Project ID: 712

Competitive Research Grant

Sub-Project Completion Report

on

Identify and Formulate Biopesticides to Control Bakanae Pathogen

Project Duration

May 2017 to September 2018

Plant Pathology Division
Bangladesh Rice Research Institute
Gazipur-1701

Submitted to
Project Implementation Unit-BARC, NATP-2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

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Acknowledgement

The execution of CRG sub-project has successfully been completed by Bangladesh Rice Research Institute [BRRI] using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thanks to the World Bank for arranging the grand fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to technology generation as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

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Acronyms

B: *Bacillus*

F.: *Fusarium*

mL: Mili Litre

P.: *Pseudomonas*

PDA: Potato Dextrose Agar

PIDG: Percentage Inhibition of Diameter Growth

R: *Rhizoctonia*

T.; *Trichoderma*

Table of Contents

Executive Summary

Bakanae caused by *Fusarium fujikuroi* is an endemic fungal disease in rice and has sporadic distribution in Bangladesh; mainly in greater Cumilla, Habigonj and Mymensingh districts. However, the incidence of bakanae is increasing and up to 51.53% yield loss has been reported from Bangladesh. Considering economic impact of bakanae, a few effective control methods are available, except the seed treatment with chemical fungicides. The use of a single chemical to control bakanae has been found to degrade the chemicals when applied singly (Kim *et al.,* 2010). Moreover, the massive and continued use of these chemicals may generate new strain of fungal pathogen which is resistant to fungicides. Because of the above limitations, more effective and environmentally sound control measures using antagonistic microorganisms and natural plant products commonly known as biopesticide might be an alternative approach to control *F. fujikuroi*. Biocontrolling agents i.e. *Bacillus* spp. *Pseudomonas* spp. and *Trichoderma* spp. have been found to control many plant pathogens. Recently, in different parts of the world, attention has been paid towards exploitation of plant products as novel chemotherapeutants in plant protection. Therefore, this project is taken to find out environmentally and toxicologically safe and more effective methods (biopesticides) to control bakanae pathogen. During the project period, 75 soil samples, 60 infected plant samples and six plant materials had been collected. It was expected to get 2-3 biocontrolling agents/plant products against bakanae pathogen from this project outcome. Forty biocontrolling bacteria, 6 *Trichoderma* spp., have been identified that can inhibit mycelia growth of bakanae pathogen *in vitro* 61-94%. Among the identified biocontrol agents two biocontrolling bacterial isolate (NS 9-4 and Bio-1) and one Trichoderma isolate (T3) were tested on seedlings as root dip method and found promising to manage bakanae disease in comparison with control (inoculated). Moreover, four plant products/ active ingredients namely neem seed extraction in ethanol, neem leaf extraction in ethanol, mehogoni extraction in ethanol and dodder plant extraction in ethanol have been identified to inhibit the mycelial growth completely (100%) of the bakanae pathogen *in vitro*. Thus, identified effective plant active ingredients/plant products will be used to formulate biopesticide singly or in accordance with the effective biocontrolling agent/s.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: Identify and formulate biopesticides to control bakanae pathogen

2. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

PI: Dr. Quazi Shireen Akhter Jahan
Principal Scientific Officer
Plant Pathology Division
BRRI, Gazipur-1701
Mobile: 01855873259; e-mail: shireenbrri@yahoo.com

Co-PI: Dr. Shamima Akter
Senior Scientific Officer
Plant Pathology Division
BRRI, Gazipur-1701
Mobile: 01552338406; e-mail: sakter@yahoo.com

3. Sub-project budget (Tk):
   3.1 Total: 1,999,890
   3.2 Revised (if any): N/A

4. Duration of the sub-project:
   4.1 Start date (based on LoA signed): 15 May, 2017
   4.2 End date : 30 September 2018

5. Justification of undertaking the sub-project:

Bakanae caused by *Fusarium fujikuroi* is an endemic fungal disease of rice and has sporadic distribution in Bangladesh mainly in greater Cumilla, Habigonj 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 of 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, a few efficient and effective control methods are available, 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 continuous use of these chemicals together may pave the way to generate numerous problems such as development of new fungal pathogen strains resistant to fungicides and the increase of waste residues and the toxic effects for human 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*. The antagonistic organisms *Bacillus* spp., *Pseudomonas* spp. and *Trichoderma* spp. have been found to control many plant pathogens including sheath blight of rice (Bhattacherjee and Dey, 2014; Kumar *et al.,* 2012). Currently, *Bacillus* spp. is identified as a successful biopesticide for controlling bakanae disease (Hossain *et al.,* 2016) and *Trichoderma* spp. is identified as a very proactive bio controlling agent for sheath blight disease management in Bangladesh (Akida *et al.,* 2015; 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 pathogen and to replace chemicals gradually with biopesticide/s which are safe to human, and non-targeted beneficial organisms and cheaper than the chemicals.

6. Sub-project goal: Enhancing rice yield through using environmental safe biopesticide.

7. Sub-project objective (s): The objectives of the research project are-

    1. To find out effective microbes (*Bacillus* spp., *Pseudomonas* spp. and *Trichoderma* spp.) along with plant active ingredients/plant products as sources of biopesticide/s for controlling bakanae disease.
    2. To find out suitable carrier material with prolong shelf life for biopesticide formulation.
    3. Strengthening research facility of Plant Pathology Laboratory of BRRI.

