



Research in

AGRICULTURE, LIVESTOCK and FISHERIES

ISSN : P-2409-0603, E-2409-9325

An Open Access Peer-Reviewed International Journal

Article Code: 0258/2020/RALF

Res. Agric. Livest. Fish.

Article Type: Research Article

Vol. 7, No. 1, April 2020: 09-15.

ENHANCEMENT OF RICE AND WHEAT SEED GERMINATION AND SEEDLING VIGOR BY BIOCONTROL AGENT, *Cladosporium cladosporioides*

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ARTICLE INFO

ABSTRACT

Received

09 March, 2020

Revised

18 April, 2020

Accepted

20 April, 2020

Online

30 April, 2019

Key words:

Rice &Wheat,
Seedling vigor,
Germination,
Seed treatment,
Biocontrol agent

The biocontrol agent, *Cladosporium cladosporioides*, strain BOU1 was used to evaluate the rice and wheat plant growth by germination percentage, germination index, vigor index- I and vigor index-II in rice and wheat seed. All the variables of germination and seedling vigor of rice and wheat were increased by the conidial suspension of *C. cladosporioides*. The rice seed treated with *C. cladosporioides* had the highest germination index and vigor index-I with average mean values of 8.2 and 4.5, respectively; while the treated wheat seed showed the highest germination percentage and vigor index- II with average mean values of 80.3 and 0.05, respectively. The research is concluded that the biocontrol agent *C. cladosporioides* improves seed germination and seedling vigor of wheat and rice, which can finally be converted into superior yield even in adverse conditions.

To cite this article: Islam MT, 2020. Enhancement of rice and wheat seed germination and seedling vigor by biocontrol agent, *Cladosporium cladosporioides*. Res. Agric. Livest. Fish., 7 (1): 09-15.



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INTRODUCTION

The *Cladosporium cladosporioides* is a widely distributed cosmopolitan and grey pigmented mold fungus. The *Cladosporium* is one of the extensively studied and the largest genera of hyphomycetes (Dugan *et al.*, 2004). It has also been isolated as saprophytes as well as associated with plants (Torres *et al.*, 2017; Saranya *et al.*, 2013; Bensch *et al.*, 2010) and insects (Habashy *et al.*, 2016; Abdel-Baky, 2000; Abdel-Baky *et al.*, 1998). Several lines of evidence suggest that *C. cladosporioides* is a potent entomopathogenic fungus and thus a potential candidate for biocontrol of insect pests (Islam *et al.*, 2019; Habashy *et al.*, 2016; Eken and Hayat, 2008). The biocontrol fungus, *C. cladosporioides* significantly improve the growth of tobacco seedlings *in vitro* when they were co-cultivated without physical contact (Paul and Park, 2013).

The benefits of seed treatment with microbial antagonists can extend well beyond plant establishment. For example, treatment of maize seed with *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* reduced populations of *Fusarium verticillioides* and associated mammalian-active mycotoxins fumonisin B1 and B2 in grain (Pereira *et al.*, 2007, 2011). Microbial antagonists can provide plant protection in situations where no chemical treatments are available, such as oilseed rape seed delivery of *Serratia plymuthica* for suppression of pathogen *Verticillium dahlia* (Muller and Berg, 2008). In this case efficacy of biocontrol agent varied with seed treatment method with bio-priming and pelleting of seed providing better plant protection than film coating, indicating the importance of developing an optimal seed treatment process for the microbial agent used (O'Callaghan, 2016). The different species of biocontrol agent *viz. Trichoderma* have attracted attention because of their effectiveness against various plant pathogens (Harman *et al.*, 2004). This biocontrol agent has shown impressive results against many phytopathogenic fungi including *M. phaseolina* (Aly *et al.*, 2007). Several recent reports indicate that the biocontrol fungi enhances tolerance to abiotic stresses during plant growth (Bae *et al.*, 2009; Yildirim *et al.*, 2006), in part due to improved root growth, improvement in water-holding capacity of plants, or enhancement in nutrient uptake (i.e., potassium); whereas, in the absence of stress, plant growth may or may not be enhanced. The goal of this study was to evaluate the ability of by biocontrol agent, *C. cladosporioides* BOU1 to improve rice and wheat germination and seedling performance as well as seedling vigor in good-quality seeds. This report describes plant growth promoting activity of a *C. Cladosporioides*, strain BOU1 in rice and wheat seeds.

