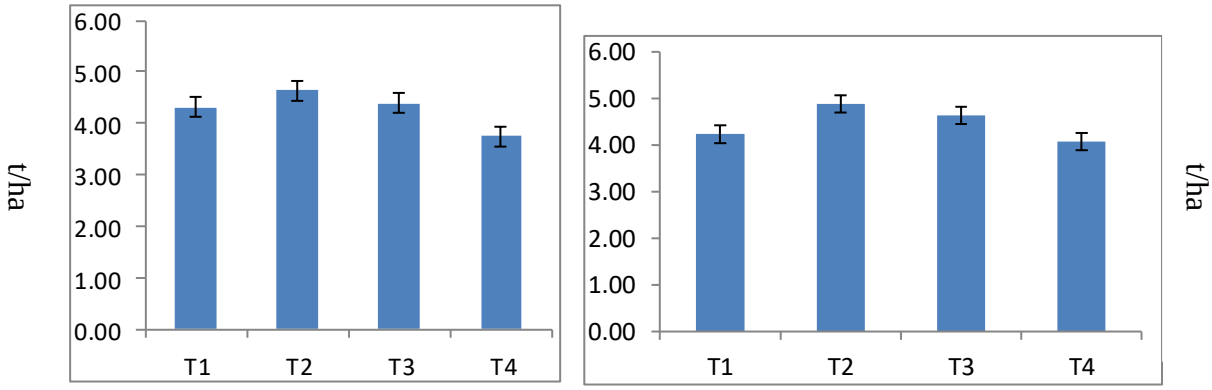


Fig.45. Comparison of yield in different treatments at Cumilla in T. Aus 2021 (a= location -1, b= location -2)

**(T1: Trichocompost @2.0 t/ha, T2: T1 +*Trichoderma* treated root dip for 30, T3: T1+Bacterial biopesticide root dip for 30 min, T4: Control)

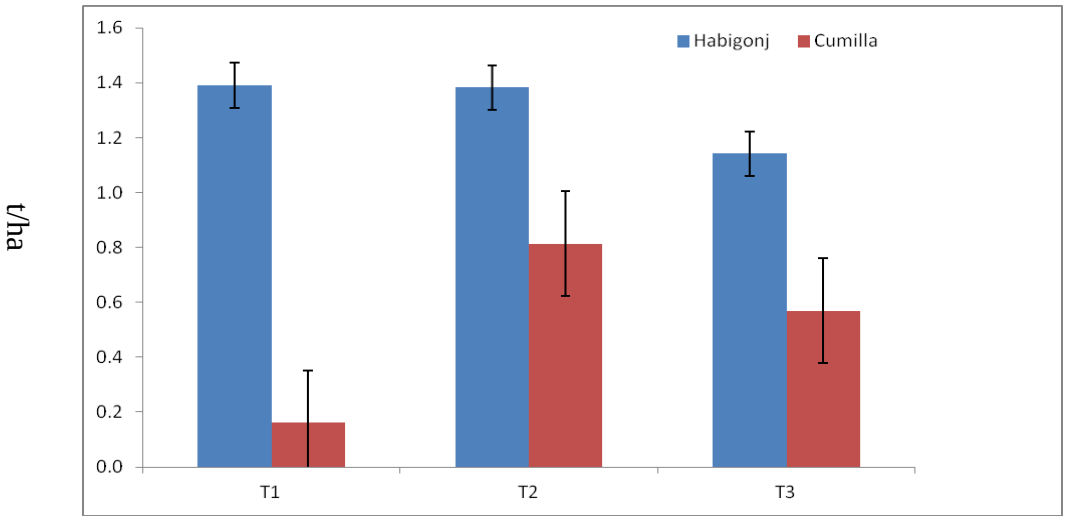


Fig.46. Yield increase (t/ha) on different treatments compared to control at Habiganj and at Cumilla in T. Aus, 2021 .

**(T1: Trichocompost @2.0 t/ha, T2: T1 +*Trichoderma* treated root dip for 30 min, T3: T1+Bacterial biopesticide root dip for 30 min)

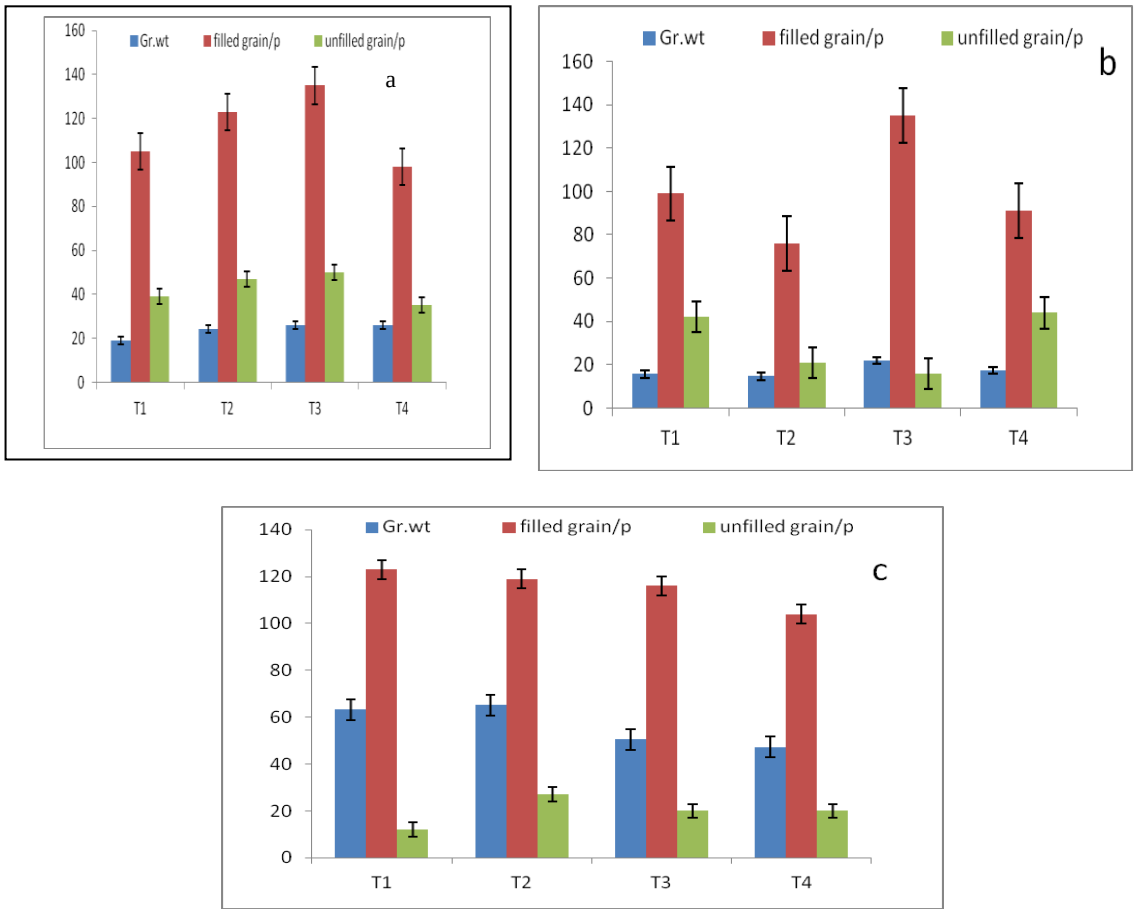


Fig.47. Comparison on yield contributing parameters at Cumilla in T. Aus, 2021(a and b) and in Habiganj (c)

**(T1: Trichocompost @2.0 t/ha, T2: T1 +*Trichoderma* treated root dip for 30 min, T3: T1+Bacterial biopesticide root dip for 30 min, T4: control)

In T. Aman 2021, disease incidence sharply decreased in T2 where bacterial biopestide was applied @ 20 ml/L H_2O after 3 weeks of treatment application whereas, after 6 weeks disease incidence was lower in T1 at Cumilla (Fig.48a). After 6 weeks of treatment application a number of diseased plants were observed in control (T4) plots followed by T1. On the other hand, it was observed that disease incidence was decreased in all treatments after 3 weeks of treatment application where disease incidence was lower (10%) (Fig.48b). This result again suggests that effectiveness of treatments is depended on degree of bakanae infecton. In T. Aman 2021 it was observed that yield was increased in all treatments compared to control in both varieties. More yield increase was observed in BRRI dhan32 compared to BR 22 (Fig 49). This higher increase in BRRI dhan32 was recorded where higher disease incidence (20%) was observed.

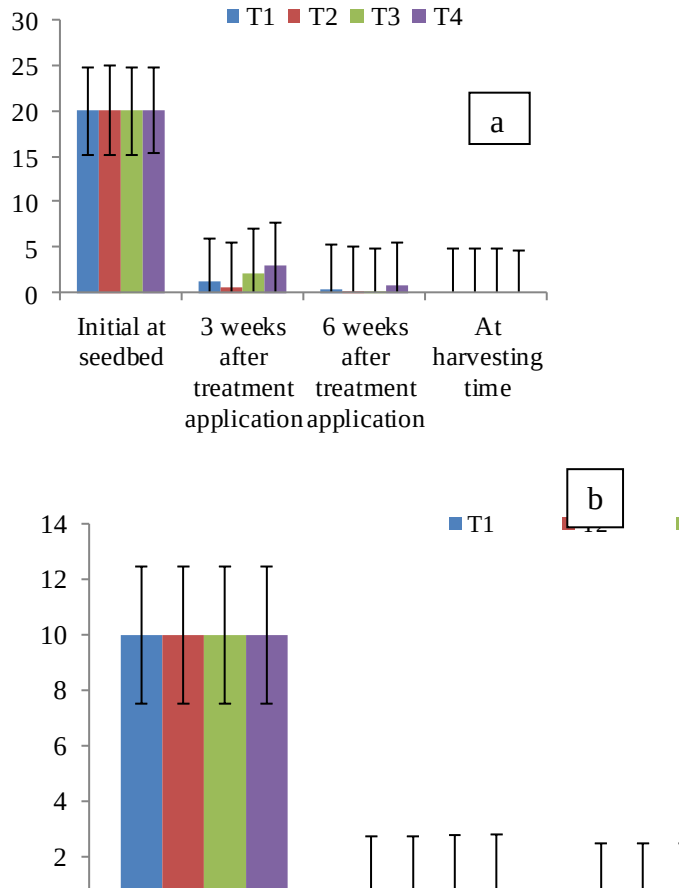


Fig.48. Senario of disease incidence in different treatments with times on BRRI dhan32(a) and on BR22(b) in T. Aman, 2021 at Cumilla

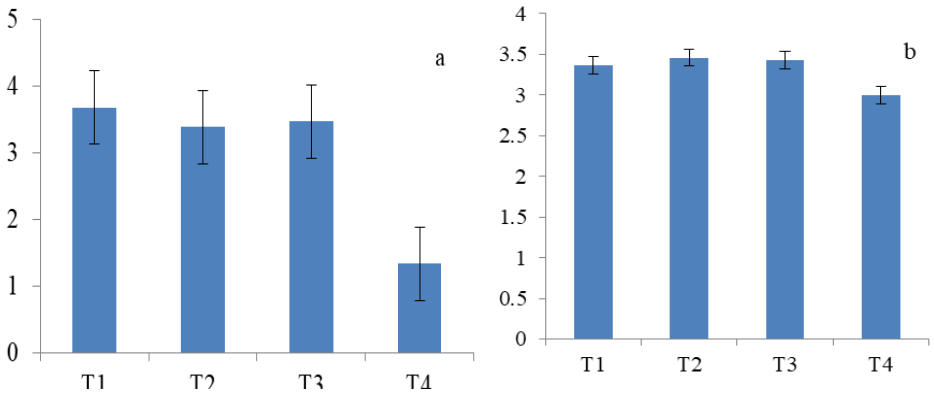


Fig 49. Comparison of yield in different treatments at Cumilla in T. Aman, 2021 (a= BRRI dhan32, b= BR22). T1: Trichocompost (1.5 t/ha), T2: bacterial biopesticide @20ml/L, T3: Trichocompost (1.5 t/ha) + bacterial biopesticide (spray), T4: control

At Habiganj disease incidence was not observed after treatment application in T. Aman 2021. Highest yield was recorded in all treatments compared to control in BRRI dhan49. Among the treatments trialed T1: Trichocompost @1.5 t/ha resulted higher yield (7.3 t/ha) followed by T3: Trichocompost @1.5 t/ha + Bacteria (spray) (6.8 t/ha) and lowest yield was observed in T4: control (5.6 t/ha) (Fig.50). This higher yield increase was due to increase of

flag leaf length and 1000 grain weight in T1 and in T3 compared to T4 (Fig.51). Considering two locations higher yield was increased (2.35 t/ha) where higher disease incidence (20%) was observed in BRRI dhan32 at Cumilla and thus a strong positive effective of treatments were found.

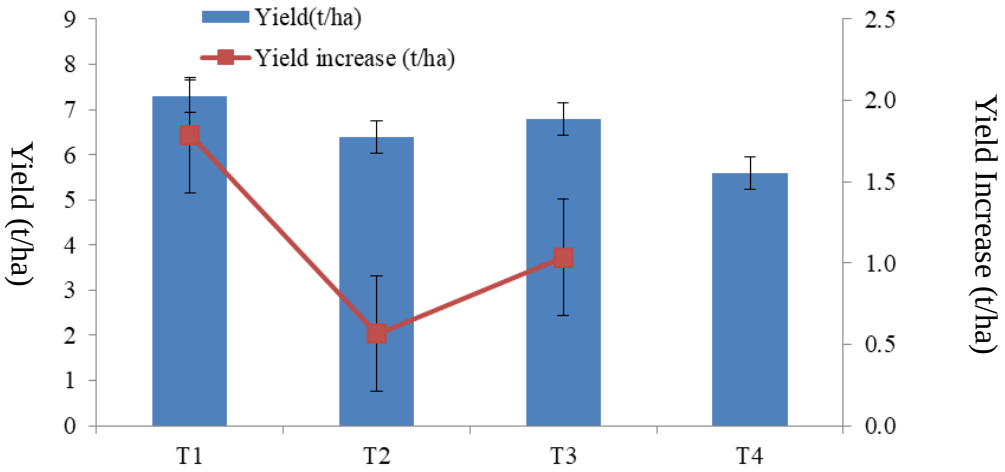


Fig. 50. Comparison of yield in different treatments at Habiganj in T. Aman, 2021 on BRRI dhan49. (T1: Trichocompost @1.5 t/ha), T2: Bacterial biopesticide spray @ 20ml/L H_2O, T3: Trichocompost @1.5 t/ha + Bacterial biopesticide spray@ 20ml/L H_2O, T4: control).

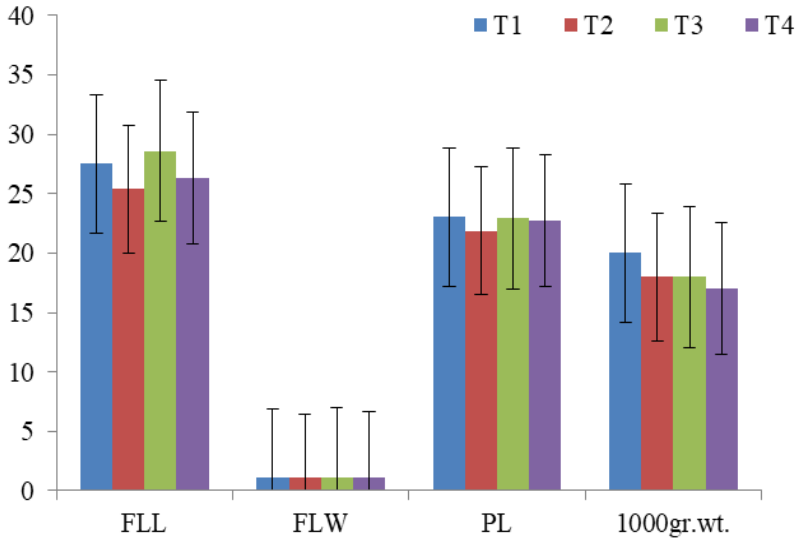


Fig.51. Comparison of yield contributing characters in different treatments at Habiganj in T. Aman, 2021 on BRRI dhan49. (T1: Trichocompost @1.5 t/ha), T2: Bacterial biopesticide spray @ 20ml/L H_2O, T3: Trichocompost @1.5 t/ha + Bacterial biopesticide spray@ 20ml/L H2O, T4: Control).

In case of nano trial in T, Aman 2021, it was observed that disease incidence decreased slowly with times up to 6 weeks after treatment application at Cumilla (Fig. 52). This might be for the time was too short to dip the roots. This result supported that the net house trial described earlier with nano treatment that 4-8 hr are required for root dip or seed treatment to get better result. Although disease incidence decreased slowly and statistically did not found

significant with treated versus non-treated but yield was increasd in nano treated plot (T1) (3.7 t/ha) compared to control (T2) plot (3.1 t/ha) at Cumilla (Fig.53).

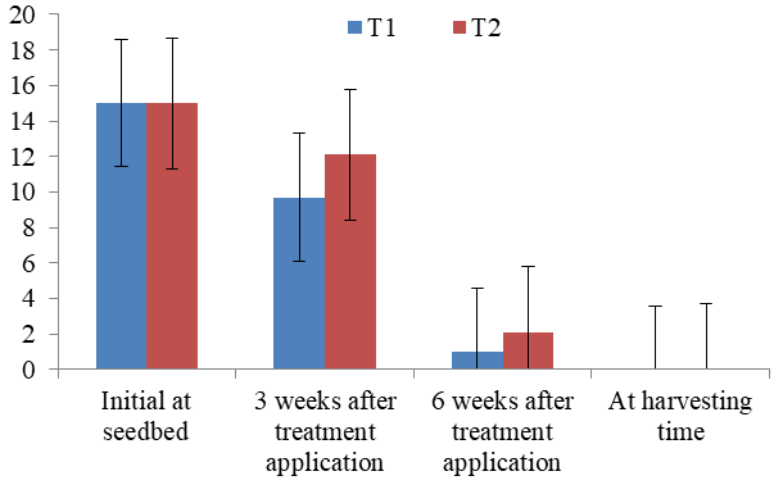


Fig.52. Scenario of disease incidence in neem leaf mediated nano treated versus non-treated plants with times on BRRI dhan32 in T. Aman, 2021 at Cumilla.

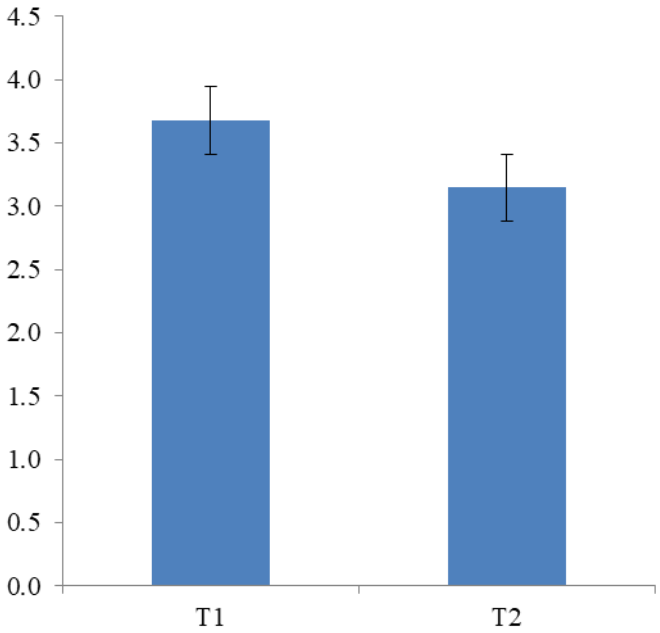


Fig.53. Yield performance after nano treatment in comparison with control in BRRI dhan32 in T. Aman 2021 (T1: Seedling root dip by in neem leaf extract mediated silver nano-particle 30 min, T2: control)

It was also observed that tiller/hill and effective tiller/hill increased in T1 (nano treated plot) whereas, decreased in T2 (untreated plot) (Fig.54.) that had ultimate effect on yield increase.

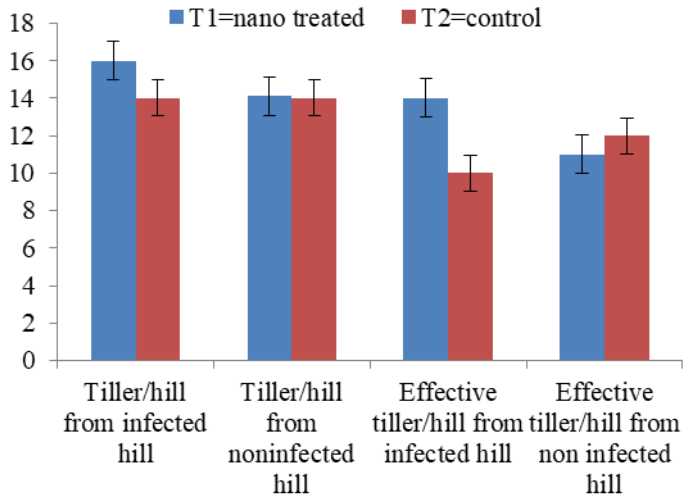


Fig. 54. Comparison on yield contributing parameters after neem leaf extract mediated silver nano-particle treatment in BRRIdhan 32 in T. Aman 2021 (average on 10 hill) at Cumilla.

In Boro 2021-22 at Trial-1, Gazipur it was observed that disease incidence decreased with times in T1 compared to T2 (Fig.55). Disease incidence was decreased up to 50% in treated plot (T1) after one week of treatment application where *Trichoderma* biopesticide based Trichocompost was applied. After four weeks of treatment applicattion it was observed that disease incidence decreased from 20% to 1%. On the other hand, disease incidence was also decreased in control plots but slowly and thus hampered the infected plants to grow. At harvest no bakanae was observed in both treated and non treated plots.

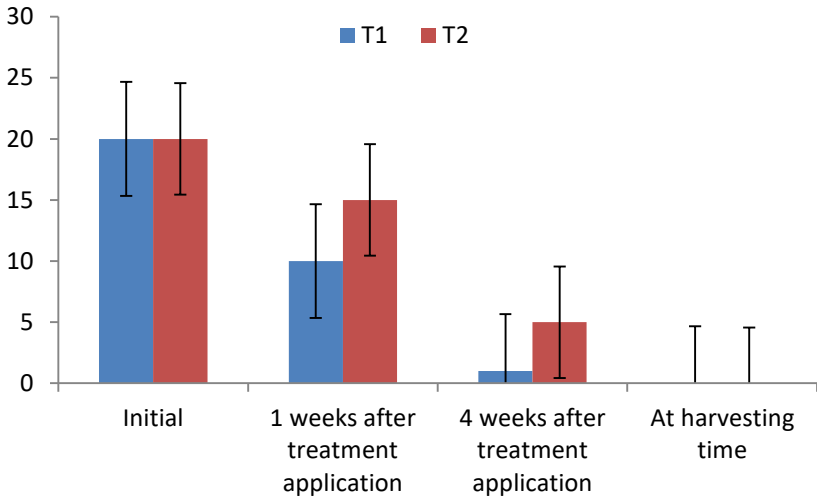


Fig 55. Scenario of bakanae incidence on different treated plants with times in Boro 2021-22, on kataribhog at Gazipur trial-1.
(T1=*Trichoderma* biopesticide based Trichocompost @1.5 t/ha, T2=control).

Higher yield was recorded in T1 (7.1 t/ha) where *Trichoderma* biopesticide based Trichocompost @1.5 t/ha used compared to T2 (5.9 t/ha) where no treatment (control) was applied (Fig. 56). Yield was increased 1.3 t/ha in T1 compared to T2.

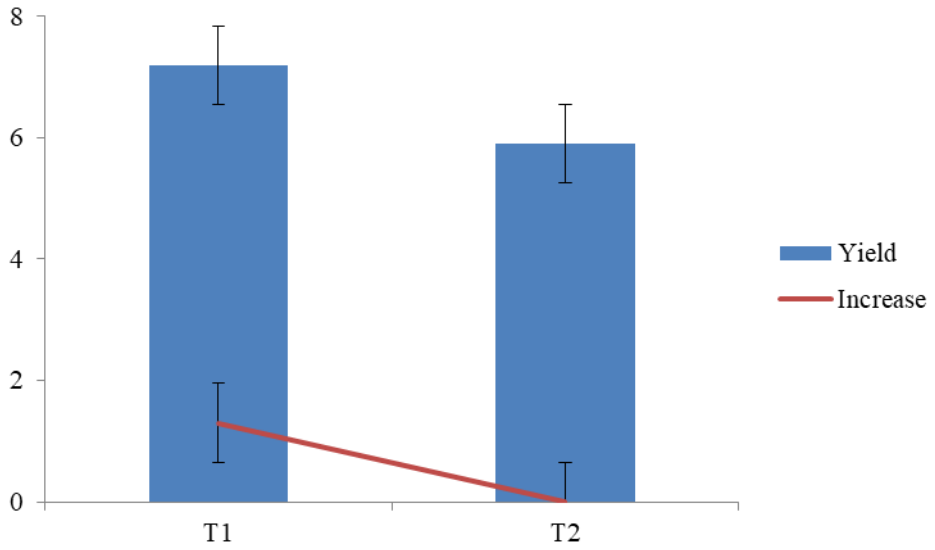
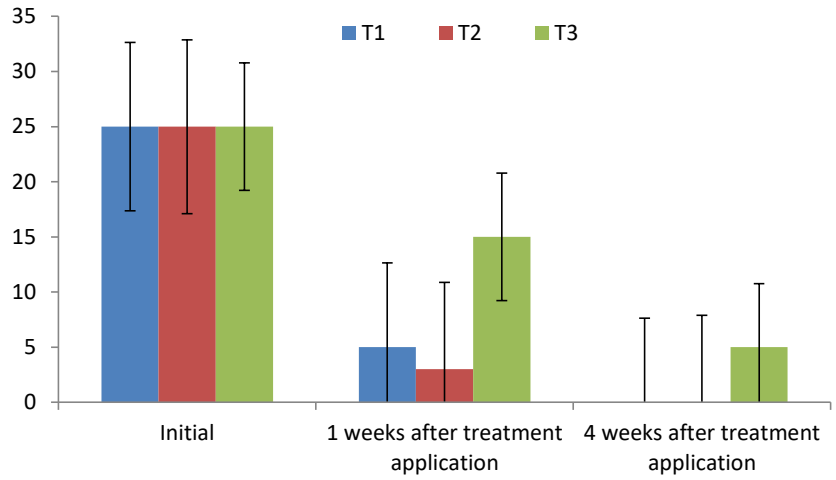


Fig 56. Yield performance in different treated plants in boro 2021-22 on kataribhog at Gazipur, Trial-1.
(T1=*Trichoderma* based biopesticide Trichocompost @1.5 t/ha, T2=control).

Hence, *T. asperelloides* has prevented pathogen development and triggered hyperparasitism on phytopathogens and thereby increased yield by managing bakanae disease. A negative relationship between *Trichoderma* and *Fusarium* richness across the land use types was obserbed by Maina *et al*., (2016). The authores also reported that higher abundance of *Fusarium* was high in the intensely cultivated lands where *Trichoderma* occurrence was low. Moreover, *Trichoderma* has ability to protect plant pathogens by stimulate different resistance mechanisms including hypersensitive resistance (HR), Induced systemic resistance (ISR) and Systemic accuired resistance (SAR) that results to increase the concentration of metabolites and enzymes like PAL, CHS (Chalcone Synthase) and phytoalexins those are related to defense mechanism (Mohiddin *et al*., 2010).