8. Implementing location (s): BRRI Plant Pathology Laboratory and Field, Gazipur.

9. Methodology in brief:
    a. Collection of samples for isolation of biocontrol agents from endemic areas: Soil samples (75) and bakanae infected plant samples (60) (Figure 1) have been collected from Cumilla, Habigonj, Mymensingh, Netrokona, Srimongal and Gazipur districts for isolation of biocontrolling bacteria and *Trichoderma* spp. against bakanae patholgen.

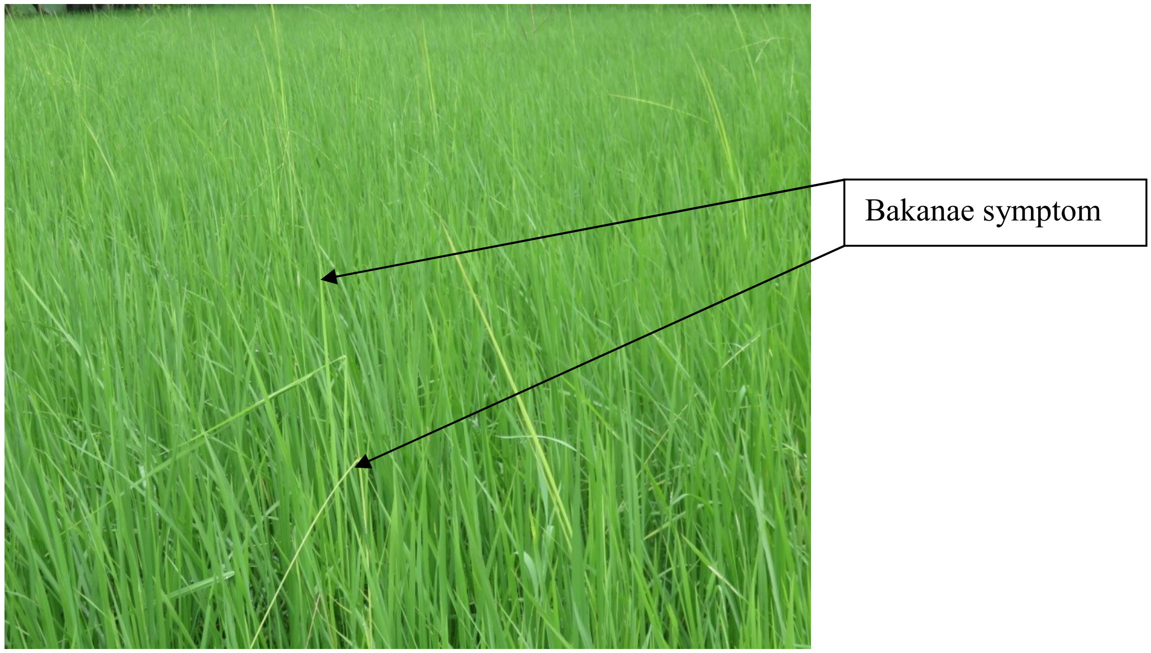

Figure 1.Bakanae infected plants.

b. Isolation, purification and morphological identification of biocontrol agents: Bacterial biocontrolling agents were isolated from collected rhizospheric soil sample using soil dilution technique (Figure 2) and also from stem region of infected rice plants.

Figure 2. Soil dilution technique

Isolation of biocontrol agent of *Trichoderma* spp. from rhizospheric soil (rhizosphere and roots) collected from different rice fields and regions were also completed using dual culture test. Then the pure cultures was maintained and preserved for further use. Morphological characters (Colony characters) of bacterial biocontrolling agents were only recorded.

Long term storage of bio-controlling bacteria: Bacterial bio-controlling agents those were found promising in dual culture test were stored at -80^{0}C for further work. At first, 50% sterile glycerol stock solution was prepared. Then aliquot 300µl glycerol solution into 2 mL tube and was autoclaved. In addition, identifying biocontrolling bacterium was grown on NA medium for two days. Then a loop full bacterial isolate was dissolved in NA broth and was vortexed and grown in broth for further two days. After that, pipette 700µl of bacterial suspension into 300µl glycerol solution into 2 mL tube and was vortexed. Thus it makes a final concentration of 15% glycerol stock solution. This stock was stored at -80^{0}C for consequent works.

c. Test of biocontrol agent/s and active ingredients against bakanae causing pathogen *in vitro*:

   In controlled condition the isolated biocontrol agents (bacteria and *Trichoderma*) were tested in growth medium following dual culture test (Kumar *et al.,* 2012). Formation of inhibition zone is an indicator of an active biocontrolling agent (Figure 3). Mycelial growth restriction or no growth is also a good sign for identifying biocontrolling agent against pathogen.

Figure 3. Identifying biocontrolling agent by formation of inhibition zone and restiction of pathogen’s mycelial growth.

The percent inhibition of diameter growth (PIDG) values were determined according to the following equation:

$$\text{PIDG (\%)} = \frac{\text{Diameter of sample} - \text{Diameter of control}}{\text{Diameter of control}} \times 100$$

d. Collection of samples and isolation of active ingredient/molecules from plant products:

Neem seed, mehogoni seed, dodder whole plant, leaf of kata mehedi (hedge plant) and green chilli were collected for extraction of plant products/ plant active ingredients and tested against bakanae causing pathogen (Figure 4). Plant extracts have been isolated that have direct conscientious antimicrobial properties (Martínez, 2012). Natural plant products i.e. active ingredients were extracted according to Gurjar *et al*., (2012) with some modifications. Briefly, Twenty gram (20 g) samples of laves/seeds/whole plant were ground with methanol and water separately using blender machine. Then the ground sample was placed into a 100 mL beaker and placed in a horizontal shaker and shaken for 30 min at 200 rpm. After that, the extract was filtered to a new sample tube using Whatman No. 1 filter paper (Figure 5). The extract with plant active ingredients were used to prepare PDA and tested against the bakanae causing pathogen in vitro.

