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Research Article

Biocontrol Activity of Soil Bacteria against *Colletotrichum melongenae* of Wheat (*Triticum aestivum* L.) Seed Germination

Md Khasrul Alam[1], Mohammad Abu Hena Mostofa Jamal[1], Abul Kalam Azad[1], Nilufa Akhter Banu[1], Md Shahedur Rahman[2] and Md Rezuanul Islam[1]*

[1]*Deaprtment of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh*

[2]*Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology, Bangladesh*

***Corresponding Author:** Md Rezuanul Islam, Professor, Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh.

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Abstract

The phytopathogenic fungus *Colletotrichum melongenae* have an adverse effect on the germination of wheat seed. It can reduce the germination rate tremendously. This negative impact of fungus on germination rate can be rescued by using of soil bacteria (*Bacillus subtilis,* RKP-2) as a biocontrol agent over the fungal infected seeds and commercial fungicides dithiocarbamates was used as control. In this study we aimed to justify the antifungal activity of *Bacillus subtilis,* RKP-2 against *Colletotrichum melongenae* to rescue the inhibition of germination comparing with commercial fungicide. The germination rate of seed in positive control was 93.33% which decreased to 43.33% when the seeds were infected with fungal pathogen. After the treatment of bacteria the germination rate was raised to 73.33% comparing with the commercial fungicide that was 80.00% which is not significantly different. The biocontrol activity of bacteria against phytopathogenic fungus is 32.14%. On the other hand the antifungal activity of commercial fungicide is 39.29% that is higher than bacterial activity. But the bacterial bio-control system is friendly to ecologically and economically. So, *Bacillus subtilis* RKP-2 may use as a bio-control agent as well as being used as active ingredients of biofertilizer.

Keywords: *Colorectal Cancer; Review; Screening; Treatment*

Introduction

Biocontrol of plant pathogens is considered as a potential control strategy in recent years, because chemical control results in accumulation of harmful chemical residues, which may lead to serious ecological problems (Sing., *et al.* 2009). Considering the deleterious effects of synthetic fungicides on life supporting systems, there is an urgent need for alternative agents for the management of pathogenic microorganisms. One of the most promising means to achieve this goal is by the use of new tools based on bio-control agents (BCAs) for pest and disease control alone or to integrate with reduced doses of chemicals in the control of plant pathogens resulting in minimal impact of the chemicals on the environment [1]. Bacteria such as *Pseudomonas, Bacillus subtilis* are the most promising bio-control agent against a range of plant pathogens under a variety of environmental conditions [2].

Wheat

Wheat (*Triticum aestivum* L.) is an important cereal crop belonging to *Gramineae* family. Among the cereals, wheat is second to rice in economic and consumption importance. Unfortunately, the production rates of these crops have failed to keep pace with some abiotic

limitations, various pests and fungus and number of storage grain molds cause considerable losses [3]. These seed borne pathogens present externally or internally or associated with seed as contaminant may cause seed abortion, seed rot and seed necrosis, reduction of germination capacity as well as seedling damage resulting in the development of disease at later stages of plant growth by systemic or local infection (Ryley., *et al*. 1989).

Colletotrichum fungus

Colletotrichum melongenae is a microscopic fungus, is known for its devastating effects on crop plants, especially on cereals and vegetables.

Bacillus subtilis RKP-2

Bacillus subtilis is a model organism for studying on plant pathogenic fungi has been frequently reported in laboratory, greenhouse, and field studies (Pusey PL., *et al*. 1984). *B. subtilis* is able to synthesize more than 60 different types of antibiotics, mainly in polypeptides, many of which possess antifungal effects and belong to the iturin family [4]. Besides the anti-fungal effects, some compounds produced by *B. subtilis* may also act as plant growth promoters [5].

Commercial fungicide

It used DITHANE M-45 as commercial Fungicide which is collected from local market of Kushtia.

Materials and Methods

Collection of *Colletotrichum melongenae*

Plant pathogenic fungus *Colletotrichum melongenae* was collected from Microbiology laboratory, department of Biotechnology and Genetic Engineering, Islamic University, Kushtia. The collected fungus was subcultured on potato dextrose agar (PDA) and allowed to grow in 27 ± 2°C. Fungal growth was observed after 5 days and stored at 4°C.