Sohel and Ghosh (2021) also reported that application of Trichocompost significantly increased organic matter, available N, P, K and S contents in soil over their initial value. Moreover, application of Trichocompost increased the nutrient holding capacity of the soil by enhancing the soil organic matter and thus had great advantage on increasing the growth and productivity of the crop and on improving the available nutrient contents in soil which is essential for sustaining crop productivity (Marimuthu *et al*., 2014).

In Boro 2021-22 in Trial-2 at Gazipur, it was observed that disease incidence was decreased with times and highest DI decreased was observed at T2 (bacterial biopesticide spray) treatment followed by T1 (dodder stem extract mediated silver nano particle) in BRRI dhan28 (Fig.57). After four weeks of treatment application, it was observed that no bakanae disease symptom was observed in T1 and T2 where dodder stem extract mediated silver nano particle and bacterial biopesticide sprayed respectively. On the other hand, after 4 weeks of treatment application bakanae symptom was visivle (5%) in control plots (T3). This decreasing DI was reflected on yield. Highest yield increased was observed in T2 (9.4 t/ha) followed by T1 (8.1 t/ha) and lowest was obserbed in T3 (7.2 t/ha) (Fig.58).



.

Fig.57. Scenario of bakanae incidence on different treated plants with times in Boro 2021-22 on BRR dhan28 at Gazipur Trial-2.

(T1=dodder stem extract mediated silver nano particle spray, T2=bacterial biopesticide spray, T3=control).

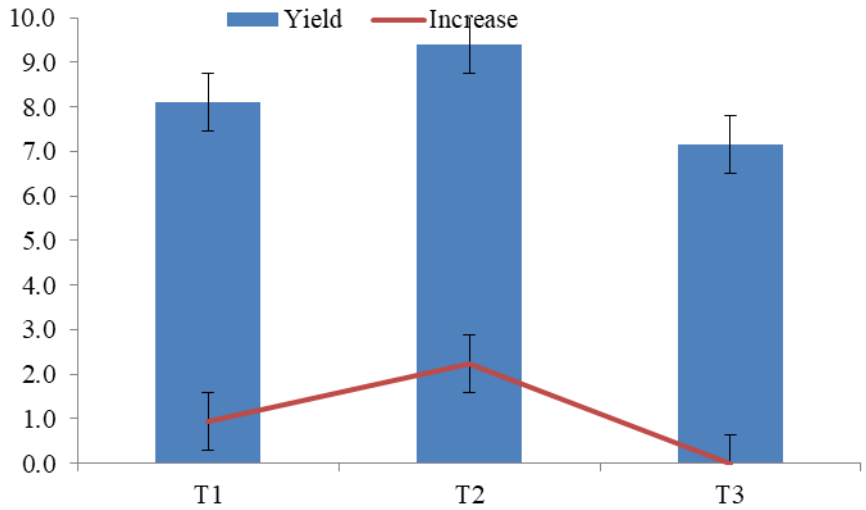


Fig.58. Yield performance in different treated plants in boro 2020-21 on BRRI dhan28 at Gazipur, Trial-2.

(T1=dodder stem extract mediated silver nano particle spray, T2=bacterial biopesticide spray, T3=control).

In Boro 2021-22 in Trial -3 conducted at Gazipur, with BRRI dhan92, it was observed that DI sharply decreased in T1 (*Trichoderma* biopesticide based Trichocompost) application followed by T2 spray (dodder based nano particle) after one week compared to control (T3) (Fig.59). After four weeks of treatment application no bakanae symptom was observed either in treated and non treated plots. Highest yield was increased in T1 (8.64 t/ha) followed by T2 (7.29 t/ha) and lowest in T3 (5.46) (Fig.60). Yield was increased due to treatments ability to reduce bakanae incidence at early stage of treatment application.

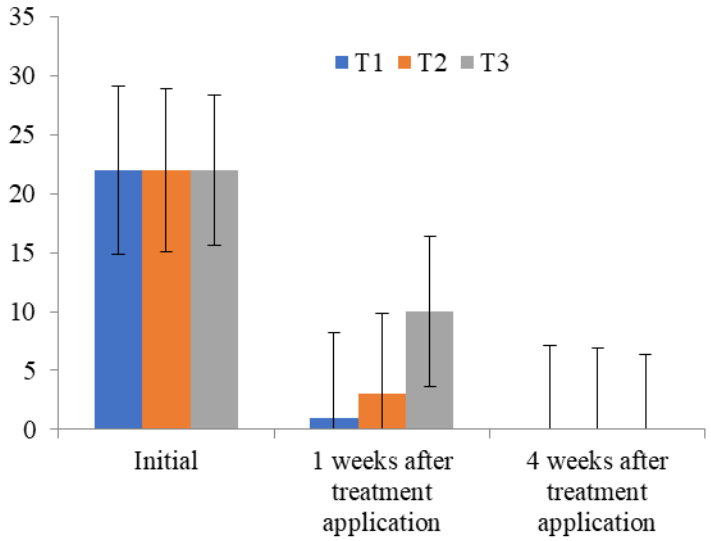


Fig. 59. Scenario of bakanae incidence on different treated plants with times in Boro 2021-22 on BRRI dhan92 at Gazipur trial-3.
(T1= *Trichoderma* biopesticide based Trichocompost, T2= dodder stem extract mediated silver nano particle spray, T3= control).

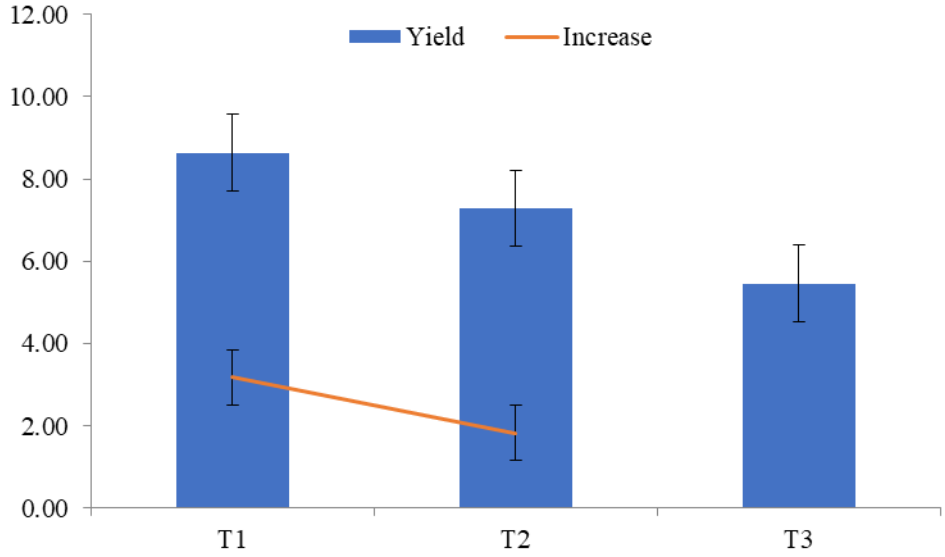


Fig.60. Yield performance in different treated plants in Boro 2021-22 on BRRI dhan92 at Gazipur, Trial-3.
(T1= *Trichoderma* based biopesticide Trichocompost, T2= dodder stem extract mediated silver nano particle spray, T3= control).

In Trial-3, it was also observed that yield increase in T1 and T2 was due to increased tiller/hill and effective tiller/hill which was due to ultimate effect by application of Trichocompost and dodder stem extract mediated silver nano particle (Fig.61).

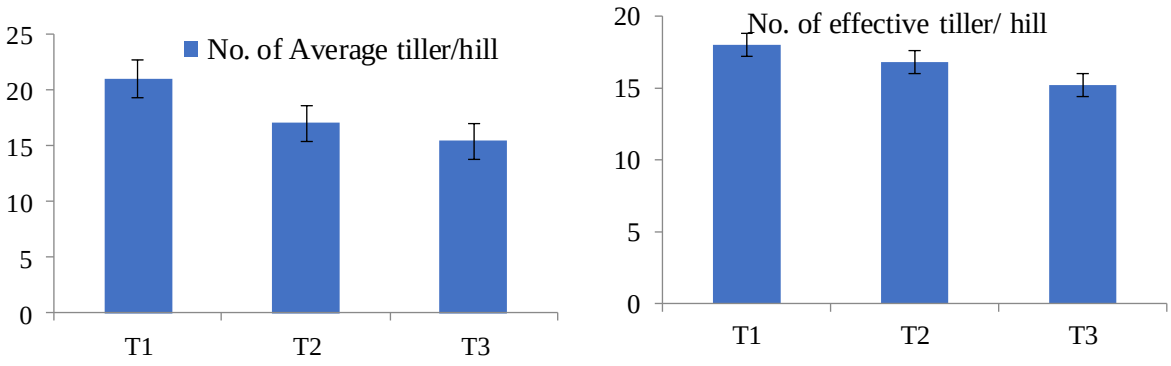


Fig.61. Scenario of average tiller/hill and average effective tiller/hill in different treated plants in boro 2021-22 on BRRI dhan92 at Gazipur, Trial-3.
(T1= *Trichoderma* based biopesticide Trichocompost, T2= dodder stem extract mediated silver nano particle spray, T3= control).

In Boro 2021-22 in Trial-4, it was observed that up to four weeks infected plants were seemed to death at both treated and no treated plots. After 8 weeks of transplanting dodder stem extract based nano particle treated plants (T1) starting to survive by exerting new tillers (>50%) whereas, non-treated plants (T2) survived a few (<10%) (Fig.62). Moreover, survival plants having delayed panicle initiation and delayed ripening. This delayed time was required for the regain energy for tiller proliferation.

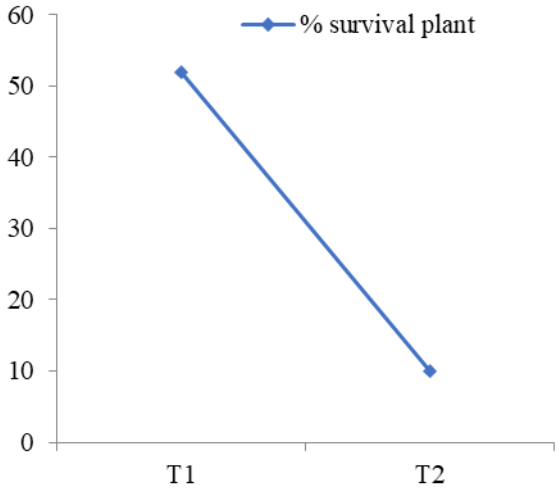


Fig.62. Survival (%) of plants after treatment with dodder stem extract mediated nano particle using root dip method in Boro 2021-22 on BRRI dhan92 at Gazipur, Trial-4
(T=Nano treated; T2= None treated).

This result is found better compared to result obtained from Habiganj trial in T. aman season 2020. Moreover, it is found that dodder stem extract mediated nano particle performed better compared to *Trichoderma* biopesticide based Trichocompost and bacterial biopesticide.

Furthermore, incidence of bakanae plants were decreased in nano treated plants (T1) compared to non treated plants (T2) with times (Fig.63). After eight weeks of nano treatment bakanae incidence was reduced from 22% to 5% whereas, in control plants incidence was found about 18% (Fig.63). At harvesting period no bakanae symptom was observed in both treated and no treated plants.

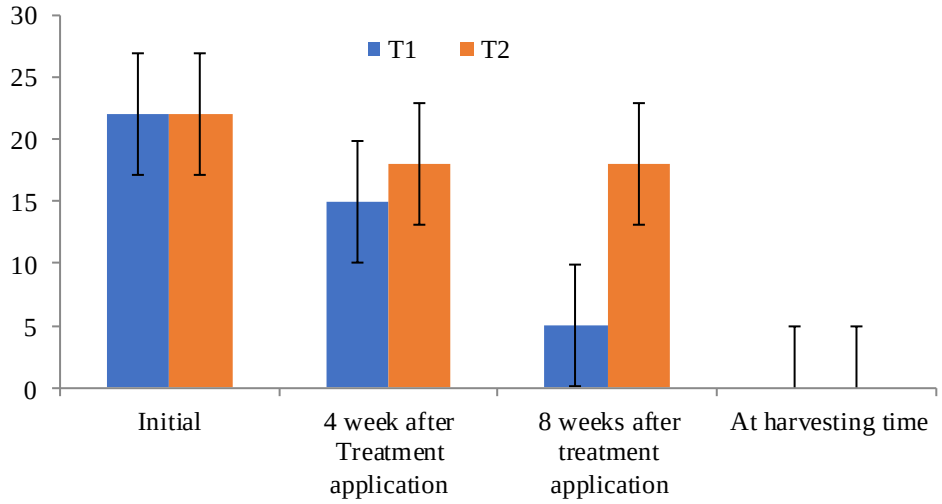


Fig. 63. Scenario of bakanae incidence on nano treated and non-treated plants with times after treatment with dodder stem extract mediated nano particle using root dip method in Boro 2021-22 on BRRI dhan92 at Gazipur, Trial-4
(T=Nano treated; T2= None treated).

Yield was also increased in dodder stem extract mediated nano treatment (T1) (12.84 g/hill) compared to control (T2) (5.70g/hill). It was observed that yield increased more than double in T1 compared to T2 (Fig.64) and this increased yield was due to increased tiller/hill and increased effective tiller/hill (Fig.65).

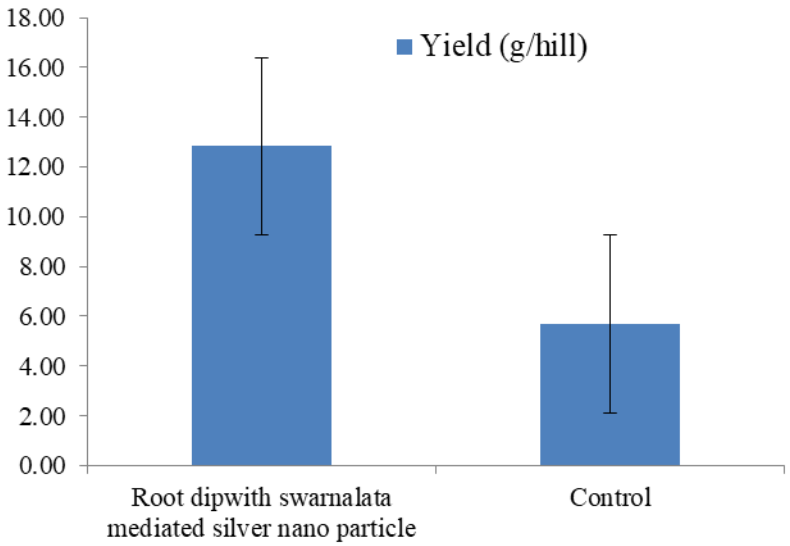


Fig.64. Yield (g/hill) performance in nano treated plant compared to non-treated plant after treatment with dodder (swarnalata) stem extract mediated nano particle using root dip method in Boro 2021-22 on BRRI dhan92 at Gazipur, Trial-4.
(T1=Nano treated; T2= None treated).

Number of average tiller/hill (21.4) and number of effective tiller/hill (20.2) was found increased in T1 (dodder stem extract mediated silver nano particle) compared to T2

(tiller/hill-12, effcetive tiller/hill= 9.3) (Fig.51). It was noticeable that almost all tillers were able to produce grain in T1 (nano treated) whereas, only 50% tillers were able to produce grain in T2 (control).

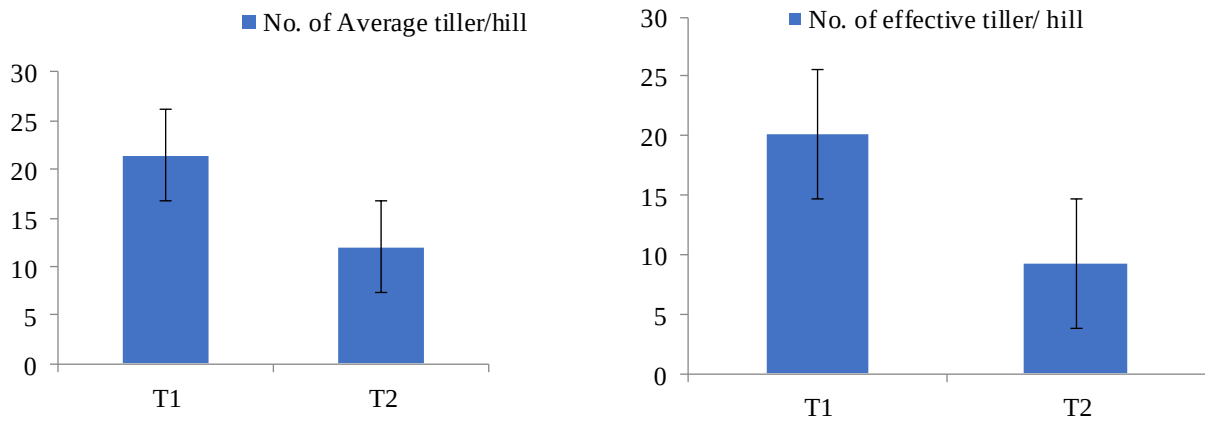


Fig.65. Number of average tiller and effecctive tiller/hill in nano treated versus none treated treatment after treatment with dodder stem extract mediated nano particle using root dip method in Boro 2021-22 on BRRI dhan92 at Gazipur, Trial-4.
(T=Nano treated; T2= None treated).

At Cumilla in Trial-1 conducted in Boro 2021-22, DI was also decreased in treated and control plots with time on BRRI dhan48 (Fig.66). After four weeks of treatment application some bakanae infection was still observed in control plots but there was no bakanae infected plants observed in treated plots. At harvest no bakanae symptom was observed in any treated plots.

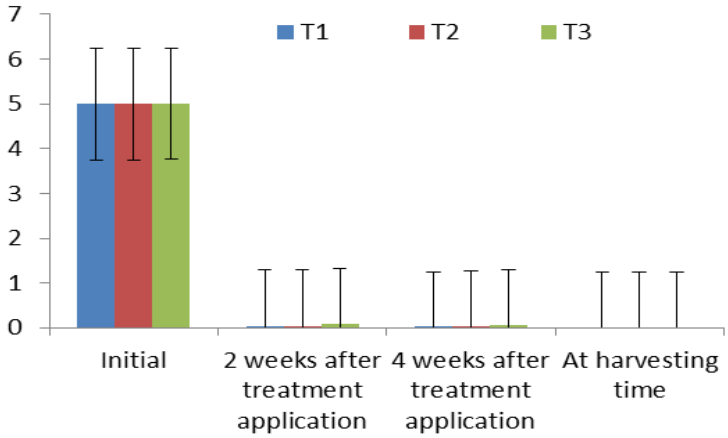


Fig.66. Scenario of bakanae incidence with times after different treatments used on BRRI dhan48 at Cumilla Trial-1 in Boro, 2021-22.
(T1= *Trichoderma* based biopesticide Trichocompost @1.5t/ha, T2= bacterial biopesticide spray @20ml/L H_2O, T3= control)

It was also observed that yield increased in T1 (7.4 t/ha) when *Trichoderma* biopesticide based Trichocompost applied followed by T2 (6. 8 t/ha) when bacterial biopesticide spray)

and lower yield observed in T3 (6.6 t/ha) when no treatment was applied (Fig.67). Yield was increased 0.2-0.8 t/ha compared to control. Yield increase was depeneded on degree on disease incidence. As disease incidence was not so much higher (5%) in seedbed, therefore, yield increase was not too high compared to control plot.

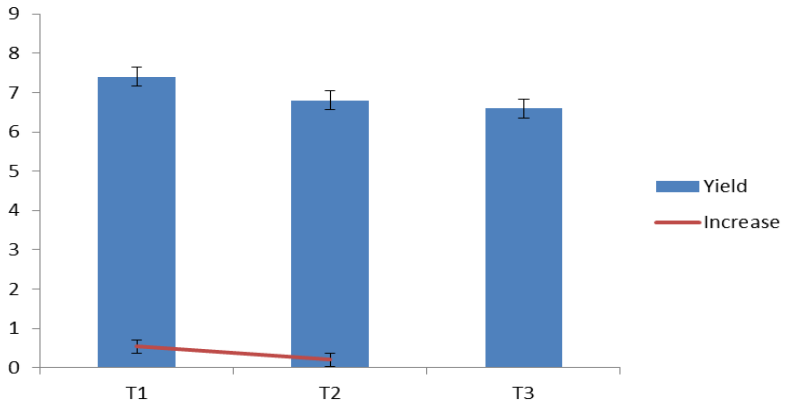


Fig.67. Yield performance of BRRI dhan48 after different treatments used on bakanae infected plants in Boro, 2021-22 at Cumilla, Trial-1.
(T1= *Trichoderma* biopesticide based Trichocompost @1.5t/ha, T2= bacterial biopesticide spray @20ml/L H_2O, T3= control)

Moreover, higher tiller/hill and higher effective tiller/hill was observed from infceted hills where treatment T1 (*Trichoderma* biopesticide based Trichocompost) and T2 (Bacterial biopesticide spray) applied compared to control (T3) (Fig. 68a). This increased tiller/hill and increased panicle/hill had effect on yield increase. It was also observed that lower tiller/hill and lower effective tiller/hill prduced from non infected hill. Higher effective tiller was produced in T1 followed by T2 (Fig.68b).

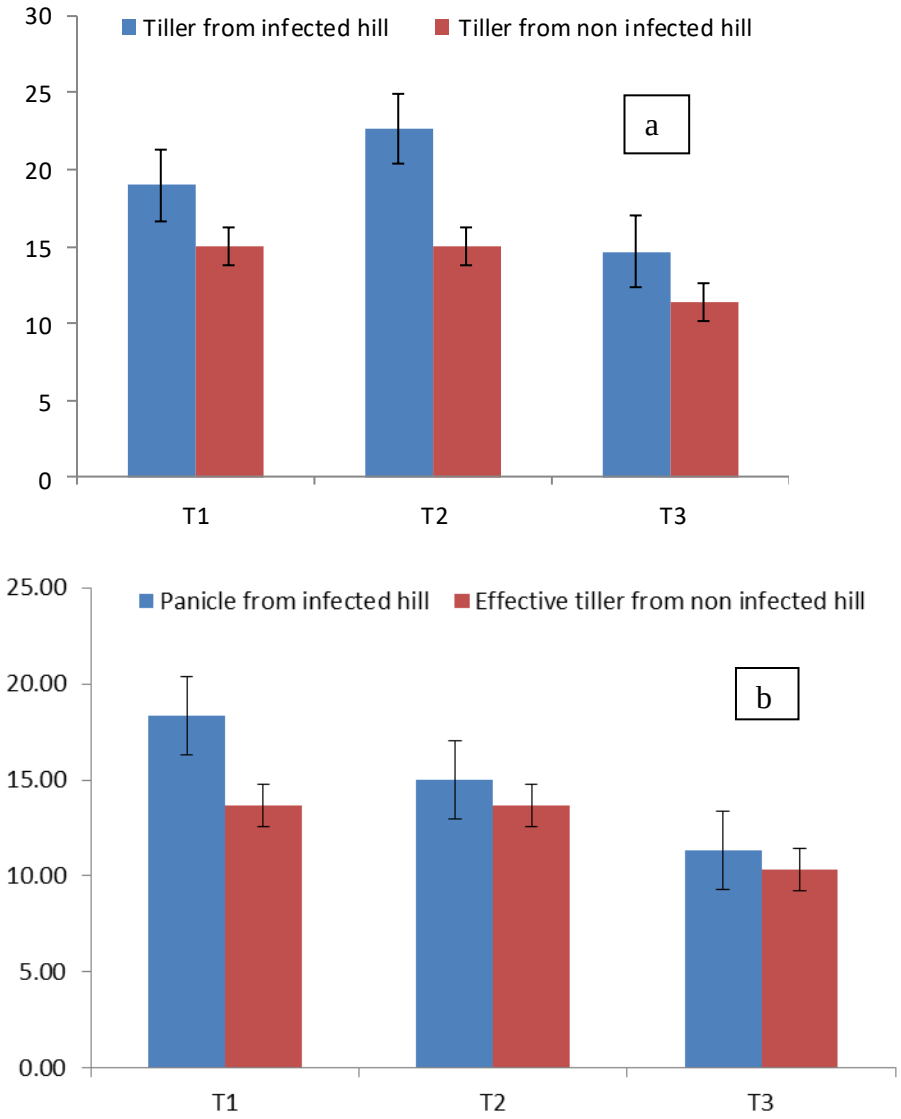


Fig. 68. Scenario of average tiller (a) and average effective tiller production (b) from infected versus non-infected bakanae plants of BRRI dhan48 on different treatments in Boro, 2021-22 at Cumilla Trial-1.

(T1= *Trichoderma* based biopesticide Trichocompost @1.5t/ha, T2= Bacterial biopesticide spray @ 20ml/L water, T3= Control)

At Cumilla in Trial-2, performance of two synthesized nano particles were evaluated compared with control. DI was also decreased with times in all treatments (Fig.69). At harvest no symptom was visible in any treated plots.

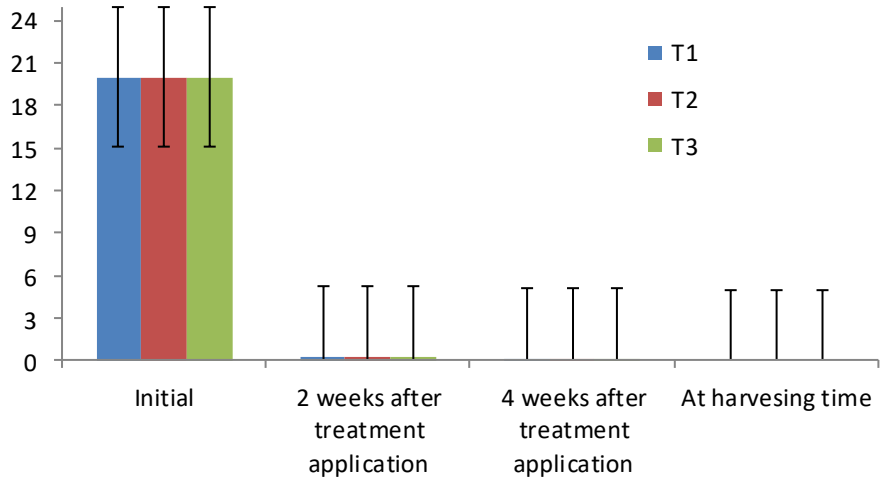


Fig.69. Scenario of bakanae incidence on different treatments with times at Cumilla Trial-2 on BRRI dhan86 in in Boro, 2021-22 at Cumilla Trial-2.
(T1= root dip with neem leaf-mediated ilver nano partcle, T2= root dip with dodder stem extract mediated silver nano partcle, T3=control).

Yield was increased in both nano treated plots (T1 and T2) compared to control plot (T3). Among the nano treated plots higher yield increased in T2 (6.36 t/ha) followed by T1 (6.16t/ha) where roots of infected plants were dip in dodder-mediated silver nano particle. Lowest yield was found in T3 (5.75 t/ha) where no treatment was applied. Yield increase was 0.4-0.61 t/ha in treated plots over control (Fig.70.).