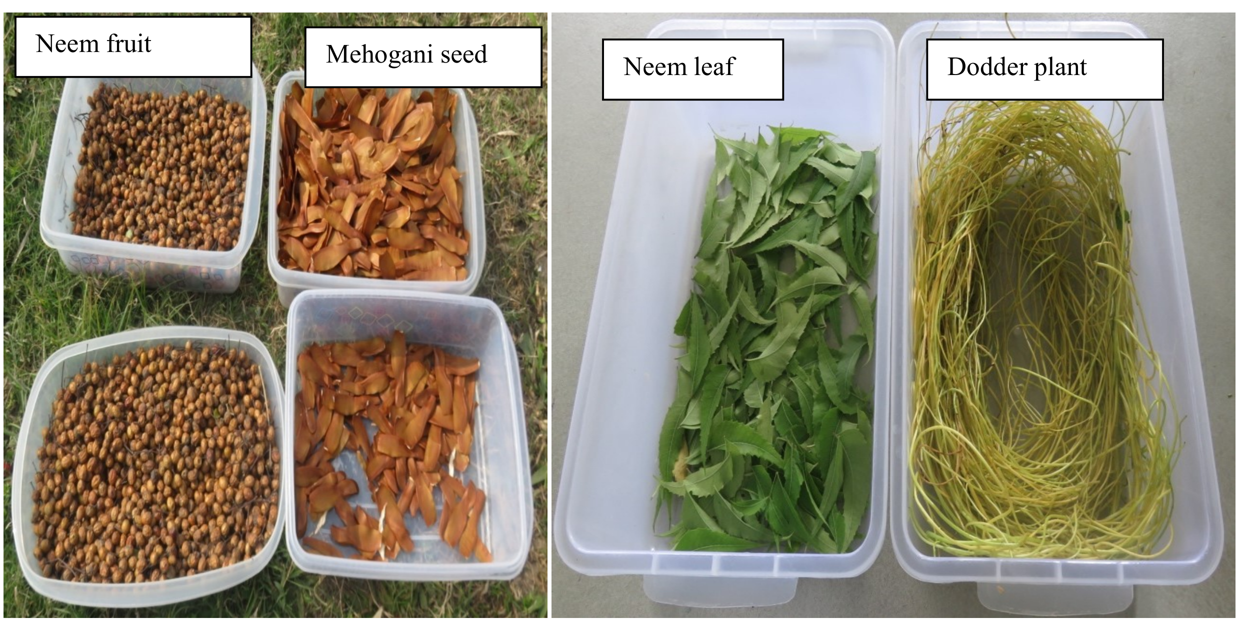

Figure 4. Collection of a variety of plant materials for isolation active ingredient to control bakanae pathogen.

Figure 5. Extraction process of plant active ingredients

e. Infected Seed collection and effectiveness test of identified biocontrolling agents:

Bakanae infected aus rice seeds of the year 2018 were collected from farmers' house from Habigonj district. Collected seeds were tested following ISTA, 2000 for seed health testing. Then, the seeds were primed with biocontrolling agents to find out their effectiveness against bakanae pathogen. The roots of the seedlings were inculated with the bakanae pathogen for 3 hours. After that, the inoculated seedlings roots were dipped in suspension of biocontrolling agents (1mL/100mL H_2O for bacteria and 10^6 conidia/mL H_2O or 10g formulated powder/Kg seeds for *Trichoderma*) for another three hours and sown on the sterilized soils on trays (20 seedlings/tray) separately. Biocontrolling agents i.e. bacteria (Bio-1, NS 9-4) and *Trichoderma* (T3) were tested to find out their effectiveness against bakanae. At the same time control seedlings roots (without pathogen inoculation) were also dipped in to sterile water for 6 hours and sown (20 seedlings/tray) separately. After 21 days of inoculation with biocontrolling agents the data were taken in comparison with control seedlings.

f. Confirmation of identified bio-control agent/s (bacteria and fungus) at molecular level: After confirmation of good performance in laboratory/net house test against bakanae pathogen, the DNA of the biocontrol agents were isolated. The isolated DNA was identified using specific molecular marker for *Bacillus, Pseuodomonas and Trichoderma (*Oskiera *et al.,* 2015; Farrag *et al*., 2015: Nair *et al.,* 2014).

10. Results and discussion:

a. Isolation, preservation and morphological identification of biocontrolling bacterial isolated:

A total of forty (40) bacterial isolates were isolated from rhizosperic soil and rice stem. Biocontrolling bacterial isolates were identified based on formation of inhibition zone and restriction of mycelia growth following dual culture technique (Figure 6).

Figure 6. Pictures of some identified biocontrolling bacteria by forming of inhibition zone and restriction of mycelia growth in comparison with control.

All of the identified biocontrolling bacterial isolates were characterized according to colony characters and the percent growth inhibition of bakanae pathogen (Table 1). Identified biocontrolling bacterial isolates performed 61-78% growth inhibition of the bakanae pathogen.

