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Promotion of Garlic Growth Characteristics Using Bioformulations Developed Based on Antagonistic Fungi*

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ABSTRACT: Twelve bioformulations were developed using six isolates of three antagonistic fungi (two isolates each) and two organic and inorganic carriers. Spore suspension of fungal isolates including Trichoderma harzianum, T. asperellum and Talaromyces flavus were prepared and were mixed with two powdery carriers including rice bran and talc following standard procedure. Prepared bioformulations were then used as garlic seed (bulb) treatment to promote three growth factors of garlic including plant height, bulb fresh weight and bulb dry weight in a green house experiment. The design of the experiment was completely randomized with 13 treatments (12 bioformulations and an untreated control) each with four replications. Results showed that all bioformulations had enhancing effects on the growth characteristics in comparison with the untreated control. According to the results, rice bran-based bioformulations performed more effectively than those of talc-based and placed in different statistical groups. Among the fungal isolates, T. harzianum, T. asperellum and Talaromyces flavus showed the most effectiveness in the enhancement of garlic height, bulb fresh weight and bulb dry weight respectively. The overall results of this study suggest that development and application of bioformulations containing antagonistic fungi may be an effective and environmentally safe method for the promotion of garlic growth.
Key words: Garlic. Growth. Trichoderma harzianum. T. asperellum. Talaromyces flavus. Rice bran. Talc

INTRODUCTION

Continuous and long-term application of chemical pesticides and fertilizers in the agriculture has resulted in the environmental contaminations and negative impacts on non-target organisms including human (Cook and Baker 1988). The use of microbial antagonists including beneficial fungi and bacteria has been shown to be a suitable and ecological-friendly candidate for the replacement of chemical pesticides and fertilizers (Cook and Baker 1988). Different fungal and bacterial antagonists have been proved to be potential biocontrol agents for controlling many plant pathogenic fungi (Metcalf et al. 2004; Heydari and Pessarakli 2010; Francisco et al. 2011; Kakvan et al. 2013; Leta, and Selvaraj 2013; Naraghi et al. 2013).

In addition to the antagonistic activities, several beneficial micro-organisms have shown their growth promotion abilities on various plants including cotton, potato and sugar beet. For example in a recent study, Naraghi et al. used some fungal antagonists to enhance the growth of cotton and potato successfully (Naraghi et al., 2013; Heydari and Naraghi 2014). In another previous study the growth of sugar beet seedlings was successfully promoted using some isolates of bacterial antagonists (Jorjani et al., 2011). According to the results of the previous studies conducted on the mechanisms and mode of action of beneficial micro-organisms the promotion of the growth of different plants could be direct or indirect. The direct mechanism includes the production

of some metabolites that have increasing effects on the growth factors such as height and weight (Naraghi et al. 2012). On the other hand, fungal and bacterial antagonists can also enhance and improve the plant health and growth indirectly by protecting them against the diseases and infections caused by different pathogens (Leta and Selvaraj 2013; Heydari and Naraghi 2014).

Trichoderma spp and Talaromyces flavus are some of the fungal antagonists which have recently been used to control various diseases including sugar beet seedling damping-off and wilt disease caused by Verticillium dahlia on various plants such as cotton, cucumber, potato and tomato (Naraghi et al., 2013). The results of the above-mentioned studies have also indicated that these fungal antagonists have the potential of increasing and enhancing the growth characteristics of the target plants by producing volatile and non-volatile metabolites including some growth regulation hormones and enzymes (Naraghi et al.. 2006, 2010, 2013).

Majority of antagonistic micro-organisms including fungi perform well in the controlled environmental conditions but fail to do so in the field due to several reasons including unsuitable method of application. One of the most important reasons for the failure of fungal antagonists in the field could have been related to the lack of proper formulation. The most practical method for application of biocontrol agents in the field is developing and preparing powdery formulations which will enable the farmers to use them as seed treatment particularly for controlling seed and root diseases. Studies have shown that the efficacy of some microbial antagonists in biological control of different plant diseases has been preserved and increased after they have been mixed with organic and inorganic carriers (Heydari and Pessarakli 2010; Kakvan et al. 2013; Samavat et al.2014).

Garlic is an important crop grown in many countries around the world including Iran (Clarkson et al., 2002; Mahdizadehnaraghi et al., 2007; Bakonyi et al., 2011). Garlic is currently being used as an important source of food and also as a medicinal plant in many countries (Clarkson et al.2002; Mahdizadehnaraghi et al.2007; Bakonyi et al. 2011). Presently, garlic is grown and cultivated in several provinces of Iran. Chemical fungicides and fertilizers are extensively applied in garlic fields to improve the health and the growth (yield) of this important crop (Mahdizadehnaraghi et al 2007). Due to the importance of garlic crop in Iran and in order to replace or reduce the application of harmful chemicals in the garlic fields, in this study we decided to develop and prepare some bioformulations using fungal antagonists including Trichoderma and Talaromyces and evaluate their effects on the growth characteristics of this plant.

MATERIALS AND METHODS

Preparation of antagonistic fungal isolates

Isolates of three fungal antagonists including Trichoderma harzianum, T. asperellum and Talaromyces flavus, were obtained from the microbial collection of beneficial microorganisms research laboratory, Iranian Research Institute of Plant Protection. These isolates were previously obtained and isolated from the soil of garlic, potato and sugar beet fields. Antagonistic activities of the isolates against some fungal pathogens including Sclerotium. cepivorum the causal agent of garlic white rot were previously evaluated and approved in In Vitro conditions in the above-mentioned laboratory (unpublished data). A list of antagonistic fungal isolates used in the study and their characteristics are presented in Table 1.