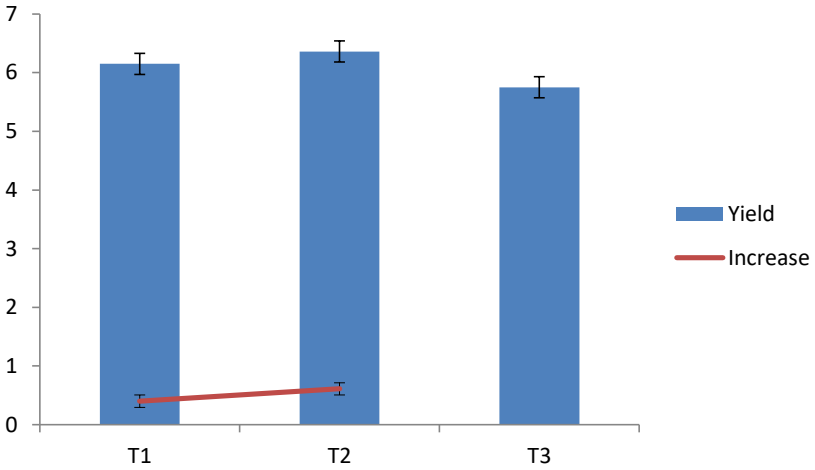


Fig.70. Yield of BRRI dhan86 on different treatments in bakanae infected plants in Boro, 2021-22 at Cumilla Trial-2.
(T1= root dip with neem leaf-mediated silver nano partcle, T2= root dip with dodder-mediated silver nano partcle, T3=control).

Higher yield in nano treated plots were due to increased higher tiller/hill and higher effective tiller/hill production compared to non treated plots from infected versus non-infected hills (Fig 71). More interestingly it was observed that although some tiller increased in non infected plants in control treatment plots but there was no variation for panicle formation in control treated plants. On the other hand, it was clearly observed that higher tiller and higher

effective tiller/panicle formed in infected plants compared to non infected plants when infected plants were treated with formulated nano particles (Fig 71).

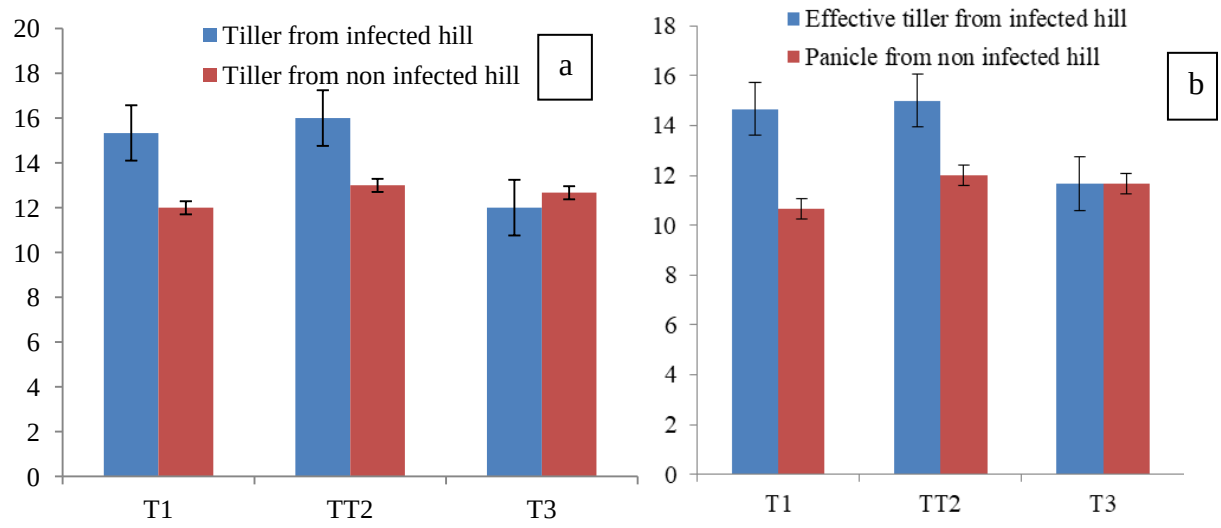


Fig.71. Scenario of average tiller and average effective tiller production from infected versus non-infected bakanae plants of BRRI dhan86 on different treatments in Boro, 2021-22 at Cumilla Trial-2.
(T1= neem leaf extract mediated silver nano particle following root dip method, T2= dodder stem extract mediated silver nano particle following root dip method and T3= control).

At Cumilla in Trial-3, DI was also decreased with times in all treated plots (Fig.72). At harvest no symptom was visible in any treated plot where T1= *Trichoderma* biopesticide based Trichococompost @1.5t/ha, T2=dodder stem extract mediated silver nano particle following root dip method, T3= bacterial biopesticide sparay @20mL/L and T4= control was applied in BRRI dhan86. At Cumilla, treatments were applied at field during transplanting after symptom appeared at seedbed.

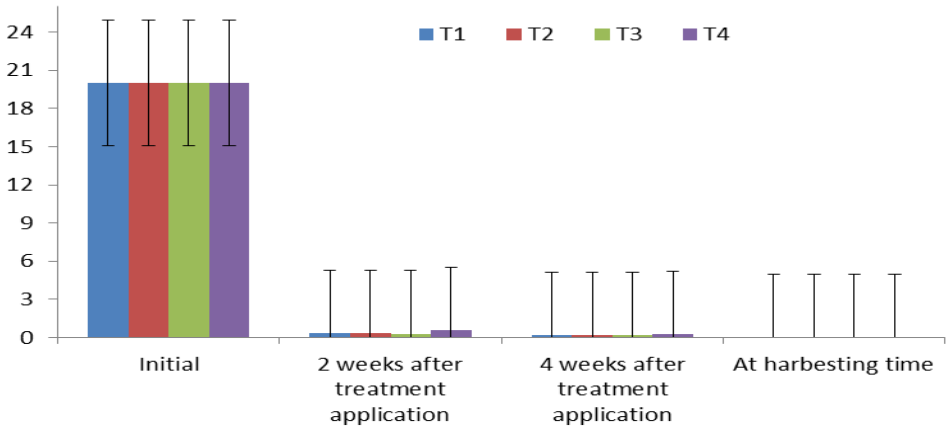


Fig.72. Scenario of bakanae incidence on different treatments with times at Cumilla Trial-3 on BRRI dhan86 in Boro, 2021-22.
(T1= *Trichoderma* based biopesticide Trichocompost @1.5t/ha, T2= dodder stem extract mediated silver nano particle following root dip method, T3= bacterial biopesticide spray @ 20ml/L water, T4= control).

Although a minimum level of disease incidence was obsereved in all treated plots after four weeks of treatment application but yield increased in all treated plots (T1, T2 and T3) compared to control plot (T4). Among the nano treated plots higher yield increased in T2 (5.85 t/ha) where roots of infected plants were dip in dodder stem extract-mediated silver nano partcle followed by T1 (5.8 t/ha) where *Trichoderma* biopesticide based Tricocompost @1.5t/ha applied during final land preparation and T3 (5.41 t/ha) where bacterial biopesticide was sprayed on infected plants after transplanting @20ml/L water (Fig.73). Lowest yield observed in T4 (5.16 t/ha) where no treatment was applied. Yield increased from 0.25-0.7 t/ha in treated plots (Fig.73). Dodder stem extract-mediated silver nano partcle and *Trichoderma* biopesticide based Tricocompost @1.5t/ha gave as similar yield without any statitical difference.

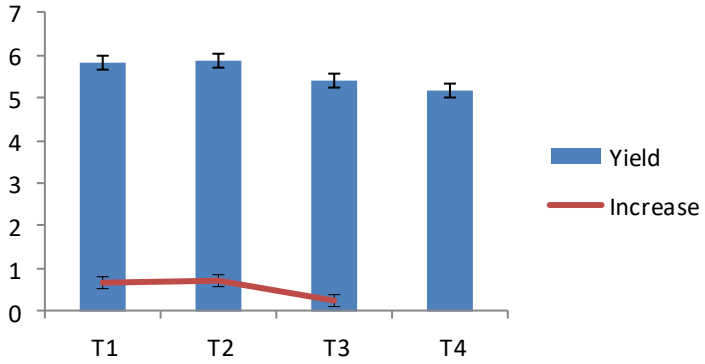


Fig.73. Yield of BRRI dhan86 on different treatments on bakanae infected plants in Boro, 2021-22 at Cumilla Trial-3.
(T1= Trichocompost @1.5t/ha, T2= dodder stem extract mediated silver nano particle following root dip method, T3= bacterial biopesticide spray@ 20ml/L water, T4= control).

Higher yield in all treated plots were due to increased higher tiller and higher panicle production compared to non treated plots (Fig 74a and Fig 74b). On the other hand, it was clearly observed that higher tiller and higher effcetive tiller/hill was formed in infected plants compared to non infected plants when infected plants were treated with formulated biopesticides (T1, T3) and dodder mediated silver nano paricle (T2) (Fig 74). Lower tiller and panicle formed in infected plants compared to non infected plants in T4 where no treatment was applied (Fig 74).

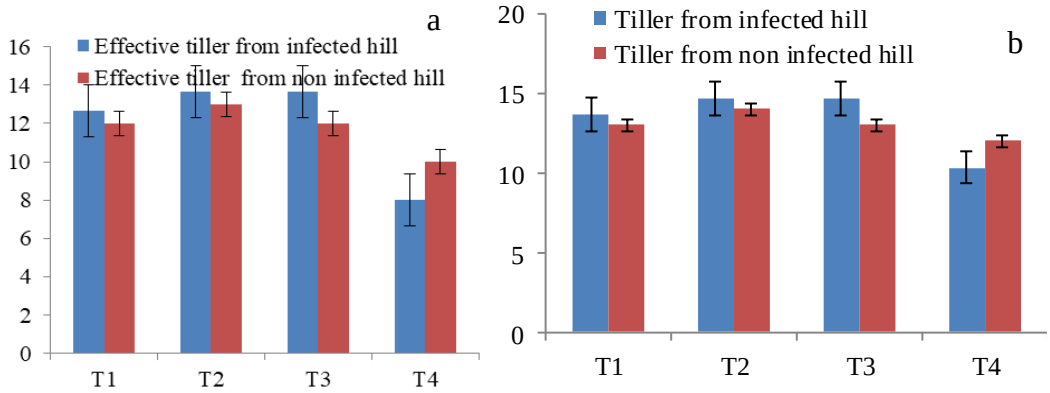


Fig.74. Scenario of average tiller and effective tiller production from infected versus non-infected bakanae plants of BRRI dhan86 on different treatments in Boro, 2021-22 at CumillaTrial-3. (T1= *Trichoderma* biopesticide based Trichocompost @1.5t/ha, T2= dodder stem extract mediated silver nano particle following root dip method, T3= bacterial biopesticide spray@ 20ml/L water, T4= control).

Leaves are the food factories of plants, so this is inspiration step for researcher and scientists to use leaves as nanofactories for production of silver nano particles. Other parts of plants as an extract for the synthesis of silver nano particles have been also studied e.g., ethanolic extract of Marigold flower (Kaur *et al*., 2012) and kinnow extract (Bansal *et al*., 2017). Silver nano particles have been reported as very effective against two plant-pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea in vitro* (Jo *et al*., 2009), *Erwinia carotovora* pv. *carotovora* and *Alternaria solani* (Abbas *et al*., 2015) and Phytophthora blight and Alternaria blight (Zakharova *et al*., 2017). Green silver nano particle using *Moringa oleifera* F. (Moringaceae) leaf extract also showed complete mortality of rice weevil at 2000 mg kg^{-1} of sorghum seeds on 15 DAT without any hazardous (Rani *et al*., 2019). The increase in Ag NPs could impregnate and adhere to fungal hyphae, thus inactivating pathogenic plant fungi or Ag NPs linked to the cell wall trigger cell lysis, causing structural damage and destroying the cell ‘s proper activity and contributing to the end of cell life of microbes (Lemire *et al*., 2013 Rai *et al*., 2009). In addition, Ag+ is thought to primarily affect the role of membranous enzymes, such as those in the respiratory chain and causing protein inhibition, bind to chromosomes, and cause chromosomal damage (Kim *et al.*,2012; Prabhu and Poulose, 2012; Kumar *et al*. 2016

Field Day and workshop

A total of six field days were organized at Cumilla, Habigonj and Gazipur districts. At each location two field days were organized. Farmers were contented to see the performance of biopesticides and nano particles to manage bakanae disease in field condition. Farmers of the respected areas were requested to availability of the biopesticides and nanoparticles to them as earielst best. Two workshops (Inception and closing) were held during the project period.

Islamic University component

1. Visual observation of AgNPs biosynthesis

The formation of AgNPs through the reduction of silver ion using neem (*A. indica)* aqueous leaf extract and dodder (*C. reflexa*) aqueous stems extract was observed primarily by the color change in the reaction solutions as shown in Fig.75 and 76 respectively. The colloidal red brown color change indicated the formation of Ag nanoparticles from which is due to the excitation of surface plasmon vibrations in the metal nanoparticles (Vanlalveni *et al,* 2021). Previous studies (Ahmed *et al*., 2016; Roy *et al*., 2017) have also reported similar changes in color due to the reaction between leaf extract and $AgNO_3$ and therefore, the complete reduction of Ag+ ions were confirmed.

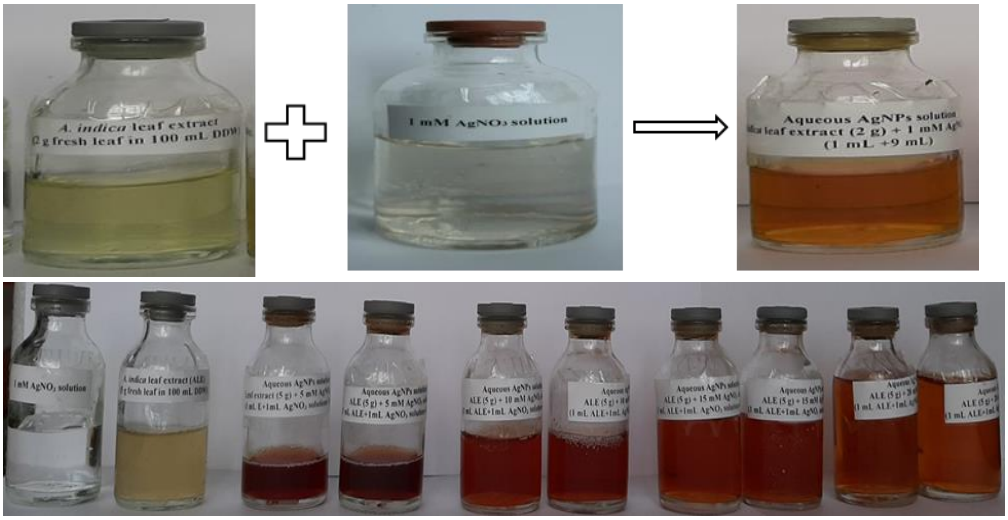


Fig. 75: Biosynthesis of the silver nanoparticles by adding neem leaves extract with $AgNO_3$ solution

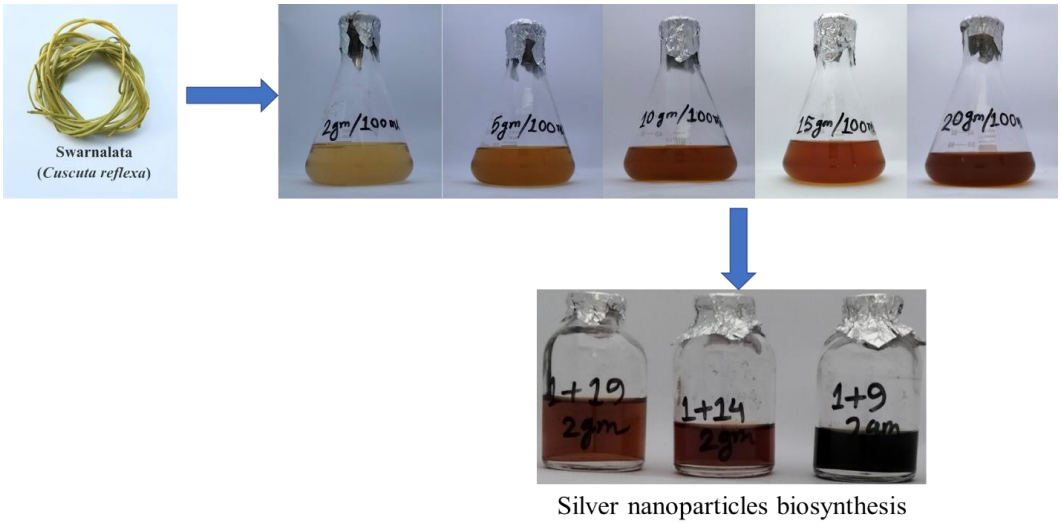


Fig.76: Biosynthesis of AgNPs using dodder (*Cuscuta reflexa)* stems extract.

2. Optimization of incubation time based on color development and UV-VIS spectral analysis of AgNPs

The photographs showed in Fig.77 presented the bioreduction of Ag+ to Ag^0 by means of color development of the reaction mixture of 1:19 ratio that was taken at different time intervals. In case of neem leaf mediated silver nano particle at 0 min, no color change was noted, and however, a yellow color appeared at 20 min. The yellow color was further changed to reddish brown and finally to dark red as the incubation time increased. Therefore, it is clear that the color intensity increases as the incubation time increased. However, the change of colour of the reaction mixture was seemed to be stayed nearly constant after 180 min of incubation which indicates the complete bioreduction of Ag+ to Ag^0. Subsequently, the UV–VIS spectroscopic absorption measurement of each of the reaction mixture at each of the mentioned interval of time was carried out to confirm the formation of AgNPs. The distinct maximum absorbance was recorded 0.291 at 419 nm

wavelength, 0.485 at 417 nm, 0.698 at 412 nm, 0.801 at 411 nm, 0.911 at 410.5 nm, 1.049 at 410 nm, 1.185 at 407 nm, 1.455 at 405.5 nm, 1.593 at 404 nm, 1.663 at 413.5 nm, and 1.724 at 421 nm after 0, 10, 20, 30, 40, 50, 60, 120, 180, 240, 300, and 360 min of reaction, respectively (Fig. 78). It is clear that that the intensity of SPR band of neem leaf mediated nano particle increased with increasing the incubation time as well as the SPR band shifted towards blue up to the incubation time of 240 min (4 hr). However, further increasing the reaction time (more than 240 min) resulted the band shifted towards red suggesting that incubation of the reaction mixture for 4 hr is the best time for the formation of monodisperse and small sized AgNPs. Moreover, the maximum absorption peak was found at 404 nm wavelength after 240 min of incubation indicates a size of approximately 20 nm. The SPR peak of the absorbance spectra of neem (*A. indica*) leaf extract-mediated AgNPs is consistent with previous studies (Paramelle *et al*., 2014; Peng *et al*., 2010), therefore, the size of AgNPs should be roughly around 20 nm. Therefore, it has been confirmed that incubation for 4 hr of the reaction mixture is the best time for the production of monodisperse, spherical and small-sized AgNPs in this study.

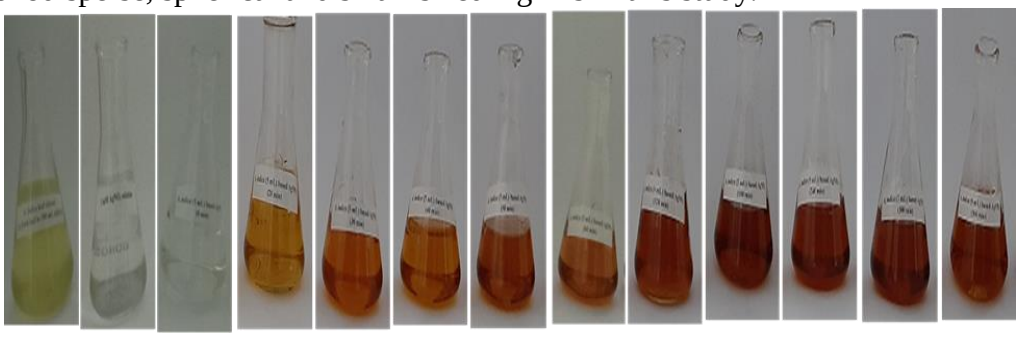


Fig. 77. Color progression of AgNPs biosynthesized from neem (*A. indica*) leaf extract of (1+19) ratio and $AgNO_3$ solution at time intervals of 0, 10, 20, 30, 40, 50, 60, 120, 180, 240, 300, and 360 min, respectively.

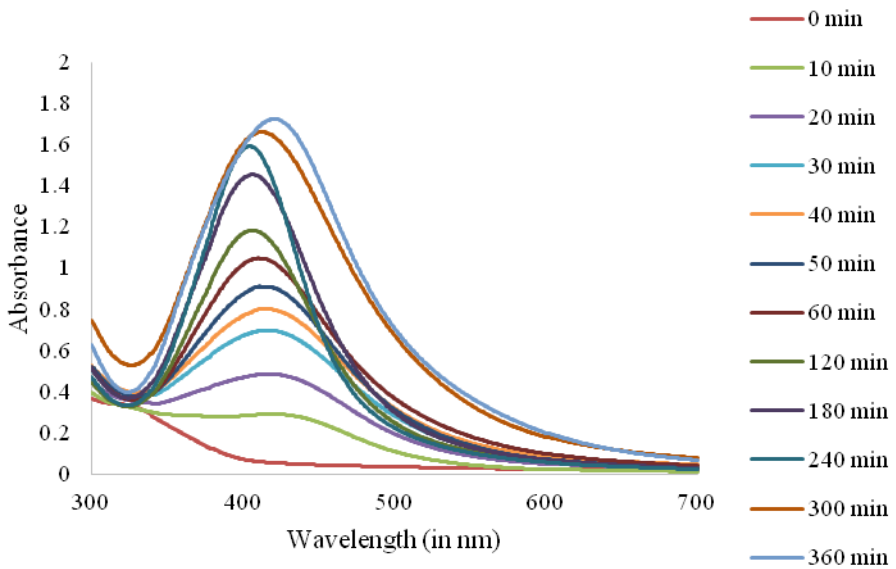


Fig 78. The UV-VIS spectra of AgNPs biosynthesized using neem (*A. indica*) leaf extract and $AgNO_3$ solution (1+19 ratio) at time intervals of 0, 10, 20, 30, 40, 50, 60, 120, 180, 240, 300, and 360 min, respectively.

On the other hand, dodder (*C. reflexa*) stems extract exhibited the sharpest and narrowest shaped SPR bands as well as the peaks shifted toward shorter wavelengths with increasing reaction time at 85°C indicated the mono-dispersity, spherical in shape and smaller sized AgNPs. The distinct maximum absorbance was recorded 1.63 at 417 nm wavelength. It has been reported that the peak position of the spectra can be used to roughly predict the size of AgNPs such as a maximum absorption peak near at 416 nm wavelength indicates a size of approximately 50 nm. AgNPs usually exhibit a SPR band due to the free electron excitation in the visible range of 400–500 nm by UV-VIS absorption spectroscopy (Sastry *et al,* 1997), however, not below than 390 nm. In all the cases, the absorption bands were exhibited within the range, therefore, confirmed the formation of AgNPs. However, as the temperature increased, the SPR bands shifted towards red as well as at extreme higher temperatures (Fig. 79) anisotropic curves were exhibited due to giving rise to two or more SPR bands indicating they are either quasi spherical or disc type (Martinez-Castanon *et al*., 2008).

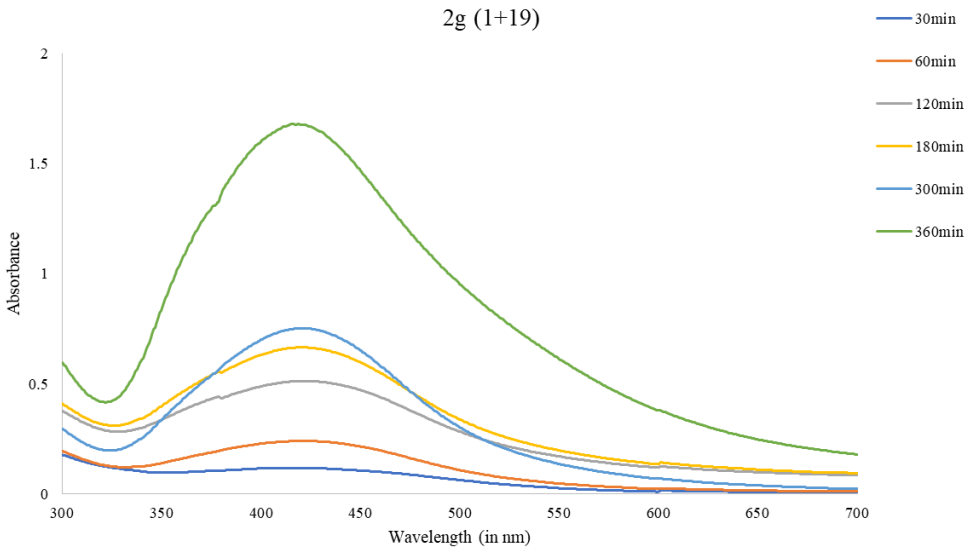


Fig. 79. UV-Visible spectra of AgNPs formed from 2g of dodder (*C. reflexa)* stem extract and $AgNO_3$ solution incubated at 85°C at the ratios of 1:9 at 30, 60, 180, 300 and 360 min respectively**.**

2. Characterization of AgNPs

Dynamic light scattering (DLS) analysis

The particle size distribution of the optimized neem leaf mediated AgNPs is presented in Fig. 80. The Z-average diameter value and polydispersity index (Pdl) were found to be 49.96 nm and 0.271 indicating the formation of small sized, monodisperse and homogenous AgNPs using neem leaf.

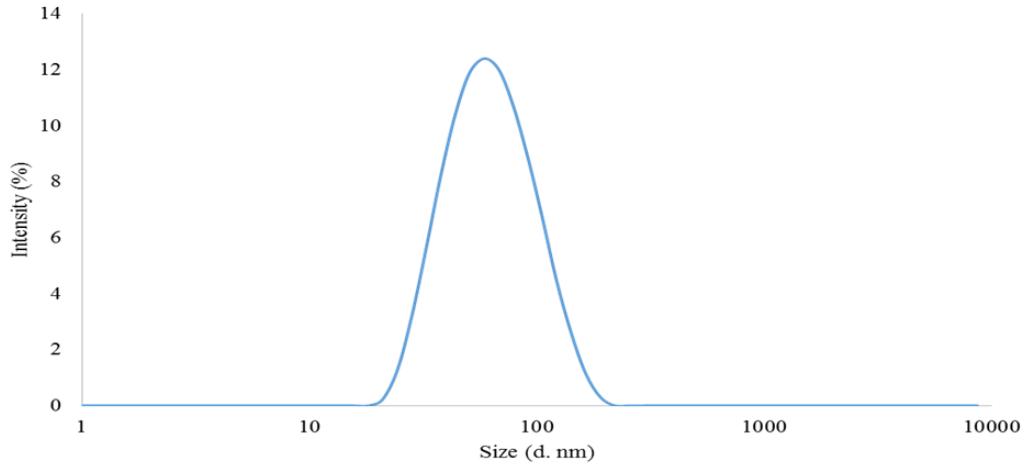


Fig. 80: Size distribution by intensity of the optimized AgNPs using a particle size analyzer.

On the other hand, the particle size distribution of the optimized dodder stem mediated AgNPs is presented in Fig. 81. Moreover, the maximum absorption peak was found at 416 nm wavelength after 240 min of incubation indicates a size of approximately 40-45 nm.