Other researchers also identified and reported a number of biocontrolling bacterial isolates those were proven effective against different plant pathogens. For instances, *P. fluorescens* was identified as effective against *R. solani* (Kamalakannan *et. al.* 2004). In Forest nurseries at Haryana, India, *P. fluorescens* was found more effective against damping off disease (Kaushik *et. al.* 2000). *Pseudomonas fluorescens*, isolated from rhizosphere of winter rape, showed antagonistic and protected germinating plants against infections by *Phoma lingam* (*Leptosphaeria maculans*), *F. acenaceam* (*Gibberella avanacea*), respectively (Ganeshan and Kumar, 2005).

b. Biocontrolling *Trichoderma* isolation and identification:

A total of six *Trichoderma* spp. (T1, T2, T3, T4, T5 and T9) were identified based on mycelia growth restriction following dual culture technique (Figure 7 and Table 2). Based on colony characters, they were assumed as *T. harzianaum* and *T. viridae*. Identified all *Trichoderma* isolates exhibited 73-94% growth inhibition of the bakanae pathogen (*Fusarium* spp.) on the 14th day of incubation in dual culture test.

Table 1. Different biocontrolling bacterial isolates were identified based on inhibition zone >1.0cm from collected regions.

Sl. No	Designation	Collection region	Source	Colony character	Inhibition zone (cm)	% growth inhibition
1	Bio-1	Hobigonj	Stem	Dull-White, elevation- flat, margin- circular and upper surface dry	1.6	74.7
2	Bio-2	Hobigonj	Stem	Surface-smooth and glossy, color-light ash, elevation- raised, fluorescent	1.3	71.1
3	HS41-1	Hobigonj	Soil	Margin-wavy, surface-rough, elevation-medium flat,color-brown	2.5	79.1
4	HS41-2	Hobigonj	Soil	Margin-entire, surface-rough and glossy, elevation-flat, color-light creamy	1.4	73.9
5	NS-9-4	Netrokona	Soil	Margin-entire, surface-glossy, elevation-raised, color-white	1.9	73.0
6	NS-15-3	Netrokona	Soil	Surface-rough and dry, elevation-flat, color-white	1.7	67.1
7	NS-15-4	Netrokona	Soil	Margin-entire, surface-flat and not glossy, color-white	1.9	74.2
8	NS10-1	Netrokona	Soil	Surface-flat and dry, elevation-little raised, color-brown	2.1	70.1
9	NS10-4	Netrokona	Soil	Surface-flat, margin-wavy, color-light creamy	2.1	72.7
10	NS10-5	Netrokona	Soil	Color-browny, surface- flat and wet, margin-circular	1.7	69.8
11	NS12-4	Netrokona	Soil	Color-creamy, margin- irregular, surface- dry, elevation- flat	1.6	71.9
12	NS 14-1	Netrokona	Soil	Color-creamy, margin- irregular, surface- dry, elevation- flat	1.9	70.8
13	NS 14-2	Netrokona	Soil	Color-white, margin- circular, Surface-flat and dry,	2.2	76.1
14	NS13-2	Netrokona	Soil	Margin-entire, elevation- flat and non-glossy, color-yellowish	1.9	73.8
15	NS13-3	Netrokona	Soil	Margin-entire, elevation-flat, surface-rough and dry, color-white	1.6	71.5
16	NS1-1	Netrokona	Soil	Color-creamy white, surface-flat, elevation-raised, margin-irregular	1.5	74.2
17	GS 31-1	Kapasia, Gazipur	Soil	Color-yelloish, surface- wet, elevator-raised, margin-circular	1.0	64.2
18	NS36-4	Netrokona	Soil	Margin-entire, surface-rough, elevation-flat, color-white	1.6	71.8
19	GS20-1	Gazipur Sadar	Soil	Creamy color, elevation-raised, surface-rough and wet, margin-irregular	1.4	68.3
20	GS20-2	Gazipur Sadar	Soil	Color-white, surface-dry, elevation-flat, margin-irregular	1.2	65.7
21	GS20-3	Gazipur Sadar	Soil	Color-creamy, elevation-raised and wet, margin-circular,	1.6	69.9
22	HS34-1	Hobigonj	Soil	Color-creamy, elevation-flat and dry, margin-irregular,	1.5	73.6
23	GS22-1	Gazipur Sadar	Soil	Color-creamy-yellowish, elevation-raised, surface- wet, margin-regular	1.5	71.2
24	GS22-2	Gazipur Sadar	Soil	Color-creamy-yellowish, elevation- light raised, margin-irregular	1.1	61.8
25	GS22-3	Gazipur Sadar	Soil	Color-creamy-yellowish, elevation- light raised, margin-irregular	1.7	73.3
26	KS35-1	Kapasia, Gazipur	Soil	Color-brownish, margin-circular, elevation-raised and wet,	2.1	73.1