MATERIALS AND METHODS

The biocontrol agent, *Cladosporium cladosporioides*, (Davidiellaceae: Capnodiales) (Fresen.) de Vries, isolate BOU1 (GenBank accession number- MG654669) was used to evaluate the growth of rice and wheat plant by germination percentage, germination index, vigor index- I and vigor index- II (Table 1). The fungal isolate was obtained from the farmer's rice field in Gazipur, Bangladesh (Islam *et al.*, 2019), which was originally isolated from the brown planthopper *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). The fungus was cultured on PDA (potato dextrose agar) medium. Conidia of *C. cladosporioides* were collected from 15-day-old cultures (maintained at 25 ± 1°C, 70 ± 10% RH, and L 12: D 12 photoperiod) and were suspended in water. The conidia were quantified using a hemocytometer and a light microscope.

The seeds of rice and wheat were obtained from BADC and BARI, respectively (Table 1). The seeds were surface sterilized with 70% ethanol followed by 5% sodium hypochlorite and finally washed with sterilized distilled water before placing for germination assay. One hundred seeds of each plant species were selected for each treatment then soaked in the respective *C. cladosporioides* in a flask containing spore suspension (1 × 10⁷ conidia/ml) for 30 min. There were 6 Petri dishes for each treatment. The seeds soaked in sterilized distilled water served as control. The treated seeds were incubated for 15 days in sterilized Petri dishes at 25 ± 2 °C fitted with filter paper and each Petri dish was irrigated with 10 ml sterilized water. The root and shoot length of the seedlings were measured by selecting fifteen seeds randomly for each treatment. Shoot length was measured from the base of the primary leaf to the base of the hypocotyls and root length was measured from the tip of the primary root to the base of hypocotyl, with all measurements expressed in cm. Shoot and root weight were examined using digital weight scales and expressed in gm. All measurements were done according to the method by Dash (2012).

Table 1. List of plant species with their scientific name, family, variety and source as used in this research

Name of Seed	Scientific Name	Family	Variety	Source
Rice	<i>Oryza sativa</i>	Gramineae	BRRRI Dhan 28	Bangladesh Agricultural Development Corporation (BADC)
Wheat	<i>Triticum aestivum</i>	Gramineae	BARI Ghom 30	Bangladesh Agricultural Research Institute (BARI)

Seed germination rate

Germination rate was the average of number of seeds that germinate over the 7 and 15 day periods. Seed germination of each species was tested as per ISTA (Anonymous, 1985) and germination percentage (GP) of each replication was worked out by using following formula:

$$GP = \frac{\text{Number of germinated seed}}{\text{Number of non-germinated seed}} \times 100 \text{----- (i)}$$

Germination Index

Numbers of seedlings emerging daily were counted from day 0 until day 15 of planting. Thereafter, a Germination Index (GI) was calculated by using the following formula as proposed by AOSA (1983):

$$GI = \frac{\text{Number of Germinated Seed}}{\text{Days of First count}} + \dots + \frac{\text{Number of Germinated Seed}}{\text{Days of Final count}} \text{----- (ii)}$$

Seedling vigor index

Seedling vigor index (SVI) was examined after 15 days of incubation using the formula given by Abul-Baki and Anderson (1973) based on germination (%) and seedling length (cm) or seedling dry weight (gm):

$$SVI - I = \frac{\text{Seedling length} \times \text{Germination Percentage}}{100} \text{----- (iii)}$$

$$SVI - II = \frac{\text{Seedling dry weight} \times \text{Germination Percentage}}{100} \text{----- (iv)}$$

Statistical analysis

Data were analyzed with a one-way ANOVA. The statistical analyses were performed using the Proc GLM procedure (SAS Studio 3.6, 2017). Means were separated using Least Significant Difference (LSD) test at 5% level of significance.