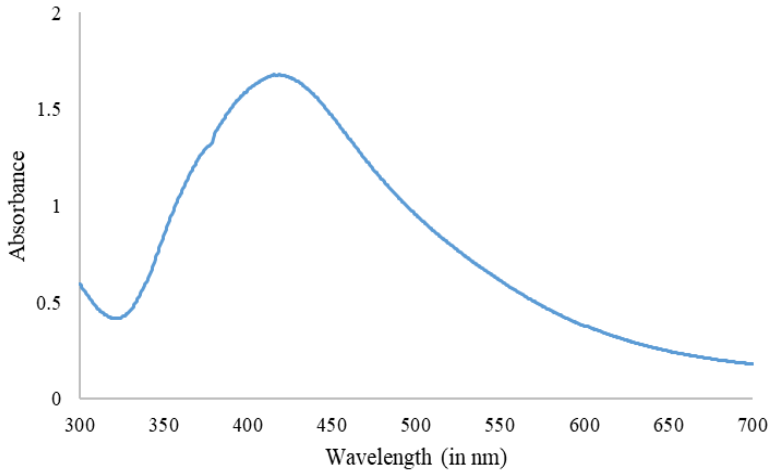


Fig. 81. The UV–Visible absorption spectrum of biosynthesized AgNPs using dodder (*C. reflexa)* stem extract. The absorption spectra of AgNPs exhibited a strong peak at 416 nm upon incubation for 85ºC for 240 min.

3. Stability test of AgNPs

The maximum absorbance of the optimized AgNPs was found to be 1.593 at 404 nm initially, while after 1 year it was 1.727 at 415.5 nm and 1.801 at 417.5 nm after 1.5 years. This indicates that the SPR band shifted slightly towards red due to the agglomeration of the particles. Moreover, a similar sharp and hight amplitude peak was noticed as like as the initial stage of the optimized AgNPs, and therefore, the biosynthesized AgNPs showed excellent stability even after 1.5 years (Fig. 82.).

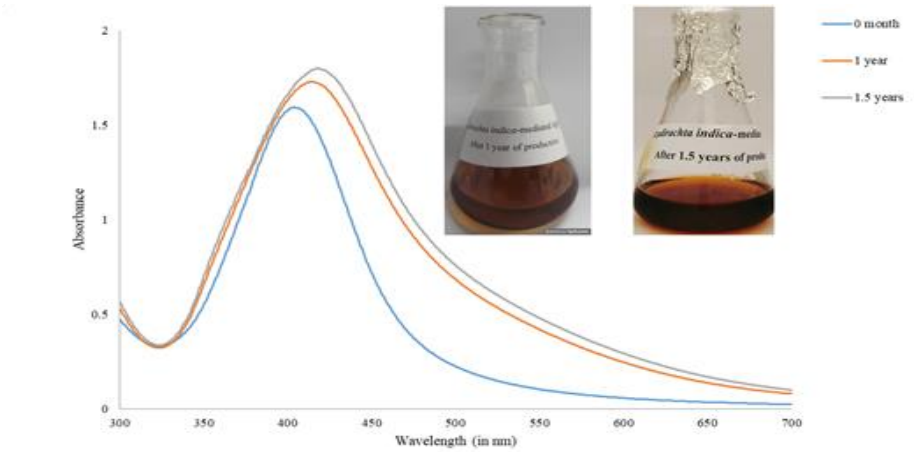


Fig. 82. Stabilization test of AgNPs using neem leaf extract.

4. *In vitro* test of nano particles derived from plant products for controlling bakanae disease

From *in vitro* trial it was observed that nano particles synthesized from neem leaf extract can successfully control the pathogen growth @12.08 mg/L when used on isolates 65 (100%) and 40 (>95%) whereas, suppress the mycelia growth slower of isolate 58 (70%) (Table 7 and Fig.83-85).

Table 7: Percent of growth inhibition of neem leaf extract–mediated AgNPs at different concentrations against different bakanae causing isolates on PDA media after 3 days incubation at 28°C

Isolates		Concentration (mg/L)						
		12.08	10.34	8.62	6.896	5.17	3.45	1.72
Fusarium fujikuroi (40)	% of growth inhibition	95.56	93.33	88.89	71.11	44.44	00	00
Fusarium proliferatum (58)	% of growth inhibition	70.00	64.44	60.00	57.78	48.89	40.00	00
Fusarium proliferatum(65)	% of growth inhibition	100.00	95.56	93.33	91.11	73.33	68.89	00

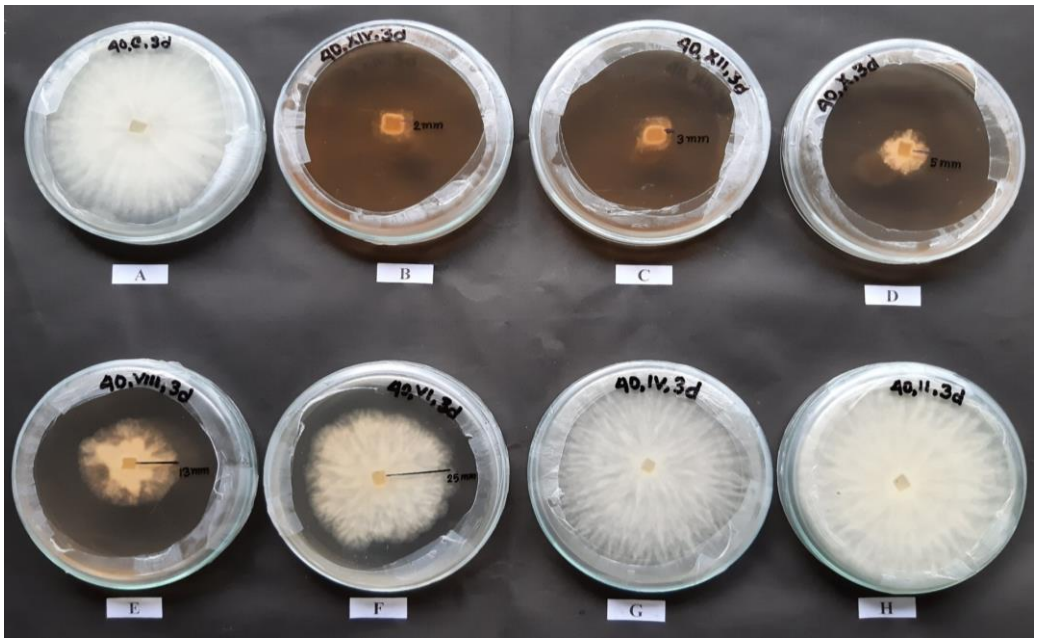


Fig.83: Antifungal activity of neem leaf extract-mediated AgNPs at different Concentrations (A: control;B: 12.08 mg/L;C: 10.34 mg/L;D: 8.62 mg/L;E: 6.896 mg/L;F: 5.17 mg/L; G: 3.45 mg/L; and *Fusarium fujikuroi* (40).

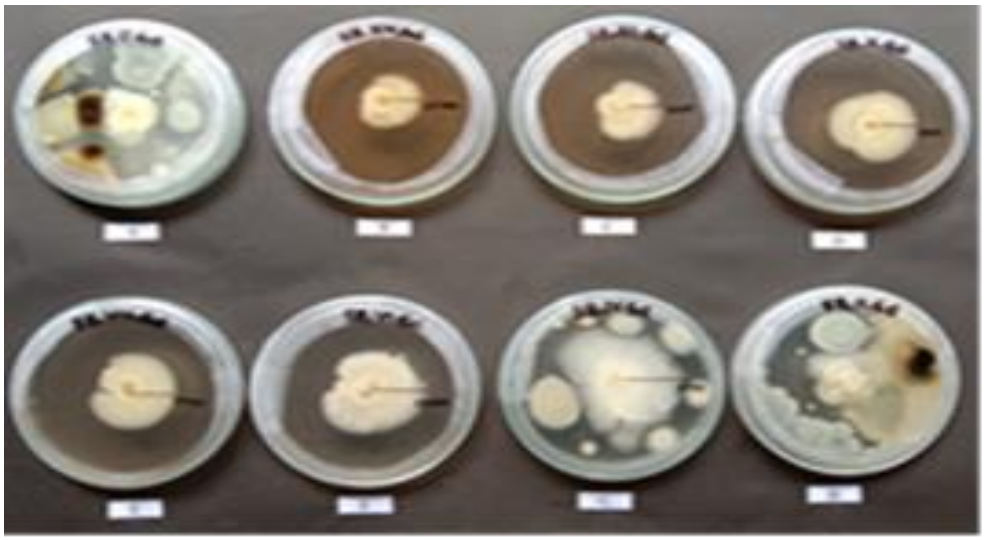


Fig. 84: Antifungal activity of neem leaf extract-mediated AgNPs at different Concentrations (A: control;B: 12.08 mg/L;C: 10.34 mg/L;D: 8.62 mg/L;E: 6.896 mg/L; F: 5.17 mg/L; G: 3.45 mg/L; and *Fusarium proliferatum* (58).

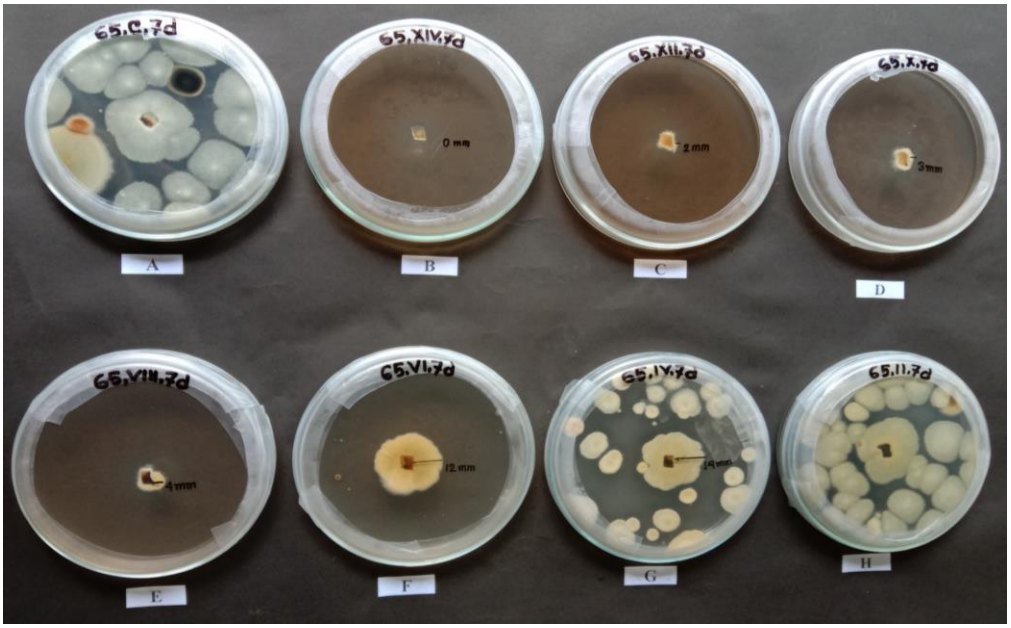


Fig. 85: Antifungal activity of neem leaf extract-mediated AgNPs at different concentrations (A: control; B: 12.08 mg/L; C: 10.34 mg/L; D: 8.62 mg/L; E: 6.896 mg/L; F: 5.17 mg/L; G: 3.45 mg/L; and H: 1.72 mg/L, respectively) against *Fusarium proliferatum* (65).

In case of nanoparticle synthesized from dodder (swarnalata) stem it was observed that it can control the pathogen growth 100% for isolate 40 followed by isolate 58 (>75%) compared to isolate 65 when sprayed @ 8.28 mg/L (Table 8 and Fig. 86-88).

Table 8: Percent of growth inhibition of dodder plant extract–mediated AgNPs at different concentrations against different bakanae causing isolates on PDA media after 3 days incubation at 28°C

Isolates		Concentration (mg/L)						
		8.28	7.09	5.91	4.73	3.55	2.37	1.18
Fusarium fujikuroi (40)	% of growth inhibition	100	100	100	86.67	66.67	33.33	00
Fusarium proliferatum (58)	% of growth inhibition	75.56	73.33	68.89	62.22	57.78	00	00
Fusarium proliferatum(65)	% of growth inhibition	64.44	60	55.56	51.11	46.67	00	00

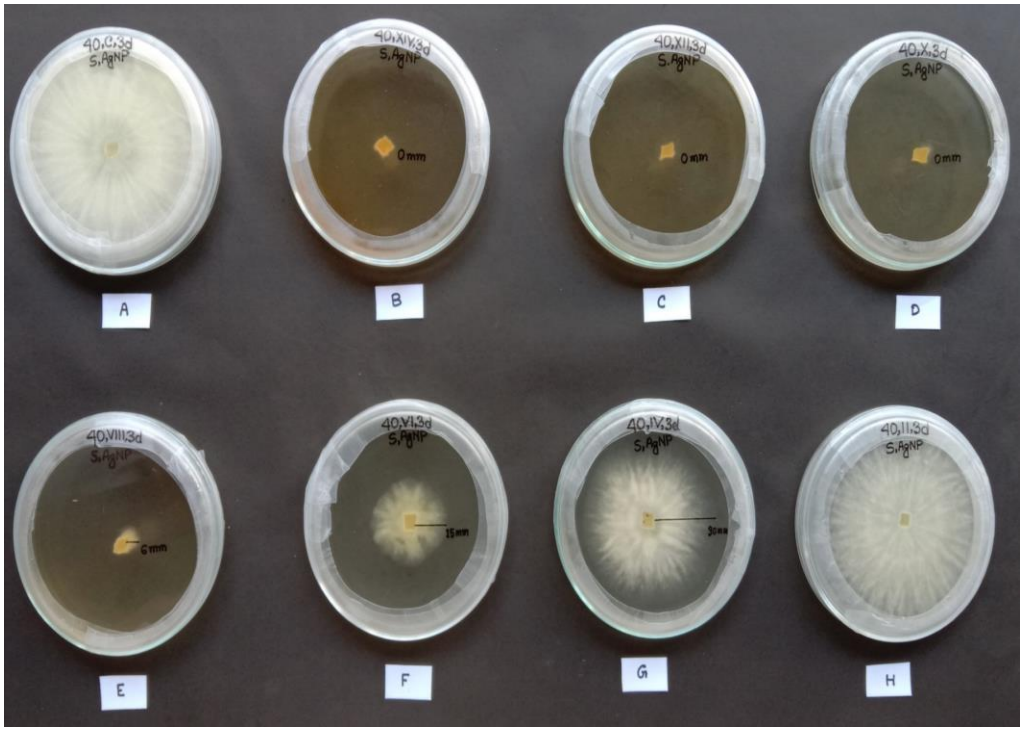


Fig. 86: Antifungal activity of dodder stem mediated AgNPs at different concentrations (A: control; B: 8.28 mg/L;C: 7.09 mg/L;D: 5.91 mg/L; E: 4.73 mg/L; F: 3.55 mg/L; G: 2.37 mg/L; and H: 1.18 mg/L, respectively) against *Fusarium fujikuroi* (40).

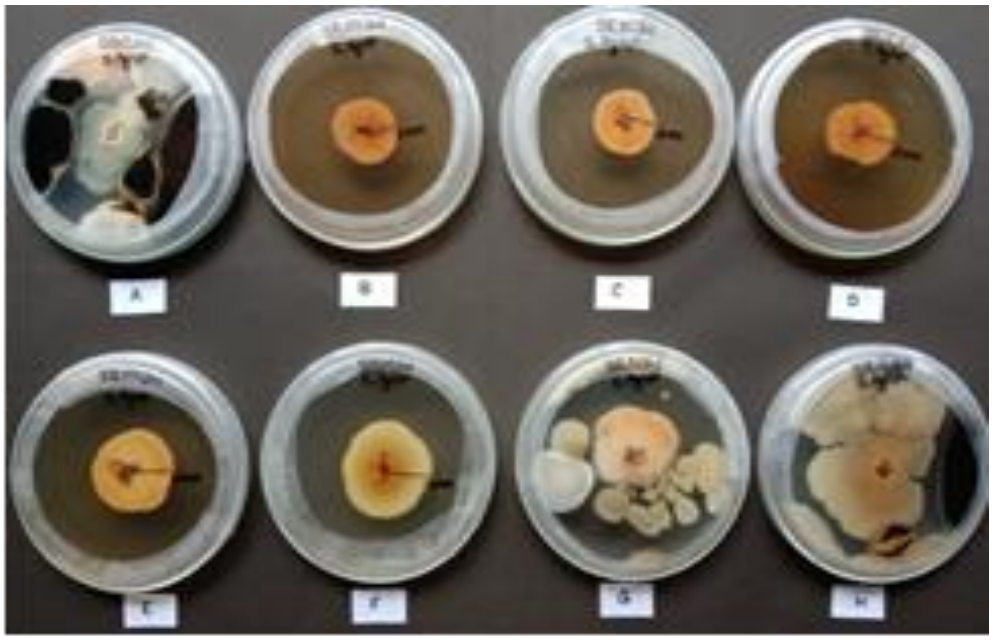


Fig. 87: Antifungal activity of dodder stem mediated AgNPs at different concentrations (A: control; B: 8.28 mg/L;C: 7.09 mg/L;D: 5.91 mg/L; E: 4.73 mg/L; F: 3.55 mg/L; G: 2.37 mg/L; and H: 1.18 mg/L, respectively) against *Fusarium proliferatum* (58).

Fig. 88: Antifungal activity of dodder stem mediated AgNPs at different concentrations (A: control; B: 8.28 mg/L; C: 7.09 mg/L; D: 5.91 mg/L; E: 4.73 mg/L; F: 3.55 mg/L; G: 2.37 mg/L; and H: 1.18 mg/L, respectively) against *Fusarium proliferatum* (65).

6. Net house trail with nano particles

It was observed that silver nano particles synthesized from neem leaf extract @ 12 mg/L and dodder stem extract @ 8 mg/L controlled bakanae disease infection when sprayed on inoculated plants. Inoculated and sprayed with nanoparticle synthesized from neem leaf and dodder plant plants showed symptomless and similar as control (un-inoculated) plants compared to infected plants (Fig. 89 & 90).

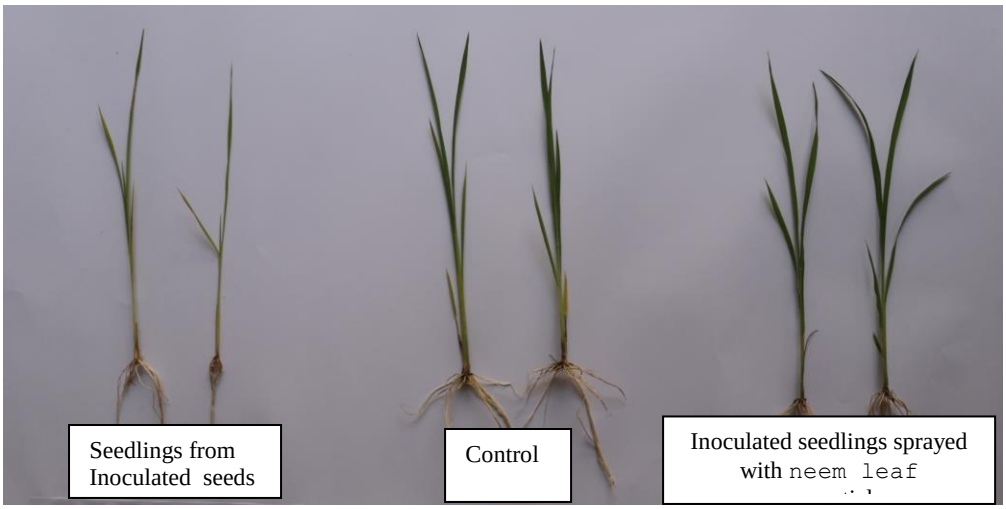


Fig.89. Green house trial with silver nano particale using neem leaf extract

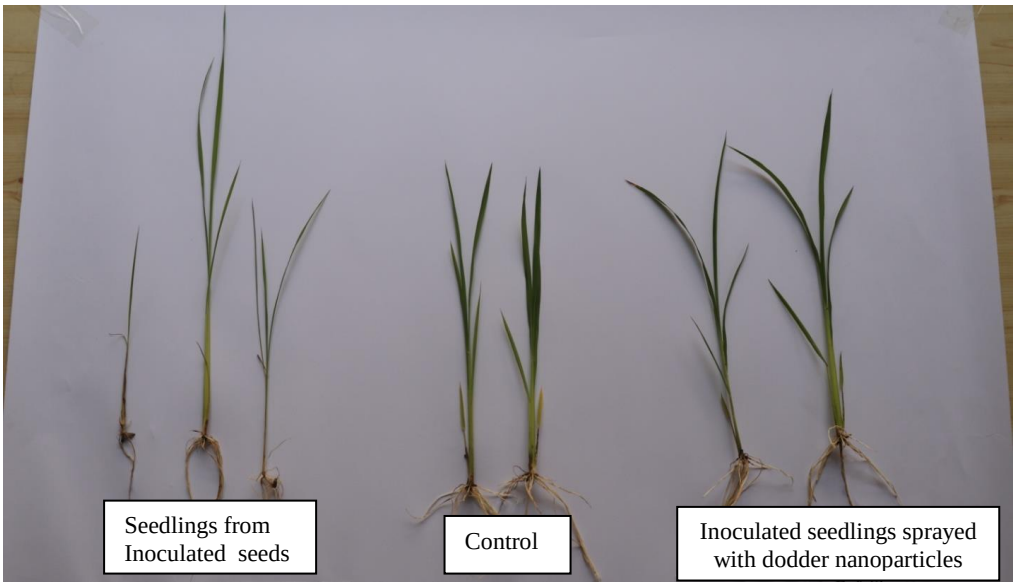


Fig.90. Green house trial with silver nano particale using dodder stem extract

12. Research highlights

Title of the sub-project: Formulation of biopesticides to control bakanae disease of rice in field condition.

Background

Bakanae disease is becoming a threat for sustainable rice production as the disease is not solely influenced by environment (Cother, 2002). Thus, bakanae is drawing the most concern in the affected rice growing areas of Asia and also becoming a threat to sustainable rice production in other parts of the rice growing world. Bakanae disease was first reported in Bangladesh in 1953 (Annonymous, 1958). Local, high yielding varieties as well as hybrid rice varieties are susceptible to this disease. The highest incidence of bakanae pathogen was observed in Bangladesh in hybrid rice of Sonar Bangla-6, BRRI hybrid dhan-2, Hira-2, ACI-1, Aloron, Modhumoti-2, and Hira-1(Ora *et al*., 2011). It was also observed that Bakanae disease incidence was much higher in farmer saved seeds and comparatively less frequent in the plots seeded with certified seeds of different agencies (Hossain *et al*., 2013).

Bakanae disease is becoming increasing as yield loss has been recorded in the range of. 3.7-50.0% in Asian region (Misra *et al.,* 1994). Moreover, in recent years bakanae disease is spreading and has been reported from new parts of Asia where previously this disease was not recorded as a greater concern. For instance, Haq *et al.* (2011) reported that the incidence of bakanae is increasing in Bangladesh and it was presumed that it might be due to an increase in the minimum temperature in that rice growing region.

In addition, Nanotechnology can offer advantages to pesticides, like reducing toxicity, improving the shelf-life, and increasing the solubility of poorly water-soluble pesticides, all of which could have positive environmental impacts. Nano particles provide themselves as (a) crop protection, or (b) nano particles as carriers for existing pesticides or other actives,

such as double-stranded RNA (dsRNA), and can be applied by spray application or drenching/soaking onto seeds, foliar tissue, or roots. Nano particles, as carriers, can provide several benefits, like (i) enhanced shelf-life, (ii) improved solubility of poorly water-soluble pesticides, (iii) reduced toxicity and (iv) boosting site-specific uptake into the target pest (Hayles *et al*., 2017). Another possible nano carrier benefit includes an increase in the efficacy of the activity and stability of the nano pesticides under environmental pressures (UV and rain), significantly reducing the number of applications, thereby decreasing toxicity and reducing their costs.

Recently, silver nanoparticles have increased in popularity, due to "green synthesis" production in plants, bacteria, fungi, or yeast (Rafique *et al.,* 2017). Silver nanoparticles have shown antifungal inhibition of *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea*, and *Curvularia lunata* by well diffusion assay (Krishnara *et al*, 2012). Leaves are the food factories of plants, so this is inspiration step for researcher and scientists to use leaves as nanofactories for production of silver nano particles. Other parts of plants as an extract for the synthesis of silver nano particles have also been studied e.g., ethanolic extract of Marigold flower (Kaur *et al.,* 2012) and kinnow extract (Bansal *et al.,* 2017). Silver nano particles have been reported as very effective against two plant-pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea in vitro* (Jo *et al*., 2009), *Erwinia carotovora* pv. *carotovora* and *Alternaria solani* (Abbas *et al.,* 2015) and Phytophthora blight and Alternaria blight (Zakharova *et al*., 2017).

A number of bakanae disease management are being used in Bangladesh as well as in other rice growing areas and reported. Besides chemical seed treatment and other physical control measures more effective and environmentally sound control measures using antagonistic microorganisms have been alternatively explored to control *F. fujikuroi*. An ecofriendly alternative to chemical pesticides is biopesticides and silver nano particle which encompasses a broad array of microbial pesticides. Biochemicals derived from micro-organisms and other natural sources such as plant parts mediated silver nano particle might be a great role to confer protection against pest damage.

Objectives

Sub-project general objective (s): Development of environmentally safe biopesticide to control bakanae disease and to increase yield of rice.

Sub-project specific objectives (component wise):

BRRI component

i) To characterize the identified effective biocontrol fungi/bacteria at species level
ii) Molecular identification of isolated biocontrol agents
iii) To find out suitable carrier material with prolong shelf life of biopesticides.
iii) To evaluate field efficacy of formulated biopesticiede and nano particle against bakanae disease of rice in field condition.

Islamic University, Kushtia component

i) Formulation of nano particle from identified effective plant product/active ingredients.
ii) *In-vitro* evaluation of nano particle against *Fusarium fujikuroi*/*F. proliferatum*.

Methodology followed

A. BRRI Component

Characterization of the bacterial biocontrol agents through biochemical tests

Certain biochemical tests were performed for the identification and characterization of Fluorescent *Pseudomonads* or *Pseudomonas* spp, or *Bacillus* spp. such as gram staining, siderophore production, cellulase production, phosphate solubilisation tests etc. as described previously by Elbeshehy *et al*. (2015).

Molecular identification of biocontrol agents

Extraction DNA from Trichoderma spp. and PCR amplification of purified DNA

Trichoderma was grown on Potato Dextrose Broth (PDB) medium. DNA of *Trichoderma* was extracted following modified CTAB method. Isolated DNA was amplified using ITS 1/ ITS 4 primers. PCR condition was as initial denaturation at 94^0C for 5 min followed by 35 cycles of denaturation was at 94^0C for 30 sec. Anneleaning temp. was at 53^0C for 30 sec, extension was at 72^0C for 1 min and final extension was at 72^0C for 7 min. *Trichoderma* isolates were identified according to the similarity of ITS region in Blast homology search.

DNA of bacterial isolates was extracted following method described by Cheng & Jiang (2005). For identification the endophytic bacterial isolates, 16S RNA gene sequence analysis was carried out using primer 91E -F GGAATTCAAAKGAATTGACGGGGGC and 13B-RCGGGATCCCAGGCCCGGGAACGTATTCAC. PCR condition was initial denaturation at 94^0C for 5 min followed by 35 cycles of denaturation was 94^0c for 30 sec, annealing at 55^0c for 30 sec, extension was at 72^0C for 1 min and final extension was at 72^0C for 7 min. Bacterial isolates were identified according to the similarity of 16S RNA genes in Blast homology search.