Sl. No	Designation	Collection region	Source	Colony character	Inhibition zone (cm)	% growth inhibition
Sl. No	Designation	Collection region	Source	Colony character	Inhibition zone (cm)	%growth inhibition
27	KS35-6	Kapasia, Gazipur	Soil	Color-white, margin-circular, elevation- flat and dry,	1.9	70.0
28	GS 5-1	Sreepur, Gazipur	Soil	Color-yellowish, margin-circular, elevation- raised, surface- wet	2.1	71.4
29	GS12-2	Netrokona, Mymensingh	Soil	Color-creamy, margin-irregular, surface-dry, elevation-slight raised	1.1	68.3
30	SS40-1	Sreepur, Gazipur	Soil	surface-rough, elevation-raised and dull, color-yellowish	3.2	78.4
31	SS40-2	Sreepur, Gazipur	Soil	Margin-round, surface-smooth and glossy, elevation-raised, colour-light brown	2.4	70.1
32	SS40-3	Sreepur, Gazipur	Soil	Margin-round, surface-smooth and glossy,elevation-raised, colour-light ash,	2.7	71.5
33	SS40-4	Sreepur, Gazipur	Soil	Margin-round, elevation-flat, surface-rough, color-white	2.9	73.3
34	MS-19-1	Mymensingh	Soil	Color-creamy, surface-sticky, glossy and flurescent, surface-regular	1.4	79.3
35	MS19-2	Mymensingh	Soil	Color-white, elevation- -flat, margin- circular , surface- rough	1.2	76.6
36	MS-6	Mymensingh	Soil	Color-browny, surface-wet margin-circular and uneven, elevation-raised	1.6	77.1
37	HS 42-1	Ghikorgacha, Hobigonj	Soil	Color-light brown, margin-circular, surface- dry, elevation-flat	1.0	68.6
38	HS 32-1	Saestagonj , hobigonj	Soil	Color- light yellow, elevation –flat, surface-dry, margin- circular	1.2	72.3
39.	HS 32-2	Saestagonj , Hobigonj	Soil	Color-brown, margin-circular, elevation- light raised, surface-wet	1.1	70.9
40.	GS-22-1	Sreepur-Gazipur	Soil	Color-deep orange, margin-circular, surface-wet, elevation- raised	1.0	69.1

Table 2. Identification of biocontrolling *Trichoderma* isolates based on % growth inhibition of bakanae pathogen.

Sl. No.	Designation*	Collection region	Source	% growth inhibition
1.	T1	Gazipur Sadar	Stem	93.7
2.	T2	Gazipur Sadar	Stem	92.1
3.	T3	Gazipur Sadar	Stem	94.6
4.	T4	Gazipur Sadar	Stem	94.1
5.	T5	Gazipur Sadar	Stem	91.4
6.	T9	Gazipur Sadar	Stem	73.2

*T=Trichoderma

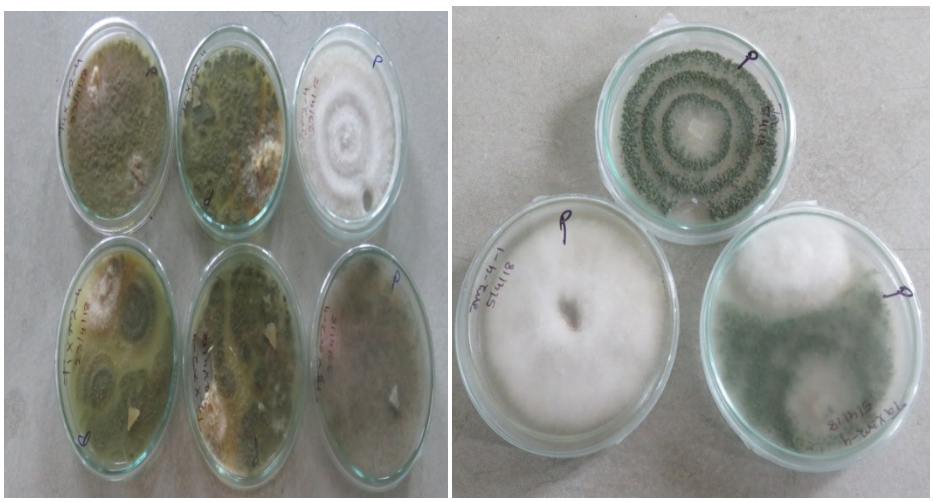

Figure 7. Pictures of some identified biocontrolling *Trichoderma* spp. by forming of inhibition zone and restriction of mycelia growth in comparison with control after 14 days of incubation.

The previous studies had also been reported that *Trichoderma* was an effective fungicide against soil born diseases such as root rot. It was particularly relevant for dry land crops such as groundnut, black gram, green gram and chickpea, which were susceptible to *Ceratocystis paradoxa* (Kannangara *et. al.,* 2017). The *Trichoderma harzianum* had been reported to had the ability to penetrate into the cell wall of resting spores and hyphae of other fungi which was attributed to the production of enzymes that catalyze the breakdown of chitin, a primary component of fungal cell walls of the pathogen (Brimner and Boland, 2003). Higher inhibition (>70%) indicated that the identified *Trichoderma* isolates was better biological control agent.

c. Effcetivity test of plant products/plant active ingredients against bakanae pathogen in laboratory condition:

Four plant products/ingredients extracted in ethanol gave excellent result for controlling bakanae pathogen (*Fusarium* spp.) by restriction of mycelia growth 100% (Table 3 and Figure 8 & 9).

Table 3. Growth inhibition (%) of bakanae pathogen using different plant products/ingredients after 7 days of incubation in *in vitro* condition.

Plant products/Ingredients	Solvent	Radial growth (cm) after 7 days	Inhibition (%) compared to control	Remarks
Neem seed	Ethanol	0	100	Excellent
Neem seed	water	8.4	39.6	Very bad
Neem leaf	Ethanol	0	100	Excellent
Neem leaf	water	9.8	29.5	Very bad
Mehogani seed	Ethanol	0	100	Excellent
Mehogani seed	water	13.5	2.9	Very bad
Dodder plant	Ethanol	0	100	Excellent
Dodder plant	water	12.8	7.9	Very bad
Control (bakanae pathogen)		13.9		

