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Evaluation of the effects of two commercial bioproducts (Biomix I and Biomix V) on the health and growth of cotton seedlings

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ABSTRACT: Nowadays in the modern agriculture, there are growing demands in the application of products to replace chemicals in managing plant pest and diseases, and fertilizing soils. In this regard, several bioproducts have recently been produced and released in different countries and have been used as biopesticide and biofertilizer. In this study two bioproducts (Biomix I and Biomix V) were tested in controlling damping-off disease and promoting the growth of cotton seedlings in comparison with a commonly used fungicide (carboxin-thiram. The study was conducted and executed in a greenhouse where seeds of a domestic cotton (Gossyppium hirsutum) variety were coated with powdery bioproducts and were sown in the pots containing pasteurized field soil pre-inoculated with Rhizoctonia solani the fungal agent of cotton seedling damping-off disease. The design of the experiment was completely randomized with five treatments (two bioproducts, commonly used fungicide, infected control and healthy control) each with four replications. The effectiveness of bioproducts in controlling cotton seedling damping-off was determined by counting the number of survived seedlings in their related pots and were compared to those of controls and fungicide. Growth promotion ability of the bioproducts was also evaluated 60 days after sowing by determining the height, fresh weight and dry weight of cotton seedlings. Results showed that both bioproducts performed effectively in controlling cotton seedling damping-off by increased the number of survived seedlings significantly in comparison with commonly used fungicide. In addition to controlling the disease, they also promoted the growth of the seedlings and caused a significant increase in seedlings height, seedlings fresh weight and seedlings dry weight.
Key words: Biomix I, Biomix V, Cotton, Damping-off disease, Growth characteristics.

INTRODUCTION

Continues application of chemical pesticides and fertilizers in the agriculture may lead to the toxic residues, development of resistance among pests and pathohens, environmental contaminations and negative impacts on non-target organisms (Heydari and Gharedaghli, 2007; Rajavel, 2000). In this context, biological control may help to develop a safe and an eco-friendly control strategy for managing plant diseases (Bharathi et al., 2004; Heydari and Gharedaghli, 2007; Heydari and Misaghi, 1998, 2003). Biological control of plant pathogens and diseases using bacterial and fungal antagonists have been the subject of numerous research studies in the recent years and have produced promising results (Heydari and Misaghi, 1998; Heydari and Misaghi, 2003; Jayaraj et al., 2005; Khodakaramian et al., 2008; Radja, 2000). The most common and effective fungi and bacteria used in the biological control of plant diseases include different species of Trichoderma Talaromyces, Pseudomonas, Bscillus, and Burkholderia (Heydari and Misaghi, 1998, 2003; Jayaraj et al., 2005; Khodakaramian et al., 2008).

Preparation and development of powdery formulation of biocontrol-active microorganisms has made their application and use easier and enabled the researchers and even farmers to use them as soil and particularly seed treatment. In several countries commercial bioformulation have been developed and produced and have been released to the agricultural market. Some of the commercial bioproducts that have been available in the market for several years and have been applied in the management of different plant diseases include Kodiak, Deny, Trichomix and Trianum P.

In addition to disease control potential, some bioformulation s contain growth hormones or some microbial ingredients such as plant growth promotion rhizobacteria known as PGPRs (Bharathi et al., 2004; Heydari and Naraghi, 2014**).** These bioproducts have the potential to be used as both biofungicide and

biofertilizer (Bharathi et al., 2004; Heydari and Naraghi, 2014) .One of the most recent and effective of these products is Biomix which has been developed and produced by Biomix Company and contain several fungal and bacterial active ingredients that are capable of protecting plants against pathogens and promoting and enhancing the plant growth factors and characteristics as well. Biomix I and Biomix V have already been used to increase the health and the growth of several crop plants such as different vegetables.

Cotton is an an important fiber crop and is grown in many countries around the world including Iran (Heydari and Misaghi,1998; Heydari and Misaghi,2003; Heydari and Gharedaghli, 2007) Like many other crop plants, cotton is susceptible to several plant pathogens including fungi . Seedling damping-off caused by Rhizoctonia solani is one of the most important and damaging disease of cotton in Iran (Heydari and Gharedaghli, 2007). The most commonly method for controlling this disease is the application of fungicide as seed treatment which is both costly and unsafe for the environment and non-target organisms, In order to search for a ecological friendly and effective strategy to manage damping-off disease and promote growth characteristics of cotton seedlings, we in this study decided to evaluate Biomix I and Biomix V in comparison with commonly used fungicide in the greenhouse conditions.