Collection of *Bacillus subtilis* RKP-2

Soil Bacteria *Bacillus subtilis* RKP-2 was collected from Microbiology laboratory, department of Biotechnology and Genetic Engineering, Islamic University, Kushtia. The Bacterial stain was subcultured on Tryptone Soya Broth (TSA) and incubated at 37°C and stored in 4°C for subsequent experimental work. Then it was again sub culture in Tryptone Soya Broth (TSB) and incubated at 37°C and stored at 4°C.

Collection of wheat seeds

Healthy Wheat (*T. aestivum* L.) seed was collected from local farmer in Kushtia. Then these were properly labeled, kept in polythene bags and stored for further studies in a freezer at -10°C until mycological testing and other processing (Fernandez., *et al.* 1985).

Commercial fungicide

Dithiocarbamates (DITHANE M-45) is a commercial Fungicide which was collected from local market of Kushtia. Recommended amount of DITHANE M-45 powder was weight by Electric Weight Balance and mixed in 250 ml water and stored at normal temperature for subsequent experimental work.

Determination of germination rate

Germination test of seeds samples was done in petridish. At first tissue paper was kept on petridish and seeds were placed on that. The work was done in four different controls. We planted wheat seeds and did not apply any fungi to use as positive control whereas the fungus was sprayed on the seeds for making negative control. Another two groups are treatment group. *Bacillus subtillis* RKP-2 was applied on fungus infected seed as well as commercial fungicides was also applied on fungus infected seeds. After 10 days the subsequent result of seed germination rate calculated by the following equation:

Seed germination (%) × 100… (Equation 1)

(Adapted by ISTA [6]).

Results

After 10 days wheat seed germination rate calculated the total number of seeds used in germination and total number of germinated seeds. In positive control germination of seeds were 93.33%. When seeds were infected by the phytopathogenic fungus (*C. melongenae)* the germination rate was decrease at 43.33%. Germination rate of fungal infected seeds with bacteria (*B. subtilis,* RKP-2) was 73.33% and the commercial fungicide (DITHANE M-45) was used the germination rate was 80.00%.

Treatment	Total seed set for germination (N)	Total germinated seed (n)	Germination rate = (n×100) / N	Activity/antifungal activity (%)
Positive control	30	28	93.33%	-
Fungus infected	30	13	43.33%	53.57%
Fungus infected and bacterial control	30	22	73.33%	32.14%
Fungus infected and fungicide control	30	24	80.00%	39.29%

Table 1: *Germination rate in different treatment including the activity of bacteria and commercial fungicide in antifungal activity.*

In order to develop an alternative approach to chemical control against phytopathogenic fungus an attempt was undertaken in this study. Here the Bio-control activity of soil born bacteria comparing with commercial fungicide was observed in the germination of Wheat seeds.

The whole result might be represented in graphically as in following.

Effect of *Collectorium melongenae* on seed germination

When seeds germinate with fungal infection the germination rate is decreased than the positive control (93.33%) (Figure 1) and the rate is 43.33%. So the percent inhibition of germination by fungus (PIGF) is 53.57% (Figure 2).

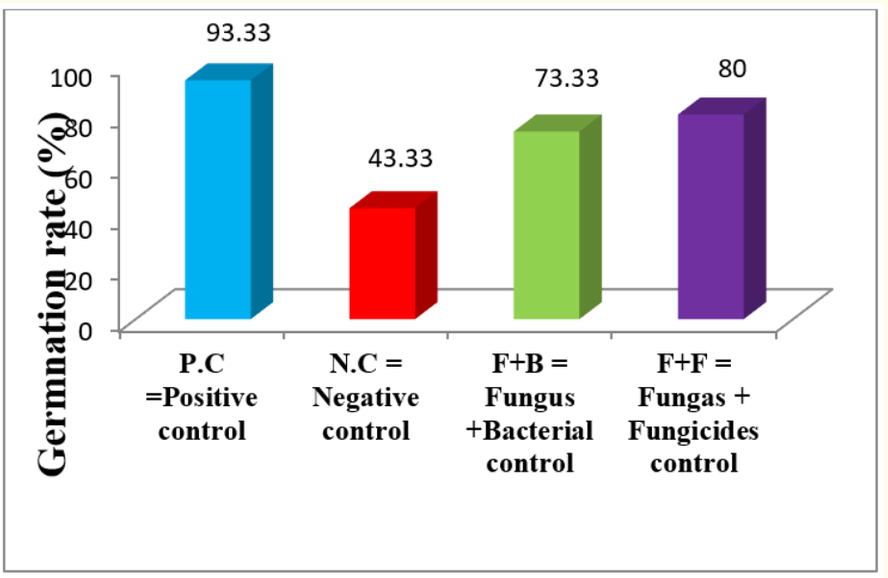

Figure 1: *Germination rate in different Treatment. P.C = Non-Treated, N.C= Fungal (C. melongenae) infected, F+B = Fungal infected seed with B. subtilisRKP-2and F+F = Fungal infected seeds with commercial fungicide (DITHANE M-45).*