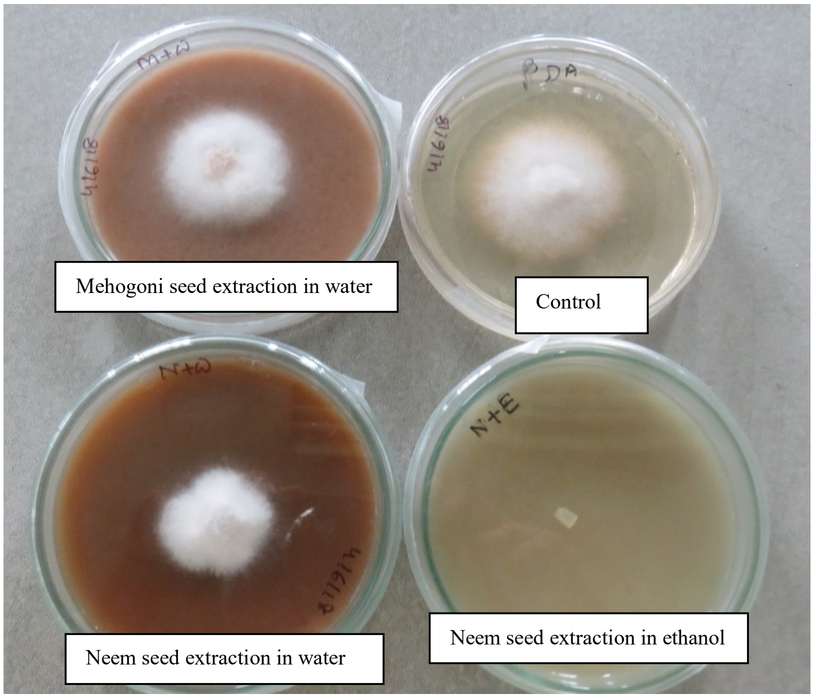

Figure 8. Pictures of some identified biocontrolling plant active Ingredients by restriction of mycelia growth of *Fusarium fujikuroi* in comparison with control after 4 days of incubation.

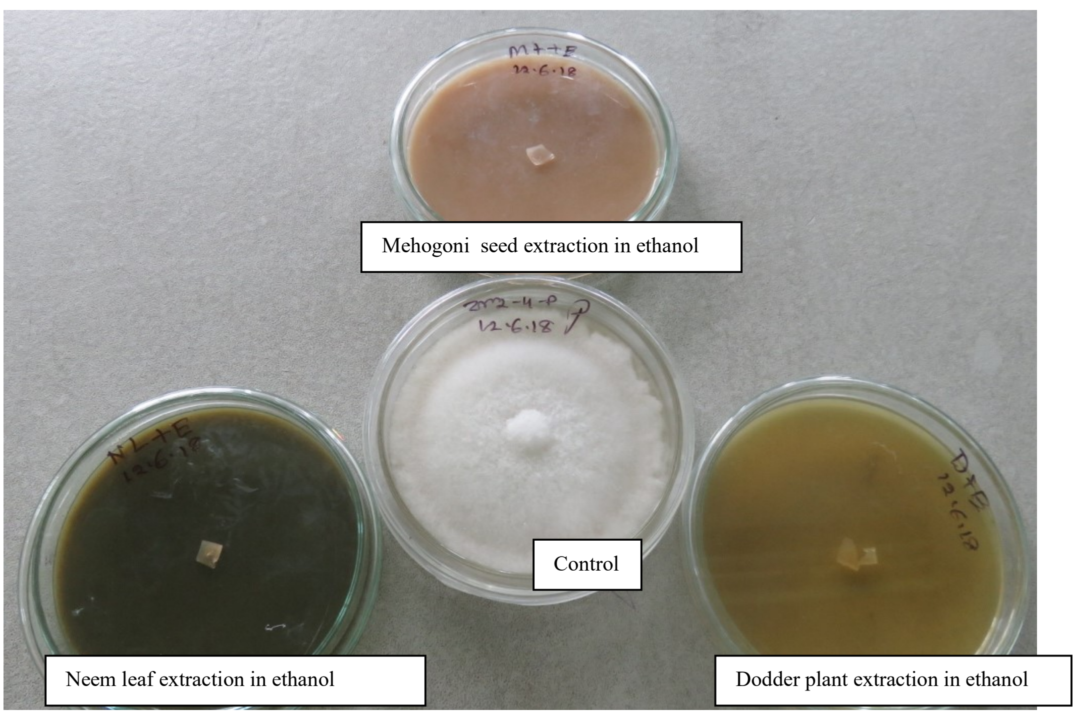

Figure 9. Pictures of some identified biocontrolling plant active ingredients by complete restriction of mycelia growth in comparison with control after 14 days of incubation.

d. Effectiveness test of identified biocontrolling agents in net house condition:

A lower percentage of bakanae pathogen was recorded from the collected bakanae infected seeds (Table 3). Therefore, the roots of younger rice seedlings (14 days old) were inoculated artificially with the pathogen @ 10^6condia/ml.