Multiplication and formulation of fungal biopesticide and shelf-life study

Trichoderma spp. was grown on PDA plates. At the same time, broken corn seeds were soaked in water for overnight, then sterilized in conical flask and cooled down to room temperature. After 5 days of incubation, when *Trichoderma* spp. was fully covered on PDA plates, then inoculated the sterilized broken corn seeds with the fungus. Further incubated the fungus in conical flask for 5-7 days for fully colonized the corn seeds. Then the colonized corn seeds with *Trichoderma* were taken out from the conical flask and dry it on trays in open air condition for 5-7days. After that, dried corn seeds colonized with *Trichoderma* were ground into powder and preserved it in plastic bag. Shelf-life study of formulated *Trichoderma* based biopesticide was carried out and found effective after >12 months of storage in polythene bag.

Formulation of bacterial biopesticide and shelh life study

The identified potential biocontrol bacterium was formulated as bacterial biopesticide on carrier material in liquid medium. Two types of formulations were prepared. Formulation-1

was formulated with and without glycerol in combination with starch in nutrient broth (100%) in conical flask and stiored in room temparature. Formulation-2 was formulated with and without glycerol in combination with starch in water (86%) and in combination with nutrient broth (12-14%).

Trichoderma biopesticide based Trichocompost

Trichoderma biopesticide based Trichocompost was prepared with water hyacinth, Cow dung and *Trichoderma* inoculum. *Trichoderma* (*Trichoderma asperelloides*) inoculum was prepared at Plant Pathology Laboratory in broken corn seeds. All materials were mixed and placed in layers in a composting pile in ratio of water hyacinth: Cow dung: *Trichoderma* inoculum: 3: 1: 0.16 (w/w). Urea solution (10%) was used for rapid decomposing. Trichocompost was prepared within 6-8 weeks. Nutrient analysis in Trichocompost produced from *Trichoderma* based biopesticide was also analysed by Soil Science Division, BRRI.

Disease protection studies under greenhouse conditions with bio-control agents

This experiment was conducted in net house condition with rice variety BRRI dhan29. The seeds were surface sterilized with 70% ethanol, washed with sterilized distilled water and then soaked for 48 hrs in sterilized distilled water. The excess water was drained out and sprouted seeds were further soaked in spore suspension (10^6 conidia/mL) of the virulent isolate of bakanae disease for 48 hr. Pre-soaked seeds in water for the healthy control treatment were soaked further in sterile distilled water for 48 hr before sowing. The seeds were then sown in sterilized soil in trays (2 kg soil/tray). After 7 days of planting both inoculated and un-inoculated seedlings were uprooted from the soil and the roots of seedlings were treated with identified biocontrol agents for 30 minutes following root dip method. Before that, five biocontrol bacterial isolates: *Bacillus cereus* (three isolates), *Leucobacter aridicollis*, and *Serratia* sp., were cultured on nutrient broth medium for 24 hr and two *Trichoderma* based biopesticides: T1 (*Trichoderma asperellum*) and T2 (*Trichoderma asperelloides*) on broken corn seed. Root dip treatment with biocontrol agents was used. The seedlings were arranged in a completely randomized design with 3 replications (40 seedlings/replication) in different trays contains sterile soil respectively. For healthy control and disease control roots of seedlings were soaked further in sterile distilled water for 30 minutes also before planting. All trays were placed in a net house at room temperature and were watered once daily with a hand sprinkler. The bakanae symptom appeared on treated seedlings were count at 14, 21 and 28 days after treatment and were expressed in % bakanae infection compared with diseased control seedlings.

Net house trial with nano particles

Two net house trials were conducted at Plant Pathology Division, BRRI. First trial with nano particles synthesized from neem leaf @ 12 mg/L was applied as seed treatment on sprouted seeds and as well as spraying on plants. Seed treatment was done on sprouting and inoculated seeds. Sprouted seeds were inoculated first with *F. fujikuroi* and then treated in synthesized nano particles for 2, 4, 6 and 8 hours as seed treatment and transplanted in trays. For spraying method, inoculated sprouted seeds were transplanted and sprayed with synthesized nano particles after 7 days of transplanting in trays. Along with seed treatment and spraying method, seeds inoculated with *F. fujikuroi* and non-inoculated seeds were

transplanted for comparison. Forty seedlings/tray and ten seedlings/rows were transplanted for each treatment. Data was recorded on % infected and % healthy seedlings after 14 days of nano treatment.

In second trial, $AgNO_3$ (1 mM) was used along with neem leaf mediated silver nano particle to observe the effect of $AgNO_3$ on bakanae disease development. Inoculation method was followed as described earlier but nano particle synthesized from neem and $AgNO_3$ treatment was lasting for overnight. Fifty seedlings/tray and ten seedlings/rows were transplanted for each treatment. Data was recorded on % infected and % healthy seedlings after 14 days of nano treatment. Plant height and root length was also noted for comparison with inoculated versus neem nano treatment and inoculated versus $AgNO_3$ treatment.

Field trial with formulated biopesticide

A total nineteen (19) field trials were carried out in different locations to find out the efficacy of formulated biopesticides and silver nano particles to manage bakanae disease in field condition. Field trial was conducted with the variety BRRI dhan49 at BRRI R/S, Habiganj farm in T. aman 2020 season. Bakanae incidence was approximate 30-40% in seedbed. In this trial, bakanae infected and non-infected (symptomless) both type of seedlings was collected from seedbed and treated with biopesticides. There were 4 treatments: T1= Trichocompost produced from *Trichoderma* based biopesticide, T2= bacterial biopesticide, T3= bacterial biopesticide and T4= Control. Trichocompost produced from formulated *Trichoderma* based biopesticide was applied @ 2.0t/ha during final land preparation. Bacterial antagonists were applied as root dip for 30 min before seedlings transplantation. Control plots were sprayed with sterilized water. Each treatment was replicated thrice. Fertilizer management was done as BRRI recommended dose.

In boro 2020-21 season, BRRIdhan 81 at Cumilla and BRRI dhan28, BRRI dhan29 were used at Habiganj for field trial. At each location two trials and thus a total of 4 trials were conducted in those locations. Three treatments were used in each trial; T1=Trichocompost was applied in seedbed (0.5t/ha) before seeding, T2= root dip with *Trichoderma* powder-based formulation (10g/L H_2O) was done before transplanting for 30 min + root dip in bacterial biopesticide formulation for 30 min before transplanting, T3= control. The bakanae incidence with visible symptom in seedbed at Habiganj was 30-40% whereas, no bakanae symptom was observed at Cumilla. Root dip was done in *Trochoderma* powder based biopesticidal solution and in bacterial biopesticide solution.

At Gazipur, two trials were conducted in highly bakanae infected fields 20-25 days after transplanting where bakanae infection was 60-70%. Seeds were supplied by Lalteer seeds Ltd and variety was hybrid Tia. In this trial two treatments were applied. In first trial T1= *Trichoderma* biopesticide based Trichococompost (2.0 t/ha) and T2= No compost (control) was applied whereas, in another trial T1= *Trichoderma* biopesticide based Trichococompost (1.0 t/ha) + bacterial biopesticide spray (20ml/L H_2O) and T2= control.

In T. Aus 2021 season, three trials were conducted. Two were at Cumilla and one at Habiganj with BRRI dhan48. Four treatments were applied at field condition as follows: T1: Trichocompost (2.0 t/ha) during final land preparation, T2: Trichocompost (2.0 t/ha) + *Trichoderma* biopesticide treated 10g/L (root dip for 30 min) before transplanting, T3: Trichocompost (2.0 t/ha) + Bacterial biopesticide @ 20ml/L H_2O (root dip for 30 min)

before transplanting, T4: control. At Cumilla no bakanae infection was observed at seedbed as well as in field condition. At Habiganj, bakanae infection was observed 5-10% at seedbed condition.

In T. Aman 2021 two trials were conducted in Cumilla and Habiganj. Four treatments were applied including control. T1= Trichocompost (1.5t/ha) during final land preparation T2= bacterial biopesticide (20ml/ha) 10-12 days after transplanting, T3= T1 + T2 and T4= control. Variety BRRI dhan49 was used in Habigonj whereas, BRRI dhan32 and BR22 were used in Cumilla. In BRRI dhan32 bakanae disease incidence was higher (25-30%) compared to BR22 (15-20%) in seedbed condition. In Habigonj disease incidence was 10-14% in BRRI dhan49 in seedbed condition.

Furthermore, another trial was conducted in T. Aman 2021, with neem mediated silver nano particle at Cumilla to find out the efficacy of neem mediated silver nano particle to manage bakanae disease of rice in field condition. Two treatments were applied including control. T1= Silver Nano particle (neem leaf extract mediated) and T2= control. Root dip method followed for overnight for nano treatment and BRRI dhan32 was used where bakanae infection was observed approximately 25-30% incidence at seedbed condition.

In boro 2021-22, four trials were conducted at Hotapara, Gazipur and three trials were conducted at Cumilla. At Gazipur, Trial-1 was conducted with Trichocompost @1.5 t/ha (T1) along with control (T2) using variety BRRI dhan28 and Trial-2 was carried out with three treatments i.e., T1= nano particle (neem lef extract mediated) following spray method @ 40ml/ L H_2O, T2= bacterial biopesticide (20 ml/L H_2O) and T3= control using local variety kataribhog. In both trials treatments were applied after transplanting when bakanae symptom was visible in field condition at farmers' field. In trial-3, three treatments were applied: T1=*Trichoderma* based biopesticide Trichocompost, T2= dodder stem extract mediated silver nano particle spray on seedbed, T3= control using BRRI dhan92. On the other hand, Trial-4 was set up based on trial-3 in Gazipur to get more conclusive results on dodder stem extract mediated siver nano particle treatment on bakanae disease management in field condition. Two treatments were used in this trial. In this trial prominent bakanae infected (internode highly elongated) plants were uprooted from the seedbed where BRRI dhan92 were seeded. Roots of 30 infected plants were soaked in dodder stem extract mediated silver nano particle for overnight and then transplanted in the field (T1). At the same time 30 infected plants were also transplanted in the field without any treatment (T2). Normal cultural practices were done in both treatments including watering and weeding. Fertilizer application was done in the field before transplanting and urea application was done once at recommended dose at 30 days after transplanting.

At Cumilla, Trial-1 was conducted with formulated biopesticides where, T1= *Trichoderma* biopesticide based Trichocompost (1.5t/ha), T2= Bacterial biopesticide (20ml/L) and T3= control using BRRI dhan48. Trial-2 was conducted at Cumilla with formulated nanoparticles along with control where T1= nano particle (neem leaf extract mediated) following root dip method, T2= nano particle (dodder stem extract mediated) following root dip method and T3= control using BRRI dhan86. In trial-3 at Cumilla four treatments along with control were used where, T1= Trichocompost (1.5t/ha), T2=nano particle (dodder stem extract mediated) following root dip method, T3= bacterial biopesticide sparay (20ml/L H_2O) and T4= control was applied on bakanae infected BRRI dhan86. At Cumilla, treatments were applied at field condition during transplanting after symptom appeared at seedbed.

Islamic University, Kushtia (Collaborating) Component

Collection and preparation of neem leaf and swarnalata stem extracts

Fresh and healthy leaves of *Azadirachta indica* (Neem) and fresh and healthy stems of *Cuscuta reflexa* (dodder) used in this experiment were collected from the campus of Islamic University, Kushtia, Bangladesh. The surface of the leaves was thoroughly cleaned under running tap water and subsequently with ddH_2O to remove any dirt or adhering contaminants. The cleaned leaves and stems were dried at room temperature, cut into small pieces which were then taken in Erlenmeyer flask followed by adding of 100ml ddH_2O. The flask was then boiled at 100ºC for 20 min and cooled down at room temperature. The cooled neem leaf extract and dodder stem extract was filtered using Whatman No.1 filter paper. Subsequently, the obtained filtrate was stored in a refrigerator at 4°C for carrying out further experiments.

Biosynthesis of AgNPs

The biosynthesis of AgNPs was carried out by following the procedures of Asimuddin *et al.*, (2020). The AgNPs was biosynthesized by mixing the aqueous neem leaf extract and aqueous dodder stem extract respectively with silver nitrate ($AgNO_3$) solution keeping the final concentration of the mixture always to 1mM. The mixture was then heated at 85°C in a hot air performance incubator (AP120, Froilabo, France). The color change of the reaction mixture from the initial colorless to yellowish color and finally to red brown color primarily indicates the completion of the reduction of silver ion (Ag+) to silver particle (Ag0) and the biosynthesis of AgNPs. Subsequently, the formation of AgNPs was further confirmed in aqueous solution using a UV-VIS spectrophotometer (U-2900 UV/VIS Spectrophotometer 200V, HITACHI, Japan) by scanning 3 ml of reaction mixtures in the range between 300 to 700 nm. The absorption spectra for each reaction mixture were recorded as a function of reaction time with a resolution of 1 nm.

Optimization of the biosynthesized AgNPs

In this study, optimization process of AgNPs biosynthesis initially started with the formation of nanoparticles from five (5) different amounts of neem leaf extracts and *Cuscuta reflexa* stem extract such as 2, 5, 10, 15, and 20g. From each of this extract of individual plant parts, 1 mL was mixed with different concentrations of $AgNO_3$ solutions at different ratios of reactants such as 1:4, 1:9, 1:14, and 1:19, (where 1mL was the volume of neem leaf/dodder stem extract and the latter was the volume of $AgNO_3$ solution) keeping the final concentration to 1 mM. The mixtures were incubated at 85°C for 1hr followed by measurement of absorption spectrum for each reaction mixture for neem leaf mediated extract whereas, the reaction mixtures were incubated at different temperatures (40, 50, 60, 70, 80, 85, 90, 95, 100, and 110 °C) for different time intervals (30min, 1hr, 2hr, 3hr, 5hr, and 6hr) followed by measurement of absorption spectrum for each reaction mixture of dodder stem mediated extract. By analyzing the obtained UV-VIS spectra of the above mixtures, further experiments were carried out to synthesize AgNPs from the neem leaf and dodder stem amounts of 2, 5, 10, and 15g. The mixtures were incubated at different time intervals in order to investigate the effects of dilution of the reaction mixtures as well as time on AgNPs biosynthesis. Subsequently, UV-VIS spectrum of each of the mixture was carried out in order to confirm the optimum amount of neem leaf, ratio of reactants and incubation time. Finally, the optimization of the incubation time of the reaction mixtures (using the optimized amount of leaf/stem extract) was carried out based on color development and UV-VIS spectra analysis. The optimized reactant ratio was further prepared keeping the final volume and concentration to 100 mL and 1mM, respectively. The mixtures were

subsequently incubated at different time intervals such as 0, 10, 20, 30, 40, 50, 60, 120, 180, 240, 300, and 360 min, respectively. The color development of each of the reaction mixture at each time interval was monitored and also the UV-VIS spectrum of each of the mixture was carried out in order to confirm the optimum incubation time. The biosynthesized AgNPs (2g) in aqueous mixture (1:19) that exhibited the maximum color development after which no color change observed as well as exhibited the sharpest, narrowest and single SPR band was selected as the optimized AgNPs in this study. The separation and purification of the optimized AgNPs from the reaction mixtures was performed by continuous centrifugation (10000 rpm/min; 20 min; 4°C) with sterile ddH_2O (Acharya *et al*., 2018). The obtained pellets were repeatedly washed (3-4 times) with ddH_2O water to ensure better separation of the AgNPs from other contaminants. After drying the pellets at 60°C in an oven (AP120, Froilabo, France), the dried AgNPs were kept at 4°C for further characterization.

Characterization of bio-AgNPs

The surface morphology, shape and particle size distribution study of the optimized AgNPs were characterized using Field Emission Scanning Electron Microscope (FESEM) equipped with EDX FESEM (JSM-7610F, JEOL Ltd. Japan). In addition, the elemental distributions of the AgNPs were investigated using EDX spectra collected from EDX detector (JSM-7610F, JEOL Ltd. Japan) operating at acceleration voltage of 15 KV. The average particle size of the produced AgNPs was determined by using a particle size analyzer (ZEN3600 Zetasizer, Malvern, U.K.). Furthermore, transmission electron microscope (TEM) image was taken to determine the morphology, size and shape of the AgNPs. TEM measurements were performed by auto focus, microtrace, autodrive, live FFT display, API (auto pre-irradiation), 120 kV accelerated voltage, multiple lens configurations, including a standard lens for unsurpassed high contrast and a class-leading UHR lens for high resolution. The TEM grid was prepared by placing a drop of the bio-reduced diluted solution on a carbon-coated copper grid and later drying it under a lamp. Besides, the crystalline nature of the AgNPs was determined employing XRD through the XRD patterns of the powder AgNPs sample by an X-ray diffractometer (PHILIPS X'Pert Pro, The Nnetherland) using Cu Kα radiation (λ = 1.54 Å), tube voltage of 33 kV, and tube current of 45 mA. The intensities were measured at 2-theta values from 10 to 90° at a continuous scan rate of 10°/min.

***In vitro* test of nano particles derived from plant products for controlling bakanae disease**

Nano particles synthesized from neem leaf and dodder stem extract were sprayed on plants inoculated with bakanae causing pathogen. At Islamic University, nano particles synthesized from neem leaf @ 12 mg/L and dodder stem extract mediated silver nano particle @ 8 mg/L were sprayed to controlled bakanae disease infection on inoculated plants. Three different isolates were isolated from bakanae infected plants and were used for inoculation to causing infection in plants. Effectiveness of the synthesized nano particles were evaluated after 21 days after inoculation. Control plants were sprayed with distilled water instead of inoculation with pathogen. Each treatment was replicated thrice following CRD.

Inoculated and sprayed with silver nanoparticle synthesized from neem leaf and dodder stem extract showed symptomless and as similar as control (un-inoculated) plants compared to infected plants Fig. 91 & 92).

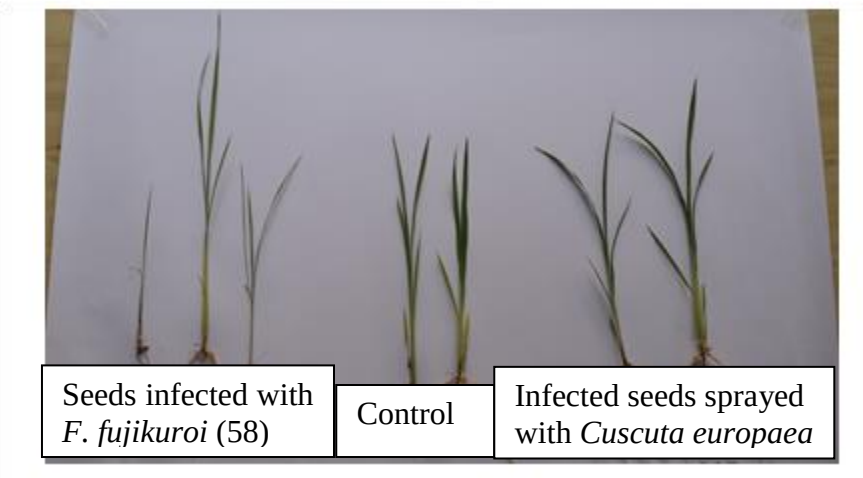


Fig.91. Green house trial with nano particale synthesized from dodder plant after 28 days of nano spray.

Fig.92. Green house trial with nano particale synthesized from neem leaf after 28 days of nano spray.

Data collection

Data was recorded as percent disease severity on artificially inoculated plants (average 40 plants/tray) at 14, 21 and 28 days after treatment application in net house condition for identifying biocontrol agents against bakanae disease. In net house condition, % infected plants in biopesticide treated trays as well as for nano treated trays were recorded in comparison with control (inoculated) plants. In field trial biopesticide were applied in natural infected fields or in uninfected seedbeds before infection. After harvest yield components (number of filled and unfilled grains per plants, grain yield and 1000 grain weight) were recorded in field condition.

Statistical analyses:

Results obtained from lab test as well as from field test were analyzed statistically.

Key findings

BRRI Component

1. Characterization and molecular identification of biocontrol agents

A total of nineteen biocontrol agents (three *Trichoderma* spp. and 16 bacterial spp.) were identified through PCR amplification and subsequently sequence analysis by Macrogen Inc. (Singapore).

2. Find out suitable carrier for formulation of biopesticide using identified biocontrol agent/s and/or active ingredient

Among the identified biocontrol agents two were used for formulation of biopesticides against bakanae disease. Other identified biocontrol agents will be used for formulation more biopesticides for future research.

Biopesticide with *Trichoderma* sp. was formulated with corn seed and packed in powder form in polythelene bag and stiored in room temparature. It was observed that *Trichoderma* sp. can survive >12 months in corn formulation. Moreover, *Trichoderma* biopesticide based Trichocompost was prepared with water hyacinth, Cow dung and *Trichoderma* based biopesticide. All materials were mixed and placed in layers in a composting pile in ratio of water hyacinth: Cow dung: *Trichoderma* based biopesticide: 3: 1: 0.16 (w/w). Urea solution (10%) was used for rapid decomposition. Trichocompost was prepared within 6-8 weeks.

Two types of formulation of bacterial biopesticides were prepared. Formulation-1 was formulated with and without glycerol in combination with starch in nutrient broth (100%) in conical flask and stored in room temperature. Formulation-2 was formulated with and without glycerol in combination with starch in water (86%) and in combination with nutrient broth (12-14%). On the other hand, it was observed that bacterial biopesticide in formulation-1 can survive up to 12 months or more whereas, in formulation-2 can survive up to 6 months or more in liqiid form (Fig 93-94). In both formulations, bacterial concentration was @ 3.0 x 10^7 CFU/mL.

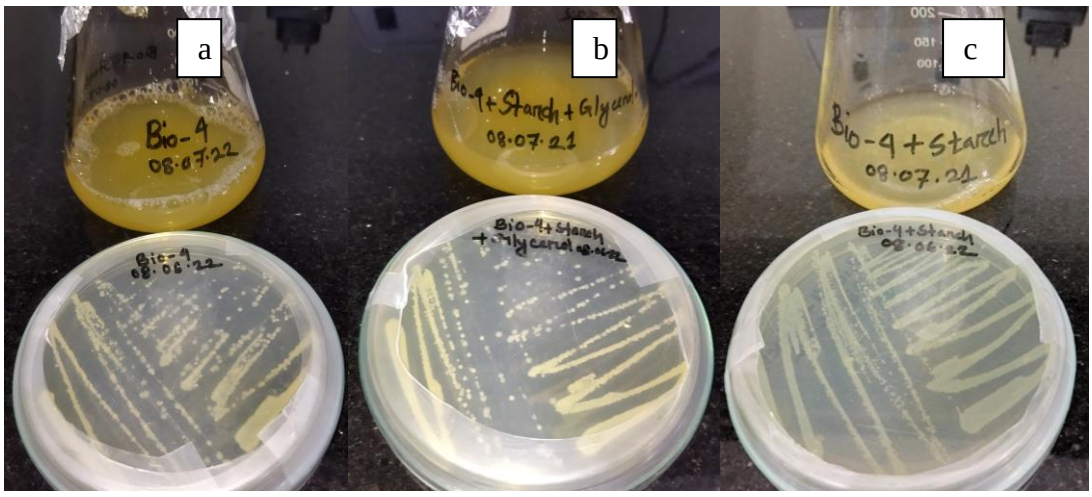


Fig.93. Shelf-life study test of formulation-1 with and without glycerol after12 month (a=nutrient broth, b= with glycerol, c= without glycerol).

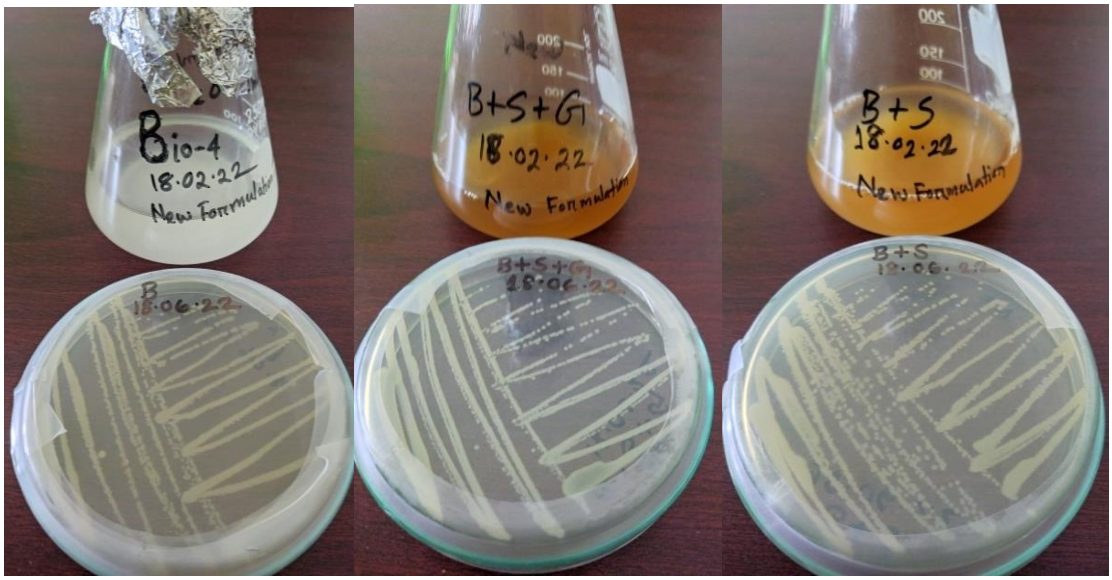


Fig.94. Shelf-life study test of formulation-2 with and without glycerol after12 month
(a= nutrient broth (14%) in water, b= nutrient broth (12%) in water with glycerol, c= nutrient broth (13%) in water without glycerol).

3. Disease protection studies under greenhouse conditions

Biopesticide produced from *Trichoderma* and biocontrol bacteria were evaluated following root dip method and bakanae infection was recorded. *Trichoderma asperelloides + Serratia* sp. performed best followed by *Bacillus cereus, Leucobacter aridicollis* and *Serratia* sp. A number of experiments using botanicals were conducted to manage bakanae disease. Garlic clove extract was found promising to control bakanae disease in Bangladesh (Riazuddin *et al*., 2013). Soaking seeds in the extract of lemon grass at 1:1dilution for 6 hr was also found to control bakanae pathogen successfully (Rahman *et al.*, 2014). The leaf extract of *Lawsonia inermis* showed maximum inhibition (60.65%) followed by roots of Asparagus racemosus (50.59 %) to control bakanae pathogen (Yasmin *et al*., 2008).

4. Net house trial with nano particle produced from neem leaf

In first net house trial it was observed that neem leaf extract-mediated AgNPs showed a very strong antifungal activity against *Fusarium fujikuroi* in in vitro condition at Plant Pathology Division, BRRI, Gazipur.It was also observed that plants infected with *F. fujikoroi* were escaped bakanae infection when sprouted seeds were treated with neem leaf mediated Ag-nano particle for 4-8 hr as seed treatment. Spraying with nano from neem leaf also promising compared to diseased control plants.