RESULTS AND DISCUSSION

In the current study, it was observed that all the seed treatments were significantly superior over control (untreated seeds). All the variables of germination and seedling vigor of rice and wheat were increased by the conidial suspension of *C. cladosporioides* BOU1 (Figure 1-4). The biocontrol agent, *C. cladosporioides* remarkably enhanced the germination and vigor index of rice and wheat indicating its growth promoting effects of plant. Significant differences were observed among the treatment of germination percentage ($F = 4.65$; $df = 3, 23$; $P = 0.0126$; Figure 1), germination index ($F = 2.98$; $df = 3, 23$; $P = 0.05$; Figure 2), seedling vigor index- I ($P < 0.0001$; $df = 3, 23$; $F = 14.51$; Figure 3) and seedling vigor index- II ($F = 55.29$; $df = 3, 23$; $P < 0.0001$; Figure 4) as compared with their respective controls. The rice seed treated with *C. cladosporioides* BOU1 had the highest germination index and vigor index- I with average mean values of 8.2 and 4.5, respectively; while the treated wheat seed displayed the highest germination percentage and vigor index- II with average mean values of 80.3 and 0.05, respectively.

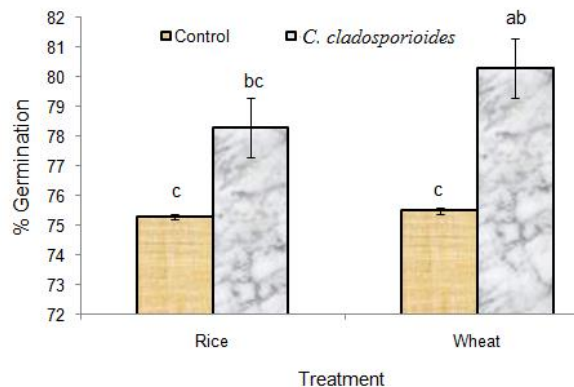


Figure 1. Mean germination percentage (\pm standard error [SE]) of rice and wheat seeds as influenced by *C. cladosporioides* BOU1. Means with the same letter within the same column are not significantly different (LSD-test, following one-way ANOVA: $P < 0.05$)

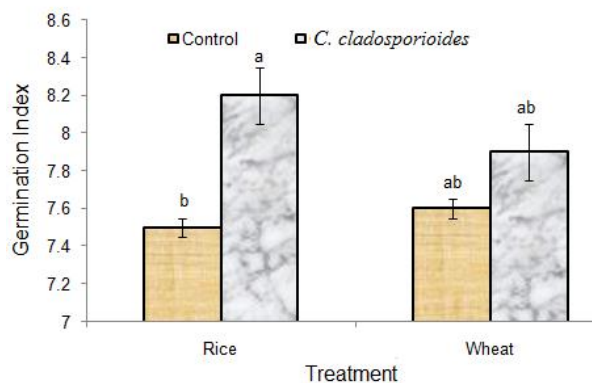


Figure 2. Mean germination index (\pm standard error [SE]) of rice and wheat seeds as influenced by *C. cladosporioides* BOU1. Means with the same letter within the same column are not significantly different (LSD-test, following one-way ANOVA: $P < 0.05$)

Biological seed treatments for control of seed and seedling diseases offer the grower an alternative to chemical fungicides. While biological seed treatments can be highly effective, it must be recognized that they differ from chemical seed treatments by their utilization of living microorganism. Storage and application conditions are more critical than with chemical seed treatments, and differential reaction to hosts and environmental conditions may cause biological seed treatments to have a narrower spectrum of use than some chemicals. Conversely, some microbial agents applied as seed treatments are capable of colonizing rhizosphere, potentially providing benefits to the plant beyond the seedling emergence stage. The microbial inoculants applied as seed treatments deliver microorganisms directly to the plant rhizosphere- the narrow zone of soil that surrounds the roots where plants interact directly with microorganisms (Philippot *et al.*, 2013). It is a zone of intense microbial activity, with growth of plants and microorganisms dependent on reciprocal provision of nutrients and a wide range of other compounds including plant growth regulators and antibiotics. Many beneficial microorganisms of agricultural importance are rhizosphere colonizing species, with ability to increase plant growth via a range of mechanisms (Babalola, 2010).