Table 3. Infected Seed collection and seed health testing

Sl.	Name of Farmers	Address	Variety	% recovery of the pathogen
1	Sumon mia	Village: Razzakpur Thana: Chunarughat, Dist. Hobigonj	BRRIdhan 48	4
2	Jamir Ali	Village: Gopalpur, Thana: Chunarughat, Dist. Hobigonj	BRRIdhan 48	0
3	Nurul Haq	Village: Gopalpur Thana: Chunarughat, Dist. Hobigonj	BRRIdhan 48	1
4	Faruq	Village: Gopalpur Thana: Chunarughat, Dist. Hobigonj	BRRIdhan 48	1
5	Titu Mia	Village: Gopalpur Thana: Chunarughat, Dist. Hobigonj	BRRIdhan 48	4
6	Torab Ali	Village: Gopalpur Thana: Chunarughat, Dist. Hobigonj	BRRIdhan 48	8
7	Shahabuddin	Village: Gopalpur Thana: Chunarughat, Dist. Hobigonj	BRRIdhan 48	0
8	Ajgor Ali	Thana: Bahubal, Dist. Hobigonj	BR 10	9
9	Ajgor Ali	Thana: Bahubal, Dist. Hobigonj	BRRIdhan 48	0
10	Milon Mia	Thana: Bahubal, Dist. Hobigonj	BRRIdhan 48	0
11	Shahid Mia	Thana: Bahubal, Dist. Hobigonj	BR 10	1

It was observed that the all three biocontrolling agents fought against bakanae pathogen successfully compared to control (Figure 10). Plants priming with biocontrolling agents looks healthy, green and no bakanae symptoms, whereas, control plants (inoculated with bakanae pathogen only) looks pale green, thin, elongated and died. Among the biocontrolling agents bacteria (Bio-1) and Trichoderma (T3) performed excellent to manage bakanae disease (>90%) in net house condition (Table 4).

Table. 4. Performance of seedlings inoculated with biocontrolling agents in net house condition

Biocontrolling agents	Total plants	Survival plant	Infected	Remarks

	inoculated	(%)	(%)	
Bacterial agent (9-4)	60 (20 /replication)	87	13	Good
Bacterial agent (Bio-1)	60 (20 /replication)	95	5	Excellent
Fungal agent (Trichoderma T3)	60 (20 /replication)	92	8	Excellent
Control (Inoculated with *Fusarium fujikuroi*)	60 (20/replication)	18	72	Very bad

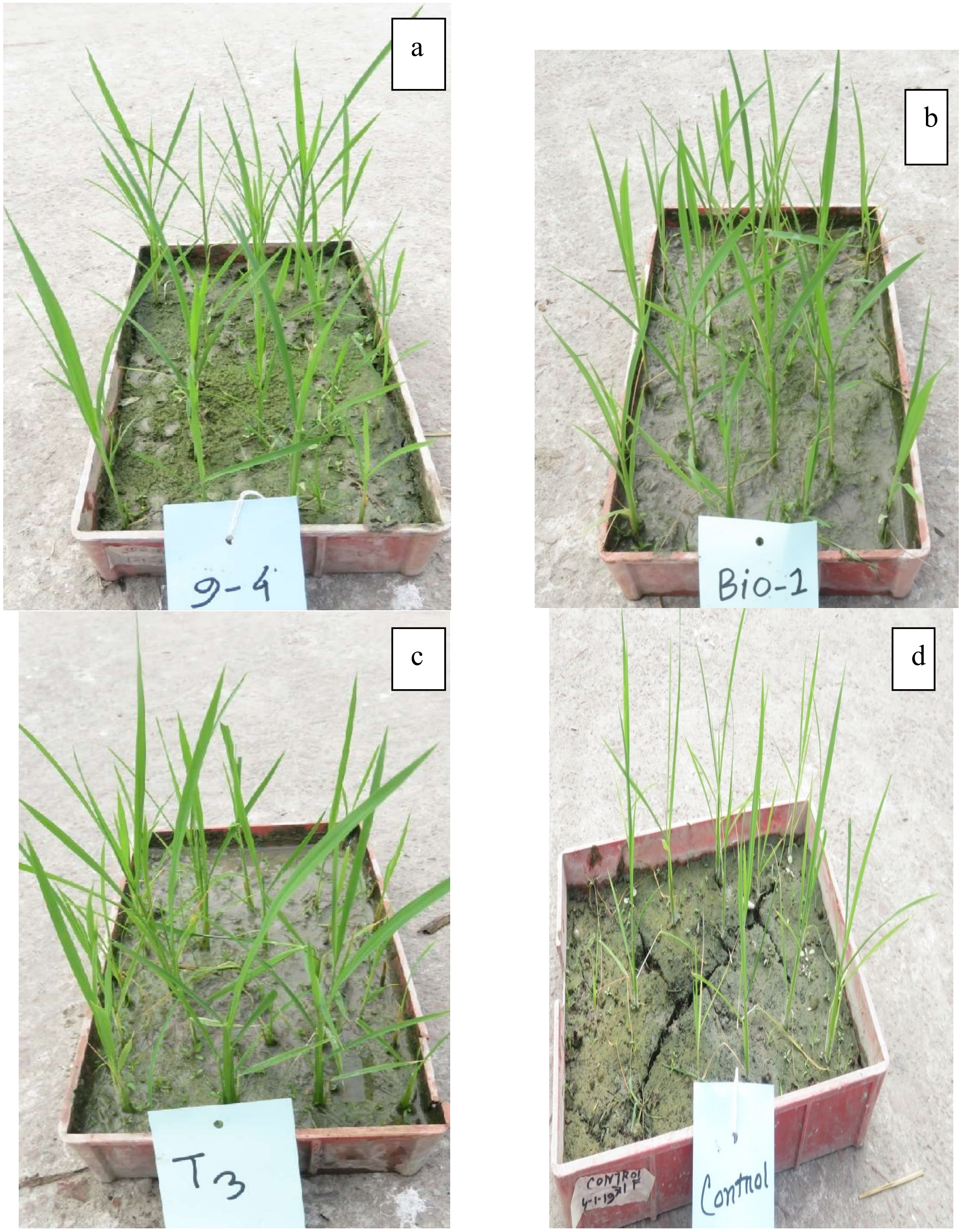

Figure 10. Performance of Biocontrolling agents against bakanae pathogen compare to control (inoculated) after 21 days of inoculation and priming.(a=bacteria, b=bacteria, c= Trichoderma and d= control).

e. Confirmation of identified bio-control agent/s (bacteria and fungus) at molecular level:

DNA of identified effective biocontrol agents were isolated and preserved. Confirmation of identification using species specific molecular marker was not done due to shortage of time.

f. Identification of suitable carrier material for *Trichoderma* formulation: *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 *Trichoderma* and incubated further for 5-7 days for fully colonized the corn seeds. Then the colonized corn seeds with *Trichoderma* were take 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 brown paper bag. It was observed that *Trichoderma* can survive on broken corn seeds for 6 months. This period for longevity/shelf life of *Trichoderma* should be further evaluated for conclusive remarks.