In second net house trial no bakanae symptom (0%) was observed when neem leaf mediated silver nano particle used and the result is similar as the healthy control. On the other hand, $AgNO_3$ treated plants showed a significant number of elongated plants as like as bakanae symtom (17.80%) and disesed control plants showed highest infection (83.75%). This result suggested that $AgNO_3$ has no role to rescue from bakanae disease symptom development. Moreover, highest plant height was increased in diseased control (8.2%) followed by $AgNO_3$ (1mM) (5.2%) treated seeds (Fig.95). Plant height was somewhat shorter (-2.4%) in silver nano (neem leaf) treated plants compared with healthy control plants (Fig.95). This plant height shortness was found in the trays also. The plant height shortness in neem leaf mediated silver nano particle compared to healthy control plants might be due to slower rate

of physiological activity occurred after application of neem leaf mediated silver nano particle.

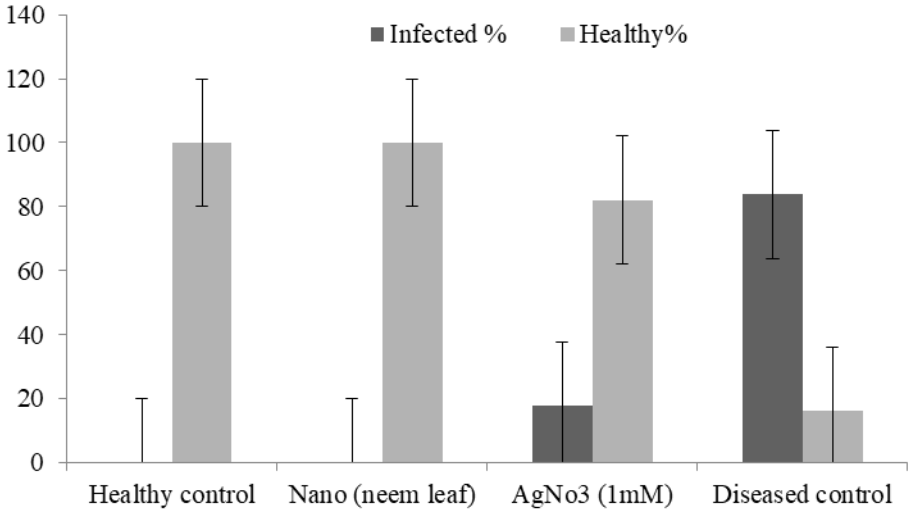


Fig.95. Bakanae symptom incidence (%) on different treatments.

4. *Trichoderma* biopesticide based Trichocompost

High nutrient value was obtained from Trichocompost produced from *Tricoderma* based biopesticide and are presented in Table 9. Organic matter was malso increased (0.1%) after application of Trichocompost.

Table 9. Nutrient analysis of Trichocompost produced from *Trichoderma* based biopesticide when applied @ 2.0 t/ha in field.

Elements	Content (%)	Kg/ha
N	0.5	10
P	0.44	8.8
K	3.59	71.6
S	0.244	4.9
Zn	0.031	0.62
Organic carbon in soil after Trichocompost application	2.5	2.4

(Nutrient analysis was done with the help of Soil Science Division, BRRI)

5. Field trial with formulated biopesticide and AgNPs for controlling bakanae disease

At Habiganj trial in T. Aman 2020, it was observed that infected seedlings were survived 25-40% when *Trichoderma* based Trichocompost and bacterial biopesticide were applied whereas; healthy seedlings were survived 98-100% after 30 days of transplanting. For all treatments it was found that total tiller (TT) and effective tiller was increased in infected plants compared to healthy plants. Higher effective tillers (31-43%) were increased where bacterial biopesticide (T2 and T3) were applied compared to *Trichoderma* based biopesticide (T1) and control (T4) treatments

In Boro 2021-22, four trials were also set up at Cumilla and Habiganj. There was no significant difference among the treatments at Cumilla as no bakanae disease incidence was observed in seedbed as well as in field condition. Yield was found higher in treatment T1 when Trichocompost was applied in seedbed @ 0.5t/ha before seeding at both locations and in T2 when root was dipped in *Trichoderma* powder formulation @10g/L H_2O before transplanting for 30 min + root dip in bacterial formulation @20 ml/L H_2O for 30 min before transplanting at location b compared to T3 (control). Although there was no disease incidence but yield was increased due to increase % effective tiller in T1 (3-20%) and in T2 (10-13%) over T3 (control).

At Habiganj trials it was observed that yield was increased in T1 in location a when Trichocompost was applied in seedbed @ 0.5t/ha before seeding and in T2 when root was dipped in *Trichoderma* powder formulation @10g/L H_2O before transplanting for 30 min + root dip in bacterial formulation @20 ml/L H_2O for 30 min before transplanting in both locations compared to control treament T3. This yield increase was due to increase grain weight/panicle in T1 (37g) and T2 (39g) compared to T3 (32g). In location-a yield performance was better in T2 compared to T1 whereas, in location-b both T1 and T2 performed better compared to T3.

In Gazipur, two trials were conducted in highly bakanae infected fields 20-25 days after transplanting where bakanae infection was 60-70% in Boro 2020-21. Higher yield (8.19 t/ha) was found in Trichocompost treated plots compared to no Trichocompost treated plot (3.59 t/ha) by producing more effective tillers in Trichocompost treated plots using hybrid Tia. In second trial at Gazipur it was observed that effective tillers were increased and thus contributed higher yield (7.75 t/ha) in bacterial biopesticide treated plots compared to control plot (6.13 t/ha). In trials at Gazipur it was observed that yield may increase 2.0-4.5 t/ha when formulated biopesticides were applied compared to control plots in highly bakanae infected (60-70%) plants in field.

In T. Aus it was observed that yield increased in different treatments compared to control in both Habiganj and in Cumilla. More yield increase was observed at Habiganj (1.1- 1.4t/ha) where bakanae infection was observed. At Habiganj yield increase was found highest in T1 (5.2 t/ha) when Trichocompost @2.0 t/ha and in T2 (5.19 t/ha) when T1 + *Trichoderma* treated root dip for 30 applied followed by T3 (4.95 t/ha) and least increase was in T4 (3.81t/ha). As there was no bakanae infection both in seedbed and in field at Cumilla therefore, yield increase was not much as in Habiganj. Higher yield increase in Habiganj was due to more grain weight, and more filled grain /panicle compared to control treatment. From this trial it is found that treatmts have more effcet on bakanae infected plants rather than non infected plants by increasing effective grain weight weight/panicle, increasing filled grain/panicle and decreasing empty grain/panicle in infected plants.

In T. aman 2022 it was observed that yield was increased in all treatments compared to control in both varieties at Cumilla. More yield increase was observed in BRRI dhan32 compared to BR22. This higher increase in BRRI dhan32 was recorded where higher disease incidence (25-30%) was observed. Variety BRRI dhan49 was used in Habiganj whereas; BRRI dhan32 and BR22 were used in Cumilla. In Habiganj higher yield was also recorded in all treatments compared to control in BRRI dhan49 where disease incidence was 10-14%. Among the treatments trialed T1: Trichocompost @1.5 t/ha resulted higher yield (7.3 t/ha) followed by T3: Trichocompost @1.5 t/ha + bacterial biopesticide spray (6.8 t/ha) and lowest yield was observed in T4: control (5.6 t/ha). This higher yield increase was due to increase of flag leaf length and 1000 grain weight in T1 and in T3 compared to T4.

In case of nano trial in T, Aman 2021, yield was increasd in nano treated plot (T1) (3.7 t/ha) where neem leaf extract mediated nano particle was applied as root dip method compared to control (T2) plot (3.1 t/ha) on BRRI dhan32 at Cumilla and bakanae was observed approximately 25-30% incidence at seedbed condition. It was also observed that tiller/hill and panicle/hill increased in T1 (nano treated plot) whereas, decreased in T2 (untreated plot) that increased yield ultimately.

In all trials at Gazipur, Boro 2021-22 it was observed that disease incidence was decreased with progression of times in all treated plots. Disease incidence was decreased up to 50% in treated plot (T1) after one week of treatment application where *Trichoderma* biopesticide based Trichocompost was applied whereas 25% decreased in control plot (T2). Higher yield was recorded in T1 (7.1 t/ha) where *Trichoderma* based biopesticide Trichocompost @1.5 t/ha used compared to T2 (5.9 t/ha) where no treatment (control) was applied. Yield was increased 1.3 t/ha in T1 compared to T2. After four weeks of treatment applicattion it was observed that disease incidence was decrease to 1%. On the other hand, disease incidence was also decreased in control plots but slowly and thus hampered the infected plants to grow. At harvest time no bakanae was observed in both treated and non treated plots.

In Trial-2 at Gazipur, it was observed that disease incidence was decreased at T2 (bacterial biopesticide spray) treatment up to 88% followed by T1 (dodder stem extract mediated silver nano particle) up to 80% in BRRI dhan28 whereas in control up to 40% decreased. After four weeks of treatment application, it was observed that no bakanae disease symptom was observed in T1 and T2 where dodder stem extract mediated silver nano particle and bacterial biopesticide sprayed respectively. On the other hand, after 4 weeks of treatment application bakanae symptom was visible (5%) in control plots. This decreasing DI was reflected on yield. Highest yield increased was observed in T2 (9.4 t/ha) followed by T1 (8.1 t/ha) and lowest was observed in T3 (7.2 t/ha).

In Trial -3 conducted at Gazipur, with BRRI dhan92, it was observed that DI was sharply decreased in T1 (*Trichoderma* based biopesticide Trichocompost) application up to 95% followed by T2 up to 87% spray (Nano particle dodder based) after one week whereas in control (T3) plot was decreased up to 55%. After four weeks of treatment application no bakanae symptom was observed either in treated and non treated plots. Highest yield was increased in T1 (8.64 t/ha) followed by T2 (7.29 t/ha) and lowest in T3 (5.46 t/ha). Yield was increased due to treatments ability to reduce bakanae incidence at early stage of treatment application. In Trial-3, it was also observed that yield increase in T1 and T2 was due to increase tiller/hill and effective tiller/hill which was due to ultimate effect by application of Trichocompost and dodder stem extract mediated silver nano particle.

In Trial-4, incidence of bakanae plants were decreased in nano treated plants (T1) compared to non treated plants (T2) with times. After eight weeks of nano treatment bakanae incidence was reduced from 22% to 5% whereas, in control plants incidence was found about 18%. At harvest period no bakanae symptom was observed in both treated and non treated plants. It was also observed that up to four weeks infected plants were seemed to death at both treated and no treated plots. After 8 weeks of transplanting dodder stem extract based nano particle treated plants (T1) starting to survive by exerted tillering (>50%) whereas, non-treated plants (T2) survived a few (<10%). Moreover, survival plants having delayed panicle initiation and delayed ripening. This delayed time was required for the regain energy for tiller proliferation. Furthermore, yield was also increased in dodder stem extract mediated nano treated plants (T1) (12.84 g/hill) compared to control plants

(T2) (5.70g/hill). It was observed that yield was increased more than double in T1 compared to T2 and this increased yield was due to increased tiller/hill (21.4) and increased effective tiller/hill (20.2) in dodder stem extract mediated nano treated plants. It is noticeable that almost all tillers showed ability to produced grain in T1 (nano treated) whereas, only 50% tillers showed ability to produced grain in T2 (control).

At Cumilla in Trial-3, DI was also decreased with times in all treated plots. Four treatments including T1= *Trichoderma* biopesticide based Trichococompost @1.5t/ha, T2= dodder stem extract mediated silver nano particle following root dip method, T3= bacterial biopesticide sparay @20ml/L and T4= control was applied in BRRI dhan86 and disease incidence was 20% in seedbed. Although a minimum level of disease incidence was obsereved in all treated plots after four weeks of treatment application but yield was increased in all treated plots (T1, T2 and T3) compared to control plot (T4). Among the nano treated plots higher yield was found in T2 (5.85 t/ha) where roots of infected plants were dip in dodder stem extract-mediated silver nano particle followed by T1 (5.8 t/ha) where *Trichoderma* biopesticide based Tricocompost @1.5t/ha applied during final land preparation and T3 (5.41 t/ha) where bacterial biopesticide was sprayed on infected plants after transplanting @20ml/L water. Lowest yield was observed in T4 (5.16 t/ha) where no treatment was applied. Yield increased was ranged from 0.25-0.7 t/ha in treated plots. Dodder stem extract-mediated silver nano particle and *Trichoderma* biopesticide based Tricocompost @1.5t/ha gave as similar yield without any statistical difference. Higher yield in all treated plots were due to increased higher tiller and higher panicle production compared to non treated plots. On the other hand, it was clearly observed that higher tiller and higher effcetive tiller/panicle was formed in infected plants compared to non infected plants when infected plants were treated with formulated biopesticides (T1, T3) and dodder mediated silver nano particle (T2). Lower tiller and panicle were formed in infected plants compared to non infected plants in T4 where no treatment was applied.

Islamic University Component

Formulation of silver nano particle using neem leaf and dodder (swarnalata) stem extract

Neem leaf mediated and dodder stem mediated silver nanoparticles are produced through neem leaf and dodder stem extraction Produced silver nano particles are confirmed by visual observation of AgNPs biosynthesis, Optimization of incubation time based on color development and UV-VIS spectral analysis of AgNPs and as well as produced AgNPs were characterized.

The maximum absorption peak of neem leaf mediated nano particle was found at 404 nm wavelength after 240 min of incubation indicates a size of approximately 20 nm. The SPR peak of the absorbance spectra of neem (*A. indica*) leaf extract-mediated AgNPs is consistent with previous studies (Paramelle *et al.*, 2014; Peng *et al*., 2010), therefore, the size of AgNPs should be roughly around 20 nm. Therefore, it has been confirmed that incubation for 4 hr of the reaction mixture is the best time for the production of monodisperse, spherical and small-sized AgNPs in this study.

On the other hand, dodder (*C. reflexa*) stems extract exhibited the sharpest and narrowest shaped SPR bands as well as the peaks shifted toward shorter wavelengths with increasing

reaction time at 85°C indicated the mono-dispersity, spherical in shape and smaller sized AgNPs. The distinct maximum absorbance was recorded 1.63 at 417 nm wavelength. It has been reported that the peak position of the spectra of AgNPs such as a maximum absorption peak near at 416 nm wavelength indicates a size of approximately 50 nm. AgNPs usually exhibit a SPR band due to the free electron excitation in the visible range of 400–500 nm by UV-VIS absorption spectroscopy (Sastry *et al*, 1997), however, not below than 390 nm. In all the cases, the absorption bands were exhibited within the range, therefore, confirmed the formation of AgNPs.

Characterization of AgNPs was done using Dynamic light scattering (DLS) analysis. The Z-average diameter value and polydispersity index (PDI) were found to be 49.96 nm and 0.271 indicating the formation of small sized, monodisperse and homogenous AgNPs using neem leaf. On the other hand, the maximum absorption peak was found at 416 nm wavelength after 240 min of incubation indicates a size of approximately 40-45 nm.

BRRI and Islamic University Component

***In vitro* and *in vivo* test of nano particles derived from neem leaf and dodder stem extract mediated for controlling bakanae disease**

Silver nanoparticles using neem leaf extract and dodder stem extract was synthesized by Islamic University. *In vitro* and *In vivo* tests were carried out using synthesized silver nano particle as described earlier in this report. It has been found and established that synthesized both silver nano particles have ability to manage bakanae disease in field condition.

Key words: Bakanae, biocontrol, biopesticde, nanoparticle, Trichocompost

B. Implementation Status

1. Procurement (component wise):

BRRI

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment	**8**	**95000**	**8**	**94900**	
1. Computer Table	2	18000	2		
2. Visitor chair	4	20000	4		
3. File cabinet	1	22000	1		
4. Steel Almir**a**	1	34900	1		
(b) Lab & field equipment	**4**	**280000**	**4**	**279000**	
1. Vortex	1	40000	1		
2. Digital weighing balance	1	160000	1		
3. Moisture meter	2	54000	2		
4. Weight balance	1	25000	1		
(c) Other capital items	**1**	**80000**	**1**	**79509**	
1. Desktop with monitor					

Islamic University

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment Procurement of Furniture: a) Computer table b) Almira	**2** 1 1	**40800** 16000 24800	**2** 1 1	**40800** 16000 24800	
(b) Lab & field equipment					
(c) Other capital items					

2. Establishment/renovation facilities:N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized:

BRRI

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	30	10	40	1 day (2 number)	2 trainings
(b) Workshop	105	35	140	1 day (2 number)	Inception & closing workshop
(c) Others (if any)					

C. Financial and Physical Progress (combined & component wise)
Combined

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance / unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	2928947	2925658	2925658	0	100	Not applicable
b. Field research/lab expenses and supplies	4791810	4765400	4765400	0	100	Not applicable
c. Operating expenses	611300	598349	598349	0	100	Not applicable
d. Vehicle hire and fuel, oil & maintenance	565050	564650	564650	0	100	Not applicable
e. Training/workshop/ seminar etc.	366883	363883	363883	0	100	Not applicable
f. Publications and printing	225000	199305	199305	0	87.12	Leaflet couldn't published
g. Miscellaneous	260590	278564	278564	0	100	
h. Capital expenses	494700	494209	494209	0	99.945	Price of some item was lower than estimated.
Total	10244280	10183693	10183693	0	99.855	Leaflet couldn't publish and price of some item was lower than estimated.

BRRI

Fig in Tk

Items of expenditure/ activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	2137390	2134101	2134101	0	100	Not applicable
b. Field research/lab expenses and supplies	3399176	3382866	3382866	0	100	Not applicable
c. Operating expenses	335241	347858	347858	0	100	Not applicable
d. Vehicle hire and fuel, oil & maintenance	444050	446750	446750	0	100	Not applicable
e.Training/workshop /seminar etc.	366883	363883	363883	0	100	Not applicable
f. Publications and printing	225000	199305	199305	0	87.12	Leaflet couldn't published
g. Miscellaneous	159820	178155	178155	0	100	Not applicable
h. Capital expenses	453900	453409	453409	0	99.89	Price of some item was lower than estimated.
Total	**7521460**	**7500002**	**7500002**	**0**	**99.71**	Leaflet couldn't publish and price of some item was lower than estimated.

Islamic University

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance / unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	791557	791557	791557	0	100	
b. Field research/lab expenses and supplies	1392634	1382534	1382534	0	100	Not applicable
c. Operating expenses	276059	250491	250491	0	100	
d. Vehicle hire and fuel, oil & maintenance	121000	117900	117900	0	100	
e.Training/workshop/seminar etc.	0	0	0	0	0	
f. Publications and printing	0	0	0	0	0	
g. Miscellaneous	100770	100409	100409	0	100	
h. Capital expenses	40800	40800	40800	0	100	
Total	**2722820**	**2683691**	**2683691**	**0**	**100**	

D. Achievement of Sub-project by Objectives (Tangible form): Technology generated/developed

General/ specific objectives of the sub-project	**Major technical activities performed in respect of the set objectives**	**Output (i.e., product obtained, visible, measurable)**	**Outcome (short term effect of the research)**
Development of environmental safe biopesticide to control bakanae disease and to increase yield of rice	1.Characterization and molecular identification of biocontrol agents	1.Nineteen biocontrol agents were identified at species level	1. Identified biocontrol agents will be used for formulation of biopesticides.
	2.Find out suitable carrier for formulation of biopesticide using identified biocontrol agent/s and/or active ingredient	2. Method of formulation for fungal and bacterial biopesticides has been completed (100%).	2. Formulated two biopestides were found effective to manage bakanae disease in field condition.
	3.Field trial with formulated biopesticide for controlling bakanae disease	3. In T. aman 2021, two trials were also set up at Cumilla and Habiganj. Experiments had been set up in field condition in boro 2021-22 at Gazipur and Cumilla with biopesticides as well as with nano particles.	3. Formulated biopesticides are suitable to manage bakanae disease and increase yield.
	4. Formulation of nanoparticles from identified effective plant product/active ingredients and find out their efficacy against bakanae disease in field condition.	4. Neem leaf and dodder plant mediated silver nano particle was produced from formulated and tested the nano particles against *Fusarium fujikuroi* in *in vitro* and *in vivo* condition.	4. Formulated nanoparticles are able to manage bakanae disease and increase yield.

E: Information/Knowledge generated/Policy generated

BRRI Component

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
1. To characterize the identified effective biocontrol fungi/bacteria at species level and formulate biopesticide.	1. Nineteen biocontrol agents were identified at species levels that were found effective to manage bakanae disease. 2. Silver nano particles were developed using neem leaf extract and dodder stem extracts, optimized and characterized.	1. Out of nineteen biocontrol agents two biopesticidal formulations were developed against bakanae disese. 2. Method of nanoparticle synthesize, optimize and characterization have been developed using organic source.	1.Two biopesticides have been formulated from the identified biocontrol agents. 2. Two silver nano particles were developed that were found effective to manage bakanae disease.
2. To evaluate field efficacy of formulated biopesticiede and nano particle against bakanae disease of rice in field condition.	A total of 19 field trials were conducted for 2 years to find out the effectiveness of formulated biopesticides to manage bakanae disease.	Formulated two biopesticides and synthesized two silver nano particles using organic sources.	Bakanae disease was managed successfully by increasing tiller and panicles/hill, increase grain number and grain weight/panicle and finally increased rice yield.

Islamic University component

General/specific objectives of the sub-project	**Major technical activities performed in respect of the set objectives**	**Output**	**Outcome (short term effect of the research)**
Formulation of nano particle from identified effective plant product/active ingredient.	Synthesized, optimized and characterized silver nano particles using neem leaf extract and dodder stem extract	Silver nano particles were developed using neem leaf extract and dodder stem extract.	Synthesized nano particles were effective against bakanae disease.
In-vitro evaluation of nano particle against *Fusarium fujikuroi/ Fusarium proliferatum.*	1.Dual cultural test was done in laboratory to find out the effectiveness of developed nano particles. Silver nano particles were developed using neem leaf extract and dodder stem extract. 2.*In vitro* test was done in net house to find effectiveness of developed nano particles against bakanae disease.	Found effective against bakanae disease.	Synthesized nano particles were effective against bakanae disease in *in vitro* condition.

BRRI and Islamic University

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
To identify the developed silver nano particles against bakanae disease in field condition	Field trials were done for two years to find out the effectiveness of developed silver nano particles in field condition.	Silver nano particle was synthesized; optimized and characterized using neem leaf extract and swarnalata (dodder) stem extract.	Synthesized nano particles were found effective against bakanae disease in field condition.

F. Materials Development/Publication made under the Sub-project

Publication	Number of publications		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	**1**		
Journal publication	7	Prepared draft for publication.	1. Characterization and Identification of biocontrol agents against bakanae disease of rice. 2. Formulation of bacterial biopesticide to manage bakanae disease of rice. 3. Silver nano particle is developed using neem leaf extract to manage bakanae disease of rice. 4. Silver nano particle is developed using swarnalata (dodder) stem extract to manage bakanae disease of rice. 5. Bakanae disease management by application of *Trichoderma* based Trichocompost in field condition.
Video clip/TV program			
News Paper/Popular Article	4		Appendix-1
Other publications,		M.Sc Thesis	Appendix-2

if any			

G. Description of generated Technology/Knowledge/Policy

(i) Technology Fact Sheet

BRRI component

1. Title of the technology: Bakanae disease management by application of *Trichoderma* biopesticide based Trichocompost under field condition.

Introduction: Bakanae caused by *Fusarium fujikuroi/F. proliferatum* in rice is sporadically distributed in Bangladesh mainly in greater Cumilla, Habiganj and Mymensingh districts. But, the incidence of bakanae is increasing in Bangladesh (Haq *et al.* 2011) and growing more concern to rice growers as yield loss 21-51.53% has been reported in Bangladesh (Hossain *et al.*, 2013; Angeles *et al*., 2006). In the present perspective of Bangladesh, it is essential to minimize yield loss due to diseases for increasing rice production in decreasing land area. Despite the considerable economic impact of bakanae, efficient and effective control methods are scantly except the seed treatment with chemical fungicides. More effective and environmentally sound control measures using antagonistic microorganisms and natural plant products commonly known as biopesticide might have an alternative approach to control *F. fujikuroi/F. proliferatum. Trichoderma* spp. has been identified as a very proactive biocontrol agent for sheath blight disease management in Bangladesh. Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Therefore, it is worth to look for environmentally and toxicologically safe and more effective methods (biopesticide) to control bakanae disease and to replace chemicals gradually with biopesticides which are safe to human, and non-target to other beneficial organisms and cheaper than the chemicals.

Description: *Trichoderma* biopesticide based Trichocompost was prepared with water hyacinth, cow dung and *Trichoderma* inoculum. *Trichoderma* (*Trichoderma asperelloides*) inoculum was prepared at Plant Pathology Laboratory in broken corn seeds. All materials were mixed and placed in layers in a composting pile in ratio of water hyacinth: Cow dung: *Trichoderma inoculum:* 3: 1: 0.16 (w/w). Urea solution (10%) was used for rapid decomposing. Trichocompost was prepared within 6-8 weeks (Fig. 96a). Nutrient analysis in Trichocompost produced from *Trichoderma* based biopesticide was also analysed with the help of Soil Science Division, BRRI. *Trichoderma* biopesticide based Trichocompost was found effcetive to manage bakanae disease in field condition when applied in field condition @ 1.5t/ha during land preparation or after visible symtom in field condition. Biopesticide with *Trichoderma* was formulated with corn seed and packed in powder form in polythelene bag and stiored in room temparature. It was observed that *Trichoderma* sp. can survive >12 months in corn formulation at room temperature condition (Fig.96b).

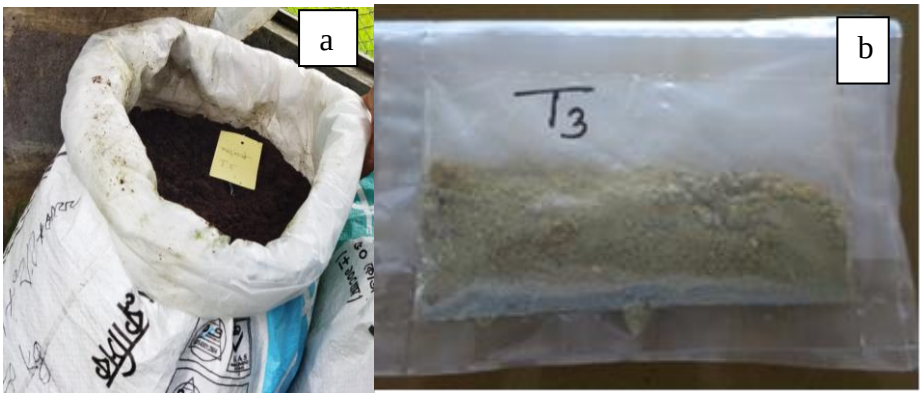


Fig.96. Prepared Trichocompost (a) and *Trichoderma* base formulated biopesticide (b).

Suitable Location: Almost all areas of Bangladesh where bakanae is prominent.

Benefits: Disease incidence was decreased and yield was increased by application of *Trichoderma* biopesticide based Trichocompost in field condition. This yield was increased by increasing total tiller/hill, total effective tiller/hill, filled grain/panicle and as well as increased grain wt./panicle. Moreover, high nutrient value was obtained from Trichocompost produced from *Trichoderma* based biopesticide and are presented in Table 10. Nutrient analysis from Trichocompost, it is suggested that cultivation cost will be reduced in accordance with bakanae disease management (Table 11). Moreover, farmers' will be financially benefitted by using Trichocompost application in field condition and are presented in simple profitability analysis (Table 12). Therefore, it is recommended to apply this technology at field level after patenting, registration and commercially large-scale production for increasing environmentally safe rice production through sustainable management of bakanae disease of rice in the concerned areas. It is cheaper and cost efefctive and environmenal friendly.