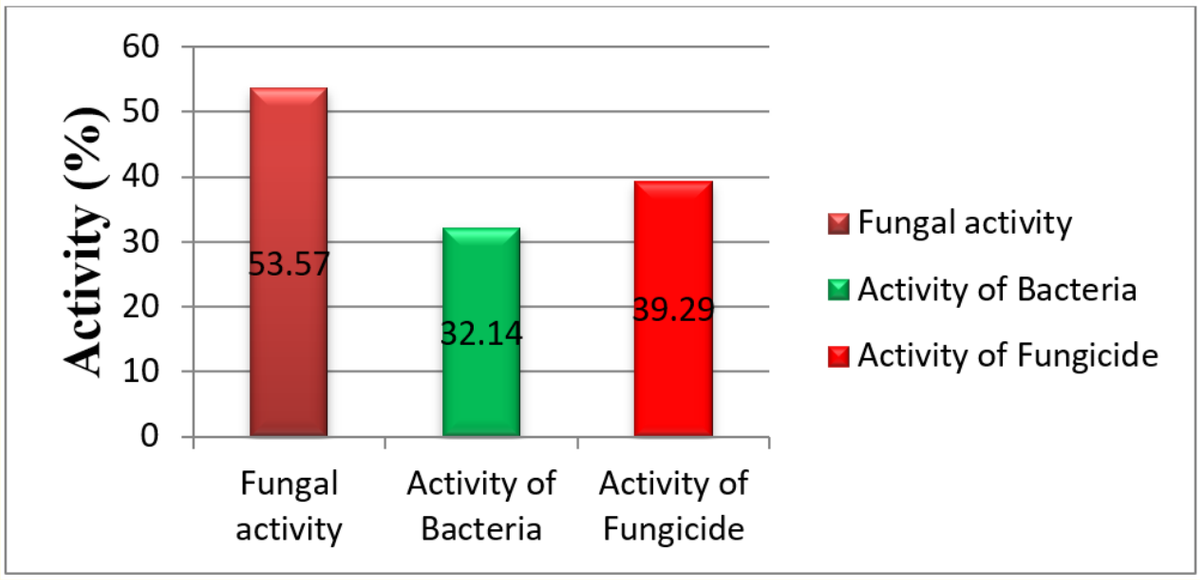

Figure 2: *Fungal (C. melongenae) activity with bacterial (Bacillus subtilis RKP-2) and commercial fungicide (DITHANE M-45).*

Effect of *Bacillus subtilis* on fungal affected wheat seed germination

When used soil Bacteria (*Bacillus subtilis* RKP-2) over the fungal treated seeds, the germination rate was raised 73.33% (Figure 1). The percent inhibition of germination by fungus with bacterial (PIG) is 21.43%. So the fungicide activity of Bacteria (biocontrol activity of Bacteria) is 32.14% (Figure 2).

Effect of commercial fungicide (DITHANE M-45) on fungal affected wheat seeds germination

When phytopathogenic fungal activity was recovered by using commercial Fungicide and that time the germination rate of wheat seeds was 80.00% (Figure 1). The percent inhibition of germination by fungus with Fungicide control (PIG) is 14.28%. So the fungicide activity of commercial fungicide is 39.29% (Figure 2).