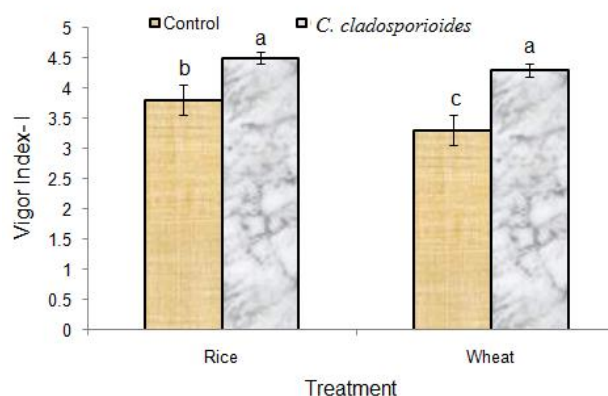


Figure 3. Mean seedling vigor index- I (\pm standard error [SE]) of rice and wheat seed as influenced by *C. cladosporioides* BOU1. Means with the same letter within the same column are not significantly different (LSD-test, following one-way ANOVA: $P < 0.05$)

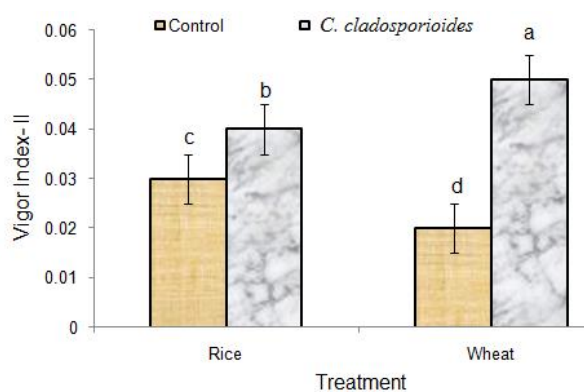


Figure 4. Mean seedling vigor index- II (\pm standard error [SE]) of rice and wheat seed as influenced by *C. cladosporioides* BOU1. Means with the same letter within the same column are not significantly different (LSD-test, following one-way ANOVA: $P < 0.05$)

Application of synthetic insecticides to control insect pest in crop field has seriously deteriorated the environment as well as increased the resistance in notorious crop pests to the insecticides. Considering the high cost and the deleterious effects of insecticides, biological control of insects through beneficial microorganisms is an eco-friendly alternative for sustainable agriculture. The fungal isolate of *C. cladosporioides* BOU1 not only controls the target insect pest, but also promotes the seed germination and seedling vigor of rice and wheat. Our current study is that the fungal isolate BOU1 significantly improved the germination and seedling vigor of rice and wheat. Growth promotion of plants by an entomopathogenic fungus is highly advantageous for its practical use in crop production. The plant-growth-promoting fungus (PGPF), *Talaromyces* sp. significantly enhanced the growth of *Brassica campestris* seedlings and their resistance to *Colletotrichum higginsianum* (Yamagiwa *et al.*, 2011). The inoculation of the rice seeds with *Trichoderma* spp. significantly increased rice seed germination rate, vigor index and speed of germination as compared with untreated control (Doni *et al.*, 2014). The seed treatments were significantly superior over control (untreated seeds). The germination percent, plumule length, radical length and vigor index in case of lentil and *Terminalia arjuna* showed better performance for all these attribute with chemical treatments (Shukla *et al.*, 2008; Cokkizgin and Cokkizgin, 2008). The information concerning the effect of soil application and seed treatment with *Trichoderma* species on seed germination shoot, root length dry root in case of chickpea was also reported earlier (Dubey *et al.*, 2011; Shahid *et al.*, 2011; Kumar *et al.*, 2014, 2015).

CONCLUSION

The present research is concluded that the biocontrol agent *C. cladosporioides* improves seed germination and seedling vigor of wheat and rice, which can finally be converted into superior yield even in adverse conditions.

ACKNOWLEDGEMENTS

The author is grateful to Bangladesh Open University for providing the study leave during this research. This study was supported by University Grant Commission (UGC) of Bangladesh as a Postdoctoral Research Fellowship.

CONFLICT OF INTEREST

There is no conflict of interest about the research.

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