Table.10. Nutrient analysis of Trichocompost produced from *Trichoderma* based biopesticide when applied @ 2.0 t/ha in field.

Elements	Content (%)	Kg/ha
N	0.5	10
P	0.44	8.8
K	3.59	71.6
S	0.244	4.9
Zn	0.031	0.62
Organic carbon in soil after Trichocompost application	2.5	2.4

(Nutrient analysis was done with the help of Soil Science Division, BRRI

Table 11. Cost estimation of fertilizers supplied form nutrient elements of Trichocompost

Elements	Kg/ha	Kg/33 decimel	Fertilizers equivalent to supplied from nutrient Kg/33 decimel	Price of fertilizers equivalent to supplied nutrient/33 decimel (Taka)
N	10	1.3	2.83 (Urea)	45
P	8.7	1.16	29(TSP)	435
K	71.1	9.48	18.96(MOP)	417
S	4.8	0.64	4.15(Zinc sulphate)	829
Total Price				1726 taka

Table 12. Simple profitability analysis in Trichocompost applied in field condition

Treatment	Treatment application rate	Yield increase over control (t/ha)	Price of paddy @ 1000tk/ mond	Cost of treatment apply/ha (taka)	Price of fertilizers equivalent to supplied fertilizer/ ha (Taka)	Benefit ration over Treatment use/ha (taka)
Trichocompost at seedbed when disease incidence 30-40%	0.5 t/ha	0.4	10000	3281 (Inoulum= 1125tk, cowdung= 281k, labour cost=1875tk)	12945	19664
Trichocompost at field condition when disease incidence 22%	1.5 t/ha	3.18	86000	9844 (Inoulum= 3375tk, cowdung= 844tk, labour cost=5625tk)	12945	89101
Trichocompost at field condition when disease incidence >60%	2.0 t/ha	4 .5 t/ha	121,621	13125 (Inoulum= 4500tk, cowdung= 1125tk, labour cost=7500tk)	12945	121441

Name and contact address of author: Dr. Quazi Shireen Akhter Jahan, Principal Scientific Officer & Head, Bangladesh Rice Research Institute, Barishal regional Station, Barishal. Mobile: +880 1855873259; Email: shireenbrri@yahoo.com

2. **Title of the technology:** Bakanae disease management by application of bacterial biopesticide formulation in field condition.

Introduction: Bakanae caused by *Fusarium fujikuroi/F. proliferatum* in rice is sporadically distributed in Bangladesh mainly in greater Cumilla, Habiganj and Mymensingh districts. But, the incidence of bakanae is increasing in Bangladesh (Haq *et al*. 2011) and growing more concern to rice growers as yield loss 21-51.53% has been reported in Bangladesh (Hossain *et al*., 2013; Angeles *et al*., 2006). In the present perspective of Bangladesh, it is essential to minimize yield loss due to diseases for increasing rice production in decreasing land area. Despite the considerable economic impact of bakanae, efficient and effective control methods are scantly except the seed treatment with chemical fungicides. More effective and environmentally sound control measures using antagonistic microorganisms and natural plant products commonly known as biopesticide might have an alternative approach to control *F. fujikuroi/F. proliferatum*. Currently, *Bacillus spp.* is identified as a successful biopesticide for controlling bakanae disease. An antagonistic bacterium, *Pseudomonas fluorescens*, has been found to be an effective biological approach to reduce bakanae disease incidence significantly under field condition (Kazempour and Elahinia, 2007). Research on seed treatment of rice with associated antagonistic bacteria gave good results for controlling bakanae disease at IRRI (Rosales and Mew, 1997). Significant reduction of bakanae disease was also observed in soil incorporated with *Pseudomonas putida* and *Pseudomonas aureofaciens* in Korea (Mew and Rosales, 1992). *Pseudomonas fluorescens* improved disease resistance and increased yield by 8.5-16.2% infected with Fusarium root rot caused by *Fusarium* species (Rajappan and Ramaraj 1999). Bakanae incidence was reduced to 18–19% when naturally infected IR42 seeds were tested with bacteria compared with benomyl treatment in farmer's field near the IRRI farm (Rosales *et al.*, 1986). Therefore, it is worth to look for environmentally and toxicologically safe and more effective methods (biopesticide) to control bakanae disease and to replace chemicals gradually with biopesticides which are safe to human, and non-target to other beneficial organisms and cheaper than the chemicals.

Description: The identified potential biocontrol bacterium (*Serratia* sp.) was formulated as bacterial biopesticide in liquid medium. Two types of formulations were prepared. Formulation-1 was formulated with and without glycerol in combination with starch in nutrient broth (100%) in conical flask and stored in room temparature. Formulation-2 was formulated with and without glycerol in combination with starch in water (86%) and in combination with nutrient broth (12-14%). It was observed that bacterial biopesticide can survive up to 12 months in both formulations (Fig.97 and 98). In both formulations, bacterial concentration was @ 3.0 x 10^7 CFU/ml. The rate of application of bacterial biopesticide 20ml/L is found effective to manage bakanae disease in field condition. Bacterial biopesticide were applied as root dip for 30 minutes before seedlings transplantation.

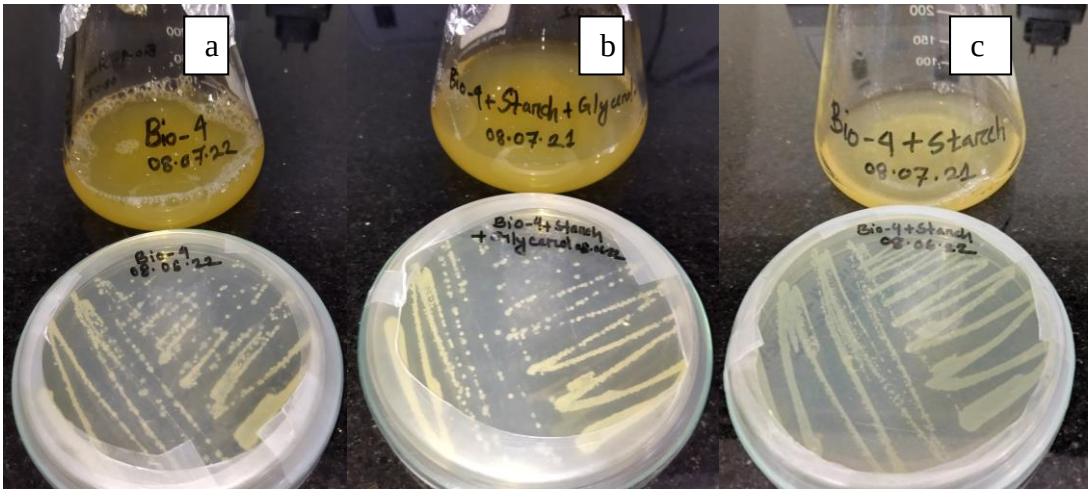


Fig.97. Shelf-life study test of formulation-1 with and without glycerol after12 month (a=nutrient broth, b= with glycerol, c= without glycerol).

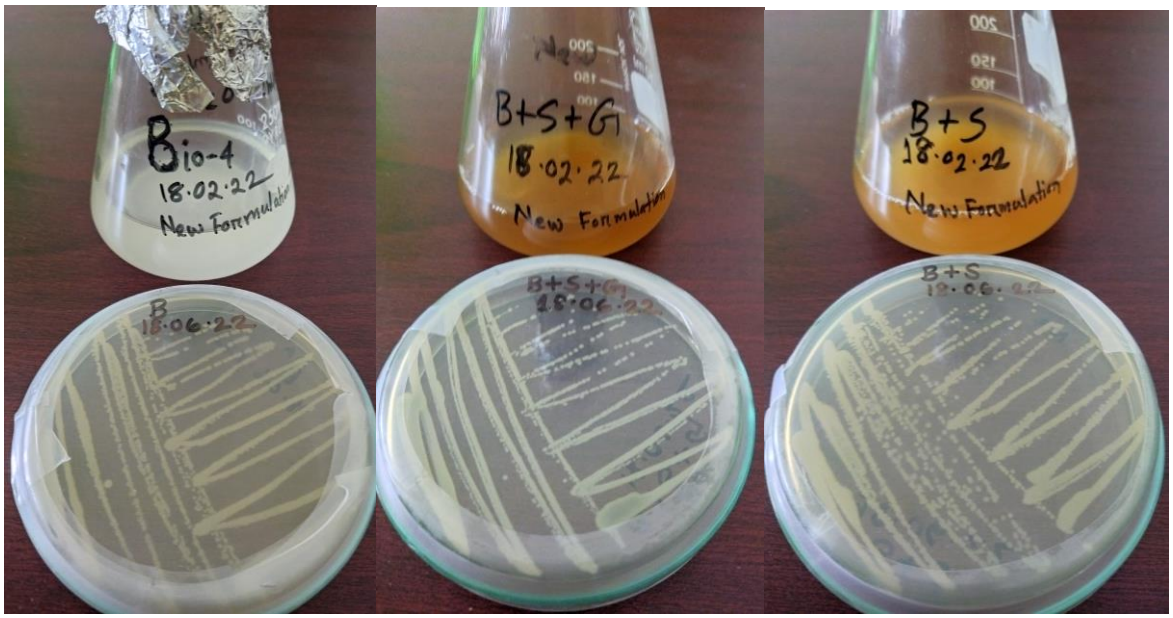


Fig.98. Shelf-life study test of formulation-2 with and without glycerol after12 month (a= nutrient broth (14%) in water, b= nutrient broth (12%) in water with glycerol, c= nutrient broth (13%) in water without glycerol).

Suitable Location: Almost all areas of Bangladesh where bakanae is prominent.

Benefits: Disease incidence was decreased and yield was inccreased by application of bacterial biopesticide in field condition. This yield was increased by increasing total tiller/hill, total effective tiller/hill, filled grain/panicle and as well as increased grain wt./panicle. Moreover, farmers will be financially benefitted by using bacterial biopesticide application in field condition that is presented in simple profitability analysis (Table 13). It is also mentioned that bacterial biopesticide produced from *Serratia* sp. has characteristic to phosphate solubilizaion and thus facilitate phosphorus fertilizer in available form from unavailable form (Zhu *et al*., 2011). Therefore, it is recommended to apply this technology at field level after patenting, registration and commercially large-scale production for increasing environmentally safe rice production through sustainable management of bakanae disease of rice in the concerned areas. It is cheaper and cost efefctive and environmenal friendly.

Table 13. Simple profitability analysis in bacterial biopesticide applied in field condition

Treatment	Treatment application rate	average yield increase over control (t/ha)	Price of paddy @ 1000tk/ mond	Cost of treatment apply/ha (taka)	Price of fertilizers equivalent to supplied fertilizer/ha (Taka)	Benefit ration over Treatment use/ha (taka)
Bacterial biopesticide Formulation-1 (DI: 5%-25%)	20ml/L	0.2-3.18	5405-85945	3320 (Inoulum= 2320tk, labour cost=1000 k)	approximately 1000 (By means of phosphate solubilizing from unavailable form to available form).	3085-83625
Bacterial biopesticide Formulation-2 (DI: 5%-25%)	20ml/L	0.2-3.18	5405-85945	1232 (Inoulum= 232tk, labour cost=1000 tk)	approximately 1000	5173-85713

Name and contact address of author: Dr. Quazi Shireen Akhter Jahan, Principal Scientific Officer & Head, Bangladesh Rice Research Institute, Barishal regional Station, Barishal. Mobile: +880 1855873259; Email: shireenbrri@yahoo.com

BRRI and Islamic University joint component:

1. **Title of the technology:** Bakanae disease management by application of neem leaf mediated nano technology in field condition.

   Introduction: Bakanae caused by *Fusarium fujikuroi/F. proliferatum* is an endemic fungal disease in rice and is sporadically distributed in Bangladesh mainly in greater Cumilla, Habiganj and Mymensingh districts. But, the incidence of bakanae is increasing in Bangladesh (Haq *et al.* 2011) and growing more concern to rice growers as yield loss 21-51.53% has been reported in Bangladesh (Hossain *et al.*, 2013; Angeles *et al.*, 2006). Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Therefore, it is worth to look for environmentally and toxilogically safe and more effective methods to control bakanae disease and to replace chemicals gradually which are safe to human, and non-target to other beneficial organisms and cheaper than the chemicals. Moreover, neem leaf extraction in ethanol, has been identified to inhibit (100%) mycelial growth of the bakanae causing pathogen *in vitro*. Recently, silver nanoparticles have increased in popularity, due to "green synthesis" production in plants, bacteria, fungi, or yeast (Rafique *et al.*, 2017). Silver nanoparticles have shown antifungal inhibition of

Alternaria alternata, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea*, and *Curvularia lunata* by well diffusion assay (Krishnara *et al.*, 2012). Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Therefore, it is worth to look for environmentally and toxilogically safe and more effective methods to control bakanae disease and to replace chemicals gradually with with alternate approaches which are safe to human, and non-target to other beneficial organisms and cheaper than the chemicals.

Description: The biosynthesis of AgNPs was carried out by following the procedures of Asimuddin *et al.*, (2020). The AgNPs was biosynthesized by mixing the aqueous neem leaf extract with silver nitrate ($AgNO_3$) solution keeping the final concentration of the mixture always to 1mM. The mixture was then heated at 85°C in a hot air performance incubator (AP120, Froilabo, France). The color change of the reaction mixture from the initial colorless to yellowish color and finally to red brown color primarily indicates the completion of the reduction of silver ion (Ag+) to silver particle (Ag^0) and the biosynthesis of AgNPs (Fig 99). Subsequently, the formation of AgNPs was further confirmed in aqueous solution using a UV-VIS spectrophotometer (U-2900 UV/VIS Spectrophotometer 200V, HITACHI, Japan) by scanning 3 mL of reaction mixtures in the range between 300 to 700 nm. The absorption spectra for each reaction mixture were recorded as a function of reaction time with a resolution of 1 nm (Fig.100).

Fig.99. Biosynthesis of the silver nanoparticles by adding neem leaves extract with $AgNO_3$ solution

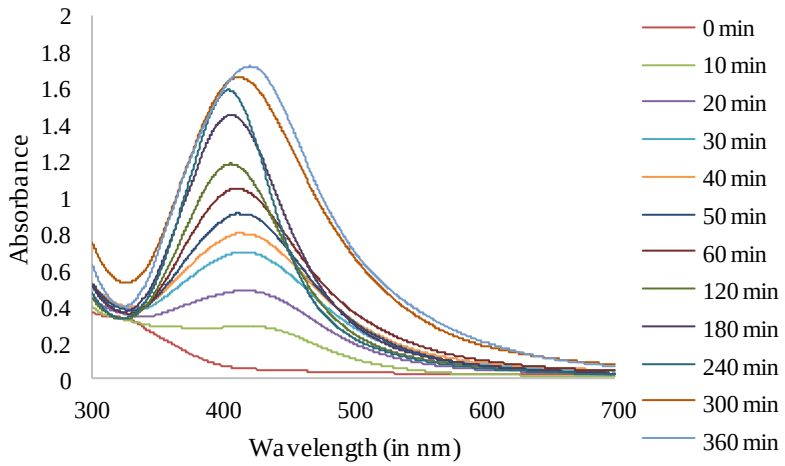


Fig 100. The UV-VIS spectra of AgNPs biosynthesized using neem (*A. indica*) leaf extract and $AgNO_3$ solution (1+19 ratio) at time intervals of 0, 10, 20, 30, 40, 50, 60, 120, 180, 240, 300, and 360 min, respectively.

Finally, the optimization of the incubation time of the reaction mixtures (using the optimized amount of leaf extract) was carried out based on color development and UV-VIS spectra analysis. The color development of each of the reaction mixture at each time interval was monitored and also the UV-VIS spectrum of each of the mixture was carried out in order to confirm the optimum incubation time. The formation of AgNPs through the reduction of silver ion using *A. indica* aqueous leaf extract and *C. reflexa* aqueous stems extract was observed primarily by the color change in the reaction solutions. The colloidal red brown color change indicated the formation of Ag nanoparticles from which is due to the excitation of surface plasmon vibrations in the metal nanoparticles (Vanlalveni *et al,* 2021). Previous studies (Ahmed *et al*., 2016; Roy *et al*., 2017) have also reported similar changes in color due to the reaction between leaf extract and $AgNO_3$ and therefore, the complete reduction of Ag+ ions were confirmed. Incubation of the reaction mixture for 4 hr has been the best time for the formation of monodisperse and small sized AgNPs. Moreover, the maximum absorption peak was found at 404 nm wavelength after 240 min of incubation indicates a size of approximately 20 nm. Therefore, it has been confirmed that incubation for 4 hr of the reaction mixture is the best time for the production of monodisperse, spherical and small-sized AgNPs in this study.

Suitable Location: Almost all areas of Bangladesh where bakanae is prominent.

Benefits: Disease incidence was decreased and yield was inccreased by application of neem leaf mediated nano particle in field condition. This yield was increased by increasing total tiller/hill, total effective tiller/hill, filled grain/panicle and as well as increased grain wt./panicle. It was also noticed that almost all tillers were able to produce grain in nano treated plants whereas, only 50% tillers were able to produce grain in control plants. Moreover, farmers' will be financially benefitted by using neem leaf-based silver nano particle application in field condition that are presented in simple profitability analysis (Table 14). Therefore, it is recommended to apply this technology at field level after patenting, registration and commercially large scale production for increasing environmentally safe rice production through sustainable management of bakanae disease of rice in the concerned areas.

Table. 14. Simple profitability analysis with silver nano particle using neem leaf extract in field condition.

Treatment	Treatment application method	Average yield increase over control (t/ha)	Price of paddy @ 1000tk/ mond	Cost of treatment application/ ha(taka)	Benefit ratio over control treatment use/ ha(taka)
Silver nano particle using neem leaf extract (Disease incidence: 20-25%)	Root dip for overnight	0. 7	18919	1428	17491

Name and contact address of author: Dr. Quazi Shireen Akhter Jahan, Principal Scientific Officer & Head, Bangladesh Rice Research Institute, Barishal regional Station, Barishal. Mobile: +880 1855873259; Email: shireenbrri@yahoo.com and
Dr. A.T.M. Mijanur Rahman, Professor, Dept. of Applied Nutrition & Food Technology, Islamic University, Kushtia. Mobile: +880-1716053597, Email: mijananftiubd@gmail.com

2.Title of the technology: Bakanae disease management by application of Swarnalata (dodder) stem extract mediated nano technology in field condition.

Introduction: Bakanae caused by *Fusarium fujikuroi/F. proliferatum* is an endemic fungal disease in rice and is sporadically distributed in Bangladesh mainly in greater Cumilla, Habiganj and Mymensingh districts. But, the incidence of bakanae is increasing in Bangladesh (Haq *et al.* 2011) and growing more concern to rice growers as yield loss 21-51.53% has been reported in Bangladesh (Hossain *et al.*, 2013; Angeles *et al*., 2006). Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Therefore, it is worth to look for environmentally and toxicologically safe and more effective methods to control bakanae disease and to replace chemicals gradually which are safe to human, and non-target to other beneficial organisms and cheaper than the chemicals. Moreover, swarnalata stem extraction in ethanol has been identified to inhibit 100% mycelial growth of the bakanae causing pathogen *in vitro*. Recently, silver nanoparticles have increased in popularity, due to "green synthesis" production in plants, bacteria, fungi, or yeast (Rafique *et al*., 2017). Silver nanoparticles have shown antifungal inhibition of *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea*, and *Curvularia lunata* by well diffusion assay (Krishnara *et al*, 2012). Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. Therefore, it is worth to look for environmentally and toxicologically safe and more effective methods to control bakanae disease and to replace chemicals gradually with with alternate approaches which are safe to human and non-target to other beneficial organisms and cheaper than the chemicals.

Description: The biosynthesis of AgNPs was carried out by following the procedures of Asimuddin *et al*., (2020). The AgNPs was biosynthesized by mixing the aqueous dodder stem extract with silver nitrate ($AgNO_3$) solution keeping the final concentration of the mixture always to 1mM. The mixture was then heated at 85°C in a hot air performance incubator (AP120, Froilabo, France). The color change of the reaction mixture from the initial colorless to yellowish color and finally to red brown color primarily indicates the completion of the reduction of silver ion (Ag+) to silver particle (Ag^0) and the biosynthesis of AgNPs. Subsequently, the formation of AgNPs was further confirmed in aqueous solution using a UV-VIS spectrophotometer (U-2900 UV/VIS Spectrophotometer 200V, HITACHI, Japan) by scanning 3 ml of reaction mixtures in the range between 300 to 700 nm. The absorption spectra for each reaction mixture were recorded as a function of reaction time with a resolution of 1 nm. Finally, the optimization of the incubation time of the reaction mixtures (using the optimized amount of leaf extract) was carried out based on color development and UV-VIS spectra analysis. The color development of each of the reaction mixture at each time interval was monitored and also the UV-VIS spectrum of each of the mixture was carried out in order to confirm the optimum incubation time. The formation of AgNPs through the reduction of silver ion using *C. reflexa* aqueous stems extract was observed primarily by the color change in the reaction solutions as shown in Fig.101. The colloidal red brown color change indicated the formation of Ag nanoparticles from which is due to the excitation of surface plasmon vibrations in the metal nanoparticles (Vanlalveni *et al*, 2021). Previous studies (Ahmed *et al*., 2016; Roy *et al*., 2017) have also reported similar changes in color due to the reaction between *C. reflexa* stems extract and $AgNO_3$ and therefore, the complete reduction of Ag+ ions were confirmed. On the other hand, *C. reflexa* stems extract exhibited the sharpest and narrowest shaped SPR bands as well as the peaks shifted toward shorter wavelengths with increasing reaction time at 85°C indicated the mono-dispersity, spherical in shape and smaller sized AgNPs. The distinct maximum absorbance was recorded 1.63 at 417 nm wavelength. It has been reported that the peak position of the spectra can be used to roughly predict the size of AgNPs such as a maximum absorption peak near at 416 nm wavelength indicates a size of approximately 50 nm.

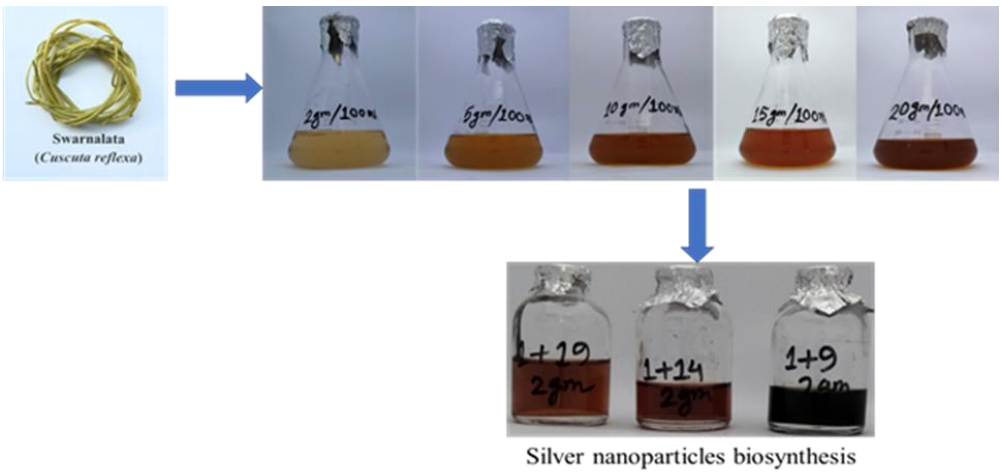


Fig.101. Biosynthesis of AgNPs using *Cuscuta reflexa* stems extract.

Suitable Location: Almost all areas of Bangladesh where bakanae is prominent.

Benefits: Disease incidence was decreased and yield was increased by application of dodder stem mediated nano particle in field condition. This yield was increased by increasing total tiller/hill, total effective tiller/hill, filled grain/panicle and as well as increased grain wt./panicle. Moreover, farmers' will be financially benefitted by using neem leaf-based silver nano particle application in field condition and are presented in simple profitability analysis (Table 15). Therefore, it is recommended to apply this technology at field level after patenting, registration and commercially large-scale production for increasing environmentally safe rice production through sustainable management of bakanae disease of rice in the concerned areas.

Table 15. Simple profitability analysis with silver nano particle using dodder stem extract in field condition.

Treatment	Treatment application rate	Average yield increase over control (t/ha)	Price of paddy @ 1000tk/ mond	Cost of treatment apply/ha(taka)	Benefit ratio over Treatment use/ha (taka)
Silver nano particle using swarnalata stem extract (Disease incidence: 20-25%)	root dip /spray	0.7-1.83	18919-49460	1428	17491-48032

Name and contact address of author: Dr. Quazi Shireen Akhter Jahan, Principal Scientific Officer & Head, Bangladesh Rice Research Institute, Barishal regional Station, Barishal. Mobile: +880 1855873259; Email: shireenbrri@yahoo.com and Dr.A.T.M. Mijanur Rahman, Professor, Dept. of Applied Nutrition & Food Technology, Islamic University, Kushtia. Mobile: +880-1716053597, Email: mijananftiubd@gmail.com

ii. Effectiveness in policy support: Not applicable

H. Technology/Knowledge generation/Policy Support (as applied)

i. Immediate impact on generated technology (commodity & non-commodity)

The formulated biopesticides and developed nanoparticles are effective to manage bakanae disease, increase yield and increase farmers' income.

ii. Generation of new knowledge that help in developing more technology in future

BRRI component

a) The formulated *Trichoderma* based biopesticides can be tested on other major rice pathogens to manage other major rice diseases.
b) The formulated bacteria based biopesticides can be tested on other major rice pathogens to manage other major rice diseases.