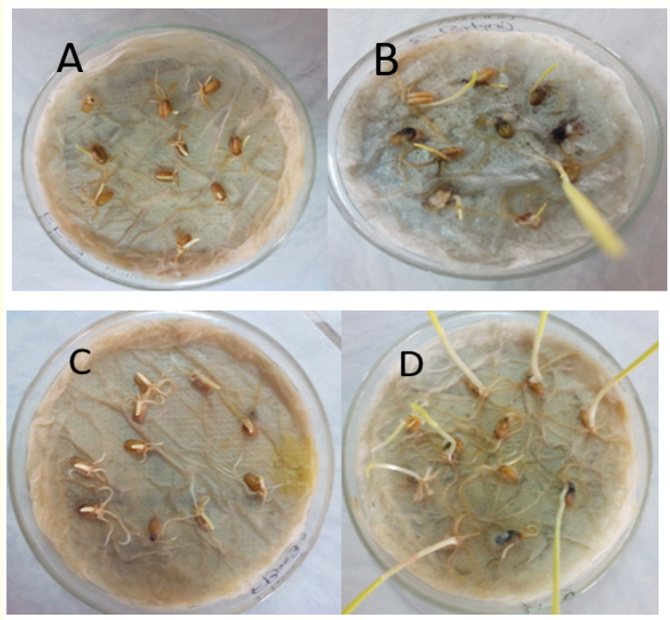

Figure 3: *Germinated seeds in different treatment. A= Non treated or positive control seeds, B= Seeds infected by fungus (C. melongenae), C= Bacterial (B. subtilis RKP-2) biocontrol of fungus infected seeds, D= Fungicide control of fungus (DITHANE M-45) infected seeds.*

Discussion

At present, there are several biocontrol products from rhizobacteria which have been developed and have been used. Among these agents, there is an increasing interest in the introduction of *Bacillus* spp. for managing fungal infection. *Bacillus* spp such as *B. subtilis, B. licheniformis, B. amyloliquefaciens and B. cereus* have been reported effective against foliar and soil-borne fungal pathogens (Hassan., *et al.* 2010 and Carneiro., *et al.* 1998). This might probably be due to their ability to induce growth and the defense response in host plant. In addition, *B*. *subtilis* non-ribosomally synthesizes several kinds of small antibiotic peptides that have antifungal activities, such as iturin (Joshi and Gardener, 2006), surfactin and fengycin. *B*. *subtilis* also secretes protease enzymes in abundance (Liu., *et al*. 2007). Competition for nutrients, especially for carbon, is assumed to be responsible for the well-known phenomenon of fungi stasis, characterizing the inhibition of fungal spore germination in soil (Alabouvette., *et al.* 2006). *B. subtilis* has an average of 4 - 5% of its genome devoted to antibiotic synthesis and has the potential to produce more than two dozen structurally diverse antimicrobial compounds (Stein, 2005).

Bacillus subtilis play role as an antifungal agent against phytopathogenic fungi because of it numerous characteristic. During this study it was experimented that germination rate of fungal infected seeds was increased by controlling with bacterial strain (*B. subtilis,* RKP-2). *B. subtilis,* RKP-2 decreased the growth of pathogenic fungus by antibiosis, nutritional competition, and possessed some enzyme that can lysis cell wall of pathogenic fungus. So, the subsequent result of this study may also be possible for this above characteristic.

We also use commercial fungicide for this same purpose. Moreover use of commercial fungicide is more preferable than soil bacteria to inhibit fungal infection at the time of seed germination. But it is more costly and unfriendly to the environment; as well as it hazardous to the environment. On the other hand using bio-control system though soil bacteria (*B. subtilis,* RKP-2); in the germination rate is not so poor, and it is cheap and eco-friendly [7-17].

Conclusion

Now a day's the report of wheat production is not so hopeful in our country and thus the production of wheat is decreasing day by day. On the other hand, some microbial Strains (fungus) which also play a crucial role to decrease the crop production by acting as a disease causing agents. Fungicides are one of major inputs in agricultural production used to protect from disease-causing agents. The excessive use of fungicides and inadequate substitution also lead to an increase in production costs and, hence, agricultural products. Furthermore the chemical fungicides are constant threat for our environment as well as it may decrease fertility of our cultivable land. One of the solution to protect food production, reduce cost and increased quality is the easily accessible natural resources. *C. melongenae* is a phytopathogenic fungus that can reduce germination capability of wheat and thus it production. So it may recognize as a natural enemy. This type of fungal infection may recovered by using commercial fungicide. It is effective but may create some problem being eco-unfriendly and raising price of production. On the other hand, *B. subtilis* RKP-2 may also be used for this purpose because it play role to inhibit fungal activity. Furthermore it is cheap and eco-friendly. This paper justifies the use of *B. subtilis* RKP-2 on the fungal infected seed germination rate comparing to the commercial fungicide (DITHANE M-45). It would be especially interesting to perceive that both the bacteria and commercial fungicide have antifungal activity. 1st one is economically and environmentally friendly but another is not.

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