MATERIALS AND METHODS

MATERIALS

Chemicals, microbial growth media and ingredients used in the study were of laboratory chemical-reagent grade and were purchased from Tehran's chemical market, Cotton seeds and commonly used chemical fungicide (Carboxin-Thiram) were obtained from the Iranian Cotton Research Institute (Gorgan, Iran). Two commercial bioproducts (Biomox I and Biomix V) were provided by Biomix Company (California, USA).

Seed treatment/coating

For seed treatment, the seeds were initially surface-sterilized with 1% sodium hypochlorite and soaked in a double volume of sterile distilled water containing the mentioned bioformulations (10 g kg^{-1} of seed). For each treatment, 48 cotton seeds (cv varamin) were coated and treated with each bioproducts separately. For the fungicide treatment seeds were treated with powdery formulation of Carboxin-thiram which is the most common chemical fungicide used in Iran.

Greenhouse studies

Commercial bioformulations were evaluated for their effects on the health (disease protection) and promotion of cotton seedlings growth characteristics under greenhouse conditions. Twelve cotton seeds from a domestic variety (Varamin) which was susceptible to Rhizoctonia solani-induced damping-off disease were sown in the plastic pots containing 3 kg pasteurized field soil pre-inoculated with the inoculums of R. solani. Cotton seeds were previously treated (coated) with powdery bioformulations and chemical fungicide as described above. The design of the experiment was completely randomized and there were five treatments (two bioformulations, fungicide, infected control and healthy control) each with four replications. Pots were placed in a greenhouse with 12 hours photo period and were watered as needed. The efficacy of different treatments on damping-ff diseases incidence and progress was determined by counting the number of healthy (survived) seedlings in each pot 15, 30, 45 and 60 days after sowing.

For the evaluation of the efficacy of bioformulations on seedling growth, at the end of disease evaluation, two seedlings were removed from each pot and were transferred to the laboratory for measuring their growth factors including height, fresh weight and dry weight.

Statistical analyses of the data

All data obtained in the experiment were first subjected to the analysis of variance (ANOVA) and means were then compared by Duncan Multiple Range Test using CO-STAT statistical soft ware (Cohort, CA, USA). Level of significance was determined at the 5% probability level.

RESULTS

Results of this study are presented in Figures 1-2 as well as Tables 1-2. Figures 1 and 2 indicate the qualitative effects of two test bioproducts on the damping-off disease and the growth of cotton seedlings. According to these figures, both the numbers and the growth of cotton seedlings have clearly been increased by Biomix I and Biomix V in comparison with the infected control and even the commonly used chemical fungicides.

Figure 1. Effectiveness of Biomix V bioproduct (right) in controlling damping-off disease and promoting the growth of cotton seedlings in comparison with infected (untreated) control (left)

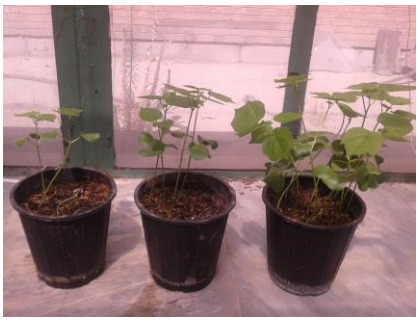

Figure2. Effectiveness of Biomix I bioproduct (right) in controlling damping-off disease and promoting the growth of cotton seedlings in comparison with common fungicide (middle) and infected (untreated) control (left)

The quantitative effects of the bioproducts on the health (diseases protection) and the growth of cotton seedlings are presented in tables 1 and 2. According to Table 1 which contains the results of the effects of bioproducts on damping-off disease of cotton seedlings, both Biomig I and BiomixV could significantly reduced the disease incidence by increasing the number of survived seedlings in comparison with infected control. According to the results, They performed even more effective than commonly used fungicide and were placed in a different statistical groups (Table 1). As the table 1 indicates, Biomix V was somehow more effective than Biomix I in its biocontrol performance.