11. Research highlight/findings:

- 40 biocontrolling bacterial isolates were isolated and preserved for further work.
- Six *Trichoderma* spp. (T1, T2, T3, T4, T5 and T9) were isolated and preserved for further work.
- Four plant products (neem seed extraction in ethanol, neem leaf extraction in ethanol, mehogoni extraction in ethanol and dodder plant extraction in ethanol) also found promising to control the pathogen in vitro.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	

(a) Office equipment	Laptop (1), Laser Printer (1), Scannar (1), UPS (1), Digital camera (1)	125000/=	Laptop (1), Laser Printer (1), Scannar (1), UPS (1), Digital camera (1)	124900/=	None
(b) Lab &field equipment	PH meter (1)	1,20000/=	PH meter (1)	1,20000/=	None
(c) Other capital items	Executive Table(1), Executive Chair(1),	30,000/=	Executive Table(1), Executive Chair (1),	30,000/=	None

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: N/A

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

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	252790	238320	238269	50.95	99	Calculation error
B. Field research/lab expenses and supplies	902100	877126	877126	0	100	
C. Operating expenses	215000	215000	203374	0	100	
D. Vehicle hire and fuel, oil & maintenance	180000	175940	175940	0	100	
E. Training/ workshop/seminar etc.	-	-	-	-	-	
F. Publications and printing	95000	-	-	-	-	Fund not received
G. Miscellaneous	80000	71414	71414	0	100	
H. Capital expenses	275000	275000	274900	100	99	Procurement process

D. Achievement of Sub-project by objectives: (Tangible form)

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)
1. To find out effective microbes (*Bacillus* spp, *Pseudomonas* spp., *Trichoderma* spp.) along with plant active ingredients/ plant products as sources of biopesticide/s for controlling bakanae disease.	Dual culture technique was use in lab condition for determining inhibition zone between biocontrolling agent and virulent isolate causing bakanae disease. Moreover trial was set up in net house to find out the effectiveness of biocontrolling agents against bakanae pathogen.	• 40 biocontrolling bacterial isolates, six Trichoderma spp. were isolated using morphological identification and preserved for further work. • Four plant active ingredients/plant products also found promising to control the pathogen in vitro. • In net house trial two bacterial and one Trichoderma biocontrolling agents performed promising to control bakanae pathogen.	Isolated biocontrolling agents and plant products/ plant ingredients will be used for formulation biopesticides and control the bakanae pathogen in large scale in future.
2. To find out suitable carrier material with prolong shelf life for biopesticide formulation.	Broken corn were used for formulation purposes.	Broken corn seed found good carrier material for *Trichoderma* but needs time for deification of good carrier material for bacteria and plant products.	More time require for getting final outcome.
3. Strengthening research facility of Plant Pathology Laboratory	PH meter is used for PH determination	Growth medium for biocontrolling agents were prepared accurately.	

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication	One (1)		
Information development	Three (3)		
Other publications, if any			

F. Technology/Knowledge generation/Policy Support (as applied):

i. **Generation of technology (Commodity & Non-commodity):**

Biopesticide is cheap and environmentally safe for pathogen management. 1mL/100mL H_2O for bacteria and 10^6 conidia/mL H_2O or 10g *Trichoderma* formulated powder/Kg rice seed is suitable for bakanae management.

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

Using the identified biocontrolling agents and plant products, biopesticide will be formulated for management of bakanae disease. Other major pathogens of rice might be managed using the identified biocontrolling agents or plant products.

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

Not yet developed.

iv. Policy Support

Policy support is required to restrict the use of chemical fungicides and minimize in accordance with aggravated to use of biopesticides for controlling safe food as well as safe environment.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring :

A total of three workshop/seminars were organized by BARC. Moreover, 5-6 times consultation meetings were done with the PIU, BARC personnel. For budget preparation, procurement process desk monitoring consultations were useful as proper guidance was paid. Moreover, in report writing, they helped to include the information in reports if any obscured.

ii) Field Monitoring (time & No. of visit, Team visit and output):

One field monitoring was done in January, 2018 (Figure 11). The monitoring team was satisfied to see the progress of the project research and encouraged a lot.

Figure 11. Visit program by Field Monitoring Team to see the progress of project research

H. Lesson Learned/Challenges (if any)

i) Biocontrolling bacterial isolation and preservation needs proper care.

ii) Procurement process took 5 months for such a short period project.

I. Challenges (if any)

1. Project period (16 months) was not enough to complete the molecular identification of the identified biocontrolling agents.
2. Sixteen (16) months was too short period to identify suitable carrier materials and formulate biopesticide.

Signature of the Principal Investigator

Date:

Seal

Counter signature of the Head of the organization/authorized representative

Date:

Seal

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