BRRI and Islamic University component

a) The formulated neem leaf mediated nano particle can be tested on other major rice pathogens to manage other major rice diseases.
b) The formulated dodder plant mediated nano particle can be tested on other major rice pathogens to manage other major rice diseases.
c) Other nano particle can be produced and formulated using other organic sources that are known to effective against plant pathogen/s.

iii. Technology transferred that help increased agricultural productivity and farmers' income

Agricultural productivity and farmers' income will be increased if generated ttechnologies are transferred.

iv. Policy support: N/A

I. Information regarding Desk and Field Monitoring

i. Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.): N/A

ii. Field Monitoring :

date	no. of visit	name and addresses of team visit	output
21.09.2021	1. (BRRI component)	(1) Dr. Md. Serajul Islam, Environmental and Social Safeguard Specialist; (2) Mohammad Assaduzzaman, Manager (Financial Management); (3)Munshi Mamunur Rahman, Documentation Associate; (4) Md. AbdurRahaman, Monitoring Associate	The ongoing subproject work is satisfactory.
30-ll-2021	2. (BRRI component)	1. Dr. NN Ali sarder, monitoring specialist, PIU-BARC, NATP-2 2. Munshi Mamunur Rahman, Documentation Associate, PIU-BARC, NATP-2 3. Md. Abdur Rahman, Monitoring associate, PIU-BARC, NATP-2 4. Deepak Kumar, Monitoring associate, PIU-BARC, NATP-2	The ongoing subproject work is satisfactory.
10.02.2022	3. (BRRI component)	1. Dr. Md. Serajul Islam, Environmental and Social Safeguard Specialist, PIU-BARC, NATP-2 2. Munshi Mamunur Rahman, Documentation Associate, PIU-BARC, NATP-2 3. A. K. M. Rakib Ullah, Resource Management Associate, PIU-BARC, NATP-2	The ongoing subproject work is satisfactory.
08.03.2022	4. (BRRI component)	1. Dr. Harunur Rashid, Project Director.	The ongoing subproject work is satisfactory.
17.02.2022	1 (Islamic University)	1. 1. Dr. Md. Serajul Islam, Environmental and Social Safeguard Specialist, PIU-BARC, NATP-2 2. 2. Munshi Mamunur Rahman, Documentation Associate, PIU-BARC, NATP-2	The ongoing subproject work is satisfactory.

iii. Weather data, flood/salinity/drought level (if applicable) and natural calamities: N/A

J. Sub-project Auditing (covers all types of audits performed)

BRRI component

Types of audits	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
GoB Audit (FAPAD)	No objection raised, found all relevant documents updated as per guideline	1791056	1st Year	Financial management & project performance found satisfactory in all the audit cases
GoB Audit (FAPAD)	No objection raised, found all relevant documents updated as per guideline	1643281	2nd Year	
Total taka		**3434337**		

Islamic University component

Types of audits	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
GoB Audit (FAPAD)	No objection raised, found all relevant documents updated as per guideline	**335804/-**	**1st Year**	Financial management & project performance found satisfactory in all the audit cases
GoB Audit (FAPAD)	No objection raised, found all relevant documents updated as per guideline	**509274.15/-**	**2nd Year**	
Total taka		**845078.15**		

K. Lessons Learned:

i) Learned how to isolate and identify the biocontrol agents against pathogens.
ii) Learned about how to make formulations and shelf-life study of formulations for preparing biopesticide.
iv) Acquire knowledge on nanoparticle and how to make nano particle/s from organic/green sources.
v) Learned about effectiveness of biopesticides and nanoparticles on bakanae disease management in field condition.
vi) Learned on how to develop inter-institutional collaboration.
vii) Learned how to design a new research proposal for achieving the ultimate goal based on the present findings.

L. Challenges (if any):

i. Difficult to conduct the sub-project activities during COVID-19 pandemic situation.
ii. System of fund release is lengthy to maintain the flow of work properly.
iii. Hampered to finish work as transferred of PI from Gazipur to Barishal at the end of the project period.

M. Suggestions for Future Planning (if any):

i. The prepared biopesticides and nano particles need to be formulated in large scale for availability to farmers as they can apply in field condition as soon as the disease symptom is visible.
ii. The identified other biocontrol agents can be used to formulate biopesticides agianst bakanae disease as well as other major diseases of rice.
iii. The prepared biopesticides and nano particles need to be tested for other major rice disease managemnt.

N. References

Abbas A, Naz SS and Syed SA (2015). Antimicrobial activity of silver nanoparticles (AgNPs) against *Erwinia carotovora* pv. *carotovora* and *Alternaria solani*. International Journal of Biosciences, 6(10), 9-14.

Acharya D, Singha KM, Pandey P. *et al.* Shape dependent physical mutilation and lethal effects of silver nanoparticles on bacteria. *Sci Rep* 8, 201 (2018). https://doi.org/10.1038/s41598-017-18590-6.

Ahmad F, Ahmad I and Khan M S (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological research, 163(2):173-181.

Ahmed S, Ahmad M, Swami BL and Ikram S 2016. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extrac,. J. Radiat. Res. Appl. Sci. 9(1): 1-7.

Alexander DB and David Zuberer D (1991). Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biology and Fertility of Soils 12(1): 39-45

Angeles, A.T., Gergon, E.B., Rillon, J.P., Rillon, G.S. and Troung, H.X. (2006). Bakanae: The "Foolish" disease of rice. Rice Technology Bulletin. 55. Philippine Research Institute.

Anonymous (1958). Annual report of the Mycology and Plant Pathology Division, Agriculture Research Institute, Gov. of East Pakistan for the year 1957-58.

Anonymous (1977). Annual report of Bangladesh Rice Research Institute for 1974–75. Mimeographed Paper, BRRI, Joydebpur, Dacca. 64 – 65.

Asimuddin M, Shaik MRa, Adil SF, Siddiqui MRH, Alwarthan A, Jamil K, Khan M (2020). *Azadirachta indica* based biosynthesis of silver nanoparticles andevaluation of their antibacterial and cytotoxic effects. J. King Saud Univ. Sci.32, 648–656

Bansal P, Kaur P and Duhan J S (2017). Biogenesis of silver nanoparticles using *Fusarium pallidoroseum* and its potential against human pathogens. Annals of Biology, 33: 180-185.

Bhattacharjee R and Dey U (2014). An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. African Journal of Microbiological Research, 8(17): 1749-1762.

Chakraborty U, Chakraborty BN and Chakraborty AP (2010) Influence of *Serratia marcescens*TRS-1 on Growth Promotion and Induction of Resistance in *Camellia sinensis* against Fomes lamaoensis. Journal of Plant Interactions, 5, 261-272

Cother, E (2002). Report on a study tour to examine symptoms of rice diseases in southern USA and California. Cooperative Research Centre for Sustainable Rice Production. Agricultural Institute, Orange NSW, 2002. (available online at: http://ses.library.usyd.edu.au/bitstream/2123/168/1/P2403STR11-02.pdf)

Cattelan AJ, Hartel PG and Fuhrmann JJ (1999). Screening for plant growth promoting rhizobacteria to promote early soybean growth. Soil Science Society American journol. 63: 1670-1680.

Cheng H-R and Jiang N (2006). Extremely Rapid Extraction of DNA from Bacteria and Yeasts. Biotechnology Letters volume 28, pages55–59.

Dewihastuti R, Lestari Y, Ussuwanto A and Rawati R (2012). Endophytic *Streptomyces* spp. As Biocontrol Agents of Rice Bacterial Blight Pathogen (*Xanthomonas oryzae* pv. *oryzae*). Journal of Biosciences. 19(4): 155-162.

Ei SL, Lwin KM, Padamyar, Khaing HO, Yu SS (2017) Study on IAA Producing Rhizobacterial Isolates and Their Effect in Talc-Based Carrier on Some Plants. J Soil Sci Plant Health 1:1.

Ejaz M, Raja NI, Mashwani ZR and Ahmad MS (2018). Effect of Silver Nanoparticles and Silver Nitrate on Growth of Rice under Biotic Stress. IET Nanobiotechnology 12(7). DOI:10.1049/iet-nbt.2018.0057

Elbeshehy,E.K.F.;Elazzazy,A.M.;Aggelis,G (2015). Silver nanoparticles synthesis mediated by new isolates of Bacillus spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus and human pathogens. Front.Microbiol, 6,453.

Gang S, Sharma S, Saraf M, Buck M and Schumacher J (2019). Analysis of Indole-3-acetic Acid (IAA) Production in *Klebsiella* by LC-MS/MS and the Salkowski Method. Bio Protocol 5; 9(9): e3230. DOI: 10.21769/BioProtoc.3230

Gangwar GP and Sinha AP. (2010). Comparative antagonistic potential of *Trichoderma* spp. Against *Xanthomonas oryzae* pv. *oryzae*. Annals of Plant Protection Sciences. 18(2): 458-463.

Gnanamanickam, S S (2009). An overview of progress in biological control. In: Biological control of rice diseases: Progress in biological control Series, ed. by S. S. Gnana-manickam, pp. 43-51. Springer, Dordrecht, Netherlands.

Gupta D and Sinha SN (2020). Production of Hydrogen Cyanide (HCN) by Purple Non-Sulfur Bacterium Isolated from the Rice Field of West Bengal. Journal of Pharmacy And Biological Sciences (IOSR-JPBS), 15(1): 16-26.

Halebian S, Harris B, Finegold SM and Rolfei RD (1981). Rapid Method That Aids in Distinguishing Gram-Positive from Gram-Negative Anaerobic Bacteria. Journal of Clinical Microbiology: 444-448

Haq M, Mia MAT, Rabbi MF and Ali MA (2011). Incidence and severity of rice diseases and insect pests in relation to climate change. In L. Ratan, V.K.S.Mannava, S.M.A.

Hayles J, Johnson L, Worthley C, Losic D (2017) Nano pesticides :A review of current research and perspectives. New Pestic. Soil Sens.,193–225.

Hossain MS, Ali AM, Mia MAT, Islam MS, and Moni ZR (2013). Estimation of Yield Loss by *Fusarium moniliforme* Caused Bakanae Disease of Rice. Eco-friendly Agril. J.6(03): 40-43.

Hossain MT, Khan A, Chung EJ, Harun-or Rashid M and Chung YR (2016). Biological Control of Rice Bakanae by an Endophytic *Bacillus oryzicola* YC7007. Plant Pathology J, 32(3): 228-241

Jahan QSA, Haque A and Latif MA (2016). Management of Sheath blight disease utilizing *Trichoderma harzianum*. Annual research review workshop 2015-16. Plant Pathology Division, BRRI, Gazipur-1701.

Jeyarajan R (2006). Prospects of indigenous mass production and formulation of *Trichroderma*, In Current Status of Biological Control of Plant diseases using antagonistic organisms in India (Eds Rabindra RJ Ramanujam B), Project Directorate of Biological Control, Bangalore, pp. 74-80, 445

JoYK, Kim BH & Jung G. (2009). Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Disease, 93, 1037-1043.

Kalbe C, Marten P, Berg G. (1996). Strains of the genus *Serratia* as beneficial rhizobacteria of oilseed rape with antifungal properties. Microbiological Research,151(4): 433-439.

Kamal MM and Shahjahan AKM (1995). Trichoderma in rice field soils and their effect on *Rhizoctonia solani*. Bangladesh Journal of Botany, 24(1): 75-79.

Kaur, P., Thakur, P., & Chaudhury, A. (2012). An in vitro study of the antifungal activity of silver/chitosan nano formulations against important seed borne pathogens. International Journal of Science, Technology, and Research, 1(6), 83---86.

Kazempour, M.N. and Elahinia, S.A. (2007). Biological control of *Fusarium fujikuroi*, the causal agent of Bakanae disease by rice associated antagonistic bacteria. Bulgerian Journal of Agriculture and Science 13: 393-408.

Kim, S.H., Park, M.R., Kim, Y.C., Lee, S.W., Choi, B.R., Lee, S.W., and Kim, I.S.(2010). Degradation of prochloraz by rice bakanae disease pathogen *Fusarium fujikuroi* with differing sensitivity: a possible explanation for resistance mechanism. Journal of the Korean Society for Applied Bioloical Chemistry, 53(4): 433-439.

Kim SM, Arockiakumar R and Park JK(2012). Effect of ECAE-processing on the thermomechanical behavior of Ti34 wt.%Nb0.14 wt.%O shape memory alloy. Materials Science and Engineering, 546: 53-58.

Krishnaraj,C.;Ramachandran,R.;Mohan,K.;Kalaichelvan,P(2012). Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Spectrochim.ActaPartAMol.Biomol.Spectrosc, 93, 95–99.

Kumar P, Thenmozhi R, Anupama P. D, Nagasathya A, Thajuddin N and Paneerselvam A (2012). Selection of potential antagonistic *Bacillus* and *Trichoderma* isolates from tomato rhizospheric soil against *Fusarium oxysporum* F. sp. *Lycoperscisi*. Journal of Microbiology and Biotechnology Research, 2 (1):78-89.

Kumar GK and Ram MR. (2014). "Phosphate Solubilizing *Rhizobia* Isolated from *Vigna trilobata*." American Journal of Microbiological Research, vol. 2, no. 3 (2014): 105-109. doi: 10.12691/ajmr-2-3-4.

Kumar N, Salar RK, Kumar R, Prasad M, Brar B, Nain V (2016). Green Synthesis of Silver Nanoparticles and its Applications—A Review. Nano Trends: A Journal of Nanotechnology and Its Applications, 19 (3), ISSN: 0973-418X (O

Lemire, J., Harrison, J. & Turner, R. Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat Rev Microbiol* 11, 371–384 (2013). https://doi.org/10.1038/nrmicro3028

Lewis JA (1991). Formulation and delivery system of biocontrol agents with emphasis on fungi Beltsville symposia. In: The rhizosphere and plant growth (Keister, D. L. and Cregan, P. B. eds.,). Agric. Res. 14:279-287.

Martinez-Castanon, G. A., N. Nino-Martinez, F. Martinez-Gutierrez, J. R. Martinez-Mendoza and F. Ruiz (2008). "Synthesis and antibacterial activity of silver nanoparticles with different sizes." Journal of Nanoparticle Research 10(8): 1343-1348.

Matin MA, Islam MN, Muhammad N and Rahman MH (2019). Impact of *Trichoderma* Enhanced Composting Technology in Improving Soil Productivity. Asian Journal of Soil Science and Plant Nutrition, 4(3): 1-19.

Maina PK, Wachira PM, , Okoth S. A., Kimenju J. W. and Mwangi J. M. (2016). Co-occurrence and Diversity of Soil Trichoderma and Fusarium species from Different Land Use Intensities in Machakos County, Kenya. Archives of Current Research International 4(1): 1-13,

Mew, T. W. and Rosales, A. M. (1992). Control of *Rhizoctonia* sheath blight and other diseases of rice by seed bacterization. In: E. C. Tjamos, G. C. Papavizaz and R. J. Cook. Biological Control of Plant (pp. 113–123). New York: Plenum Press.

Misra, J.K., Merca, S.D. and Mew, T.W. (1994). Fungal pathogens In T.W. Mew and J.K. Misra. A manual of Rice Seed Health Testing (pp: 75-84). Los Baños, Laguna, Philippines: IRRI, International Rice Research Institute.

Miyaki T, Tateishi H, Sakuma Y and Saishoji T (2012). A novel soil-type biopesticide KNB422-soil against rice seedling diseases. Journal of Pesticide Science, 37(2): 129-134

Mohiddin FA, Khan MR, Khan SM and Bhat BH (2010). Why Trichoderma is considered super hero(Super fungus) against the evil parasites? Plant Pathology Journal, 9(3):92-102

Modi K K.and Jacob F F.(2017). Isolation and Characterization of Phosphate Sollubilizing *Bacillus* Consortia for Plant Growth Promotion. Int. J. Pure App. Biosci. 5 (3): 278-283.

Mohamed EAH, Farag G and Youssef SA(2018). Phosphate Solubilization by *Bacillus subtilis* and *Serratia marcescens* Isolated from Tomato Plant Rhizosphere. Journal of Environmental Protection, 9, 266-277.

Marimuthu, S., Surendran, U. & Subbian, P. (2014). Productivity, nutrient uptake and postharvest soil fertility as influenced by cotton-based cropping system with integrated nutrient management practices in semiarid tropics. Arch. Agron. Soil Sci., 60(1), 87–101.

Ora N., Faruq A.N., Islam M.T., Akhtar N.and Rahman M.M. (2011). Detection and Identification of Seed Borne Pathogens from Some Cultivated Hybrid Rice Varieties in Bangladesh. Middle-East Journal of Scientific Research 10 (4): 482-488.

Panchal KJ (2021). Identification of Cellulase Enzyme Involved in Biocontrol Activity. Practical Handbook on Agricultural Microbiology pp 317–322.

Paramelle D, Sadovovy A, Gorelik S, FreeP J. Hobley J, and D. G. Fernig DG. (2014). Rapid method to estimate the concentration of citrate capped silver nanoparticles from UV-visible light spectra. The Analyst. DOI:10.1039/c4an00978a

Prabhu S and Poulose EK (2012) Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett 2(1):1–10.

Peng, H., Wang, N., Hu, Z. R., Yu, Z. P, Liu, Y. H., Zhang, J. S., and Ruan, R. (2012). "Physicochemical characterization of hemicelluloses from bamboo (Phyllostachys pubescens Mazel) stem," Ind. Crop. Prod. 37(1), 41-50. DOI: 10.1016/j.indcrop.2011.11.031.

Rahman MZ, Ali MA, Hossain MS, Monsur MA, Zaman MZ (2014). Effect of lemon grass (*Cymbopogen citratus*) extract on seed health of rice. Bangladesh Rice Journal, 17:1-2.

Rafique M, Sadaf, I, Rafique MS, Tahir MB (2017). A review on green synthesis of silver nanoparticles and their applications. Artif. Cells Nanomed. Biotechnol, 45:1272–1291.

Rani SS, Justin CGL, Gunasekaran K and Roseleen SSJ (2019). Efficacy of green synthesized silver nanoparticle, plant powders and oil against rice weevil *Sitophilus oryzae* L. (Coleoptera: *Curculionidae*) on sorghum seeds. Journal of Pharmacognosy and Phytochemistry; 8(5): 38-42.

Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27(1):76–83.

Rao MS, Umamaheswari R, Chakravarthy AK, Grace GN, Kamalnath M and Prabu P (2015). A frontier area of research on liquid biopesticides: the way forward for sustainable agriculture in India. Current Science, 108(9): 1590-92

Rajappan K and Ramaraj B (1999). Evaluation of fungal and bacterial antagonists against *Fusarium moniliforme* causing wilt of cauliflower. Ann Plant Protect Sci 7:205_207.

Rezzonico, F., Zala, M., Keel, C., Duffy, B., Moënne-Loccoz, Y., & Défago, G. (2007). Is the ability of biocontrol fluorescent *pseudomonads* to produce the antifungal metabolite 2, 4-diacetylphloroglucinol really synonymous with higher plant protection. New Phytologist, 173(4), 861-872.

Riazuddin M, Rahman H, Haque A.H.M.M., Rashid M.H. and Siddiqui M.N. A (2013). Eco-friendly management of seedling blight of rice. Bulletin of Institute of Tropical Agriculture, 36:1-7.

Rosales, A.M. and Mew, T.W. (1997). Suppression of *Fusarium moniliforme* in rice by rice-associated antagonistic bacteria. Plant Disease 81:49-52.

Rosales, A.M., Mew, T.W. and Nuque, F.L. (1986). Biological control of bakanae disease of rice (*Oryza sativa*) by antagonistic bacteria [Philippines]. Paper presented in 17 Anniversary and Annual Convention of the Pest Control Council of the Philippines,), 8-10 May 1986. Iloilo City, Philippines, 1986.

Roy P, Das B, Mohanty A and Mohapatra S 2017. Green synthesis of silver nanoparticles using *Azadirachta indica* leaf extract and its antimicrobial study, Appl. Nanosci. **7:** 843-850.

Saha D, Purkayastha GD and Saha A (2012). Biological Control of Plant Diseases by *Serratia* Species: A Review or a Case Study. In Frontiers on Recent Developments in Plant Science.eds. Akash Goyal Canada and Priti Maheswari. Pp: 99-115 (17) Doi: 10.2174/978160805403911201010099

Schwyn, B. and Neilands, J.B., (1987). Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 160, 47–56.

Sastry, M., Mayya, K.S. and Bandyopadhyay, K. (1997). "pH Dependent Changes in the Optical Properties of Carboxylic Acid Derivatized Silver Colloidal Particles", Colloids Surf. A, 127, 221-228.Sethi S,Datta A, Gupta L,1and Gupta S(2013). Optimization of Cellulase Production from Bacteria Isolated from Soil. Biotechnology,1-7.http://dx.doi.org/10.5402/2013/985685.

Sharma, A.; Johri, B.N. Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. Microbiological Research, v.158, p.243-248, 2003.

Siddiqui, I. A., Shaukat, S. S., Sheikh, I. H., & Khan, A. (2006). Role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. World Journal of Microbiology and Biotechnology, 22(6), 641-650.

Sujatha N and Ammani K (2013). Siderophore production by the isolates of fluorescent *Pseudomonads*. International journal of current research and review, 5 (1-7).

Sohel MH and Ghosh MK (2021). Effect of Compost, Vermicompost, Trichocompost and NPKS Fertilizers on the Growth, Yield and Yield Components of Capsicum (*Capsicum annum* L). Effect of compost, vermicompost, trichocompost and NPKS fertilizers on the growth, yield and yield components of capsicum (*Capsicum annum* L.). EBAUB J., 3, 29-35.

Souza R.d., Ambrosini A., Passaglia L.M. Plant growth-promoting bacteria as inoculants in agricultural soils. Genet. Mol. Biol. 2015; 38:401–419. doi: 10.1590/S1415-475738420150053

Vanlalveni C, Lallianrawna S, Biswas A, Selvaraj M, Changmai B, Rokhum SL (2021). Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. RSC Adv. 11:2804–2837.

Yasmin M, Hossain KS, Bashar MA (2008). Effects of some angiospermic plant extracts on in vitro vegetative growth of *Fusarium moniliforme*. Bangladesh Journal of Botany, 37(1): 85-88.

Zakharova, O. V., Gusev, A. A., Zherebin, P. M., Skripnikova, E.V., Skripnikova, M. K., Ryzhikh, V. E., & Krutyakov, Y. A. (2017). Sodium tallow amphopolycarboxyglycinate-stabilized silver nanoparticles suppress early and late blight of *Solanum lycopersicum* and stimulate the growth of tomato plants. Bio Nano Science.

Zhu F, Qu L, Hong X and Sun X (2011). Isolation and Characterization of a Phosphate Solubilizing Halophilic Bacterium *Kushneria sp*. YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China. Evidence-Based Complementary and Alternative MedicineVolume 2011, Article ID 615032, 6 pages. doi:10.1155/2011/615032

Signature of the Coordinator
Date …….

Seal

Counter signature of the Head of the organization/authorized representative
Date ……..
Seal

Appendix-1: News Paper News

1. Amar Somoy

আমার সময়

গাজীপুরে ফসল কর্তন ও মাঠ দিবস অনুষ্ঠিত

2. **Ajker jonbani**

দৈনিক আজকের জনবাণী

গাজীপুর সদরে ফসল কর্তন ও মাঠ দিবস অনুষ্ঠিত

মানিক মিয়া, গাজীপুর সদর প্রতিনিধি

গাজীপুর সদর উপজেলায় বায়োপেস্টিসাইড ও অর্গানিক ন্যানোপার্টিক্যাল ব্যবহারের মাধ্যমে ধানের বাকানী রোগ দমন শীর্ষক ফসল কর্তন ও মাঠ দিবস অনুষ্ঠিত হয়েছে।

বুধবার (২৭ এপ্রিল) সকালে কৃষি সম্প্রসারন অধিদপ্তরের সহযোগীতায় ও বাংলাদেশ ধান গবেষণা ইনস্টিটিউট উদ্ভিদ রোগতত্ত্ব বিভাগের ব্যাস্তবায়নে সদর উপজেলার ভাওয়ালগড় ইউনিয়নের রূদ্রপুর গ্রামের বিভিন্ন ধান ক্ষেতে ফসল কর্তন ও মাঠ দিবস অনুষ্ঠিত হয়। গাজীপুর সদর উপজেলা কৃষি কর্মকর্তা হাসিবুল হাসানের সভাপতিত্বে প্রধান পৃষ্ঠা ২ কলাম ১

3. **Ajker potrika**

আজকের পত্রিকা

মাঠ দিবস গাজীপুর সদরের ভাওয়ালগড় ইউনিয়নে রুদ্রপুর গ্রামে গতকাল জৈব বালাইনাশক ব্যবহার করে ধানের বাকানি রোগ দমনের ওপর মাঠ দিবস অনুষ্ঠিত হয়েছে। ছবি: আজকের পত্রিকা

4. Dainik Notun khobor


19:46 53.3K/s

মানিক মিয়া গাজীপুর প্রতিনিধি:

গাজীপুর সদর উপজেলায় বায়োপেস্টিসাইড ও অর্গানিক ন্যানোপার্টিক্যাল ব্যবহারের মাধ্যমে ধানের বাকানী রোগ দমন শীর্ষক ফসল কর্তন ও মাঠ দিবস অনুষ্ঠিত হয়েছে।

বুধবার (২৭ এপ্রিল) সকালে কৃষি সম্প্রসারন অধিদপ্তরের সহযোগীতায় ও বাংলাদেশ ধান গবেষণা ইনস্টিটিউট উদ্ভিদ রোগতত্ত্ব বিভাগের ব্যাস্তবায়নে সদর উপজেলার ভাওয়ালগড় ইউনিয়নের বক্রপুর গ্রামের বিভিন্ন ধান ক্ষেতে ফসল কর্তন ও মাঠ দিবস অনুষ্ঠিত হয়।

গাজীপুর সদর উপজেলা কৃষি কর্মকর্তা হাসিবুল হাসানের সভাপতিত্বে প্রধান অতিথি হিসেবে উপস্থিত ছিলেন, বাংলাদেশ ধান গবেষণা ইনষ্টিটিউট (ব্রি) এর ডিরেক্টর রিসার্চ ডঃ খালেকুজ্জামান।

কৃষক আরমান হোসেনের সঞ্চালনায় বিশেষ অতিথি হিসেবে বক্তব্য রাখেন, বাংলাদেশ ধান গবেষণা ইনস্টিটিউট উদ্ভিদ রোগ তত্ত্ব বিভাগের ডঃ আব্দুল লতিফ, NATP,-2 PBRG প্রকল্প পরিচালক, বাংলাদেশ ধান গবেষণা ইনস্টিটিউট প্রধান বৈজ্ঞানিক কর্মকর্তা ডঃ কাজী শিরীন আখতার জাহান,বৈজ্ঞানিক কর্মকর্তা আমেনা খাতুন, উপ সহকারী কৃষি অফিসার লিজয়া রাণী নন্দী ও বিভিন্ন কৃষি কর্মকর্তা সহ নয়নপুর ব্লক এর কৃষকরা।

অনুষ্ঠানে শতাধিক কৃষকের উপস্থিতিতে মাঠ দিবস অনুষ্ঠানে বক্তারা বলেন, ধানখেতে ক্ষতিকর বাকানী (ছত্রাক) রোগ দমনে এবারই তাঁরা পরীক্ষামূলকভাবে ধানের আবাদ করেন, যাতে কৃষকরা বড় ধরনের ক্ষতির মুখে না পড়ে।বায়োপেস্টিসাইড ও অর্গানিক ন্যানোপার্টিক্যাল (নিম পাতা সংশ্লেশিত) ব্যবহারের ব্যাপক সফলতা পাওয়ায় মাঠপর্যায়ে ছড়িয়ে দিতে পারলে কৃষকদের উৎপাদন ব্যয় কমবে এবং ধানের ফলন বাড়বে বলেও আশা করেন বক্তারা।

এসময় কৃষক বিল্লাল হোসেন, এই পদ্ধতি ব্যবহারের

Appendix-2: MSc. Thesis

1. Ziniya Sultana (Roll:171629). Management of Rice Bakanae Disease using *Azadirachta indica* Leaf Extract Mediated Silver Nanoparticles in Field Condition. Reg. No: 1234, Session :2017-18 (M.Sc). Department of Applied Nutrition and Food Technology.Islamic University, Kushtia.

2. Md. Tarek Aziz, Roll (M.Sc) : 181607. Optimization and characterization of green biosynthesis of silver nanoparticles from *Cuscuta reflexa* stem extract. Session :2018-19 (M.Sc), Department of Applied Nutrition and Food Technology. Islamic University, Kushtia

3. Tayeba Tasnima (Roll:181624). Application of *Cuscuta reflexa* stem-mediated Silver Nanoparticles for the control of *Fusarium fujikuroi* in vitro and Rice Bakanae Disease in Field. Reg. No: 1238, Session :2018-19 (M.Sc), Department of Applied Nutrition and Food Technology. Islamic University, Kushtia.