Table 2 presents the results of the effects of bioproducts on three growth characteristics of cotton including seedling height, seedling fresh weight and seedling dry weight. As this Table indicates, both bioproducts increased and promoted all three growth characteristics of cotton seedlings significantly in comparison with the untreated control and the commonly used fungicide. According to the results presented in this Table, seedlings height, fresh weight and dry weight in the treatments containing Biomix I and Biomix V were much higher than those of others and are placed in separate statistical groups (Table 2).

Table 1. Effects of Biomix I and Biomix V on the incidence of cotton seedling damping-off disease caused by Rhizoctonia solani

Treatments	Number of healthy seedlings 15 days after sowing	Number of healthy seedlings 30 days after sowing	Number of healthy seedlings 45 days after sowing	Number of healthy seedlings 60 days after sowing
Healthy control	12.00 a	12.00 a	12.00 a	12.00 a
Infected control	5.00 c	3.50 c	3. 00 cd	3.00 cd
Fungicide	8.00 b	7.75 b	7.00 b	7.00 b
Biomix I	9.75 ab	9.25 ab	9.00 ab	9.00 ab
Biomix V	11.00 a	10.50 a	10.50 a	10.50 a

In each column, values marked with the same letters are not statistically different according to Duncan Multiple Range Test ($p>0.05$).

Table 2. Effects of Biomix I and Biomix V on some growth characteristics of cotton seedlings in the green house conditions.

Treatments	Seedlings height (cm)	Seedlings fresh weight (g)	Seedlings dry weight (g)
Healthy control	60.25 b	6.35 b	2.15 b
Infected control	37.50 d	2.10 d	0.65 d
Fungicide	57.50 b	6.85 b	2.21 b
Biomix I	75.00 a	10.85 a	3.32 a
Biomix V	78.50 a	11.20 a	3.55 a

In each column, values marked with the same letters are not statistically different according to Duncan Multiple Range Test ($p>0.05$).

DISCUSSION

The overall results of this study indicate that it may be possible to enhance and promote the health and growth of cotton through the application of some bioproducts which are available in the market. Both bioproducts (Biomix I and Biomix V) which were used in this research study as seed treatment performed very effective by decreasing the incidence of cotton seedling damping-off disease as well as increasing the growth of cotton by promoting the measured growth characteristics.

In the first part of the study commercial bioproducts were used against cotton seedling damping-off disease caused by a fungal plant pathogen (Rhizoctonia solani) along with the commonly used chemical fungicide (Carbixin-thiram). Based on the results obtained in this part of the greenhouse experiment Biomix I and Biomix V could significantly increased the number of survived seedlings and performed more effective than the commonly used chemical fungicide in controlling and reducing the incidence of damping-off which is a major disease of cotton in the world including Iran. The results obtained in this part of the study are in the agreement with those of some previous studies in the effectiveness of some bioproducts in the management of different plant diseases ((Beatty and Jensen, 2002; Selim et al. 2005).

Commercial bioproducts usually contain microbial active ingredients such as fungal and bacterial antagonists which have different mechanisms and mode of actions such as mycoparasitism, nutrient deprivations, antibiosis, etc. By employing these mechanisms they are capable of combating different plant pathogens and controlling various plant diseases spores (Bharathi et al. 2004; Emmert and Handelsman, 1999; Heydari and Naraghi, 2014; Jayaraj et al., 2005; Viswanathan and Samiyappan, 2001).

In the second part of our study we investigated the growth promotion ability of the bioproducts on cotton seedlings. In this phase, three growth characteristics including seedling height, seedling fresh weight and seedling dry weight were determined in different experimental treatments . According to the results of obtained in this section, Biomix I and Biomix V bioproducts performed effective and promoted the growth of cotton seedlings by enhancing and increasing the above-mentioned growth characteristics. As was mentioned previously, biological control active microorganisms particularly antagonistic bacteria secrete and produce some enzymes and growth hormones which enable them to act both as antagonist and growth promoter which have been reported previously (Chung et al., 2005; Khodakaramian et al. 2008; Saravanakumar et al. 2007).

The results of the present study are promising and may have practical applications in the management of cotton seedling damping-off which is a major disease of cotton in Iran and around the world. Application and the use of biological control methods in controlling and combating plant pathogens are both effective and safe strategies which can gradually replace or reduce the application of harmful chemical pesticides. In this regard development and production of commercial bioproducts such as Biomix I and Biomix V may help the farmers and growers of different agricultural crops to protect their crops against harmful pests and pathogens, increase the yield, protect the environment and natural resources and achieve a sustainable agricultural system.

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