Development and preparation of bioformulations

Twelve bioformulations were developed and prepared using six isolates of the above-mentioned fungal antagonists and two organic and inorganic carriers including rice bran and talc. The powdery compounds of the carriers were selected based on their use in previous studies. They were steam-sterilized at 121°ᶜ for 30 min, and dried aseptically in glass trays before use.

The fungal isolates were first grown on potato dextrose agar (PDA) culture medium for purification and were then incubated for about three weeks for sporulation (spore production). The spores in the Petri plates were washed out by adding 10 ml of distilled water to each plate. A spore suspension of each fungal isolate was prepared at 10^7 spore ml^{-1} using a hemocytometer.

For preparation of bioformulations, 10 ml of each fungal spore suspension was added to a plastic bag containing 50 g of each carrier. The bags were then placed in an incubator at 30 ° C for three weeks until the fungi covered the surface of the carriers. The contains of the bags were then evacuated and dried out in the laboratory conditions, blended and were used for seed treatment. For seed treatment, five g of each bioformulations was mixed with 15 ml distilled water in a glass tray. This combination was used for treatment of 12 garlic seeds (bulbs). The coating and treating process was performed by rolling the garlic bulbs in the powdery bioformulations for 10 min and were allowed to dry for 60 min.

Greenhouse experiments

Greenhouse experiments were conducted at completely randomized design (CRD) with 13 treatments (12 bioformulations and an untreated control) each with four replicates. A replicate consisted of a plastic pot containing two kg of pasteurized garlic field soil and three garlic bulbs treated (coated) with each bioformulation. Control pots were also included in the experiment containing soil sown with untreated garlic bulbs. Pots were placed in a greenhouse with 12 hours photo period and were watered as needed. The effects of different bioformulations on the growth characteristics of garlic including height, fresh weight and dry weight were determined 90 days after sowing.

Statistical analysis

Data obtained in the greenhouse experiment were first subjected to analysis of variance (ANOVA) and means were then compared using Duncan Multiple Range Test by Statistical Analysis System software (SAS) version 9.

RESULTS

Results of this study are presented in Tables 2 and 3. Table 2 shows a list of bioformulations developed in the study using six isolates of three antagonistic fungal species including Trichoderma harzianum, T. asperellum and Talaromyces flavus. As this Table indicates, 12 bioformulations were developed and prepared using the above-mentioned fungal isolates and two organic and inorganic carriers including rice bran and talc. These bioformulations were then evaluated for their effects on the growth characteristics of garlic plants in a greenhouse experiment.

Results of the greenhouse study on the effects of different bioformulations on the growth characteristics of garlic plant including height, bulb fresh weight and bulb dry weight are shown in Table 3. According to this Table, in the greenhouse study all bioformulations promoted and enhanced garlic growth factors with different rates. As the results indicate, bioformulations developed based on rice bran organic carrier and all six isolates of antagonistic fungi increased the three growth criteria more than those bioformulations contained inorganic talc carrier and most of them were placed in different statistical groups.]. However, in comparison with the untreated control all 12 bioformulations resulted in the significant increase in the height, fresh weight and dry weight of garlic plant, although the growth promotion activity of rice bran-based bioformulations were most evident (Table 3).

Table 1. Antagonistic fungal isolates used in the study and their characteristics

Isolate code	Isolate identity	Isolation host	Isolation location
Tr.a-1	Trichoderma asperellum	Potato	Hamadan Province
Tr.a-2	Trichoderma asperellum	Potato	Hamadan Province
Tr.h-1	Trichoderma harzianum	Garlic	Hamadan Province
Tr.h-2	Trichoderma harzianum	Garlic	Hamadan Province
Ta.f-1	Talaromyces flavus	Sugar beet	Alborz Province
Ta.f-2	Talaromyces flavus	Sugar beet	Alborz Province

Table 2. Description of different bioformulations (treatments) in the greenhouse experiment

Treatment code	Treatment description
Control	Untreated seeds + soil
R.B.-Tr.h-1	Seeds treated with Rice bran-Trichoderma harzianum (isolate 1) bioformulation
R.B-Tr.h-2	Seeds treated with Rice bran-Trichoderma harzianum (isolate 2) bioformulation
R.B.-Tr.a-1	Seeds treated with Rice barn-Trichoderma asprellum (isolate 1) bioformulation
R.B-Tr.a-2	Seeds treated with Rice barn-Trichoderma asprellum (isolate 2) bioformulation
R.B- Ta.f-1	Seeds treated with Rice barn-Talaromyces flavus (isolate 1) bioformulation
R.B- Ta.f-2	Seeds treated with Rice barn-Talaromyces flavus (isolate 2) bioformulation
Talc-Tr.h-1	Seeds treated with Talc-Trichoderma harzianum (isolate 1) bioformulation
Talc-Tr.h-2	Seeds treated with Talc-Trichoderma harzianum (isolate 2) bioformulation
Talc-Tr.a-1	Seeds treated withTalc-Trichoderma asperellum (isolate 1) bioformulation
Talc-Tr.a-2	Seeds treated withTalc-Trichoderma asperellum (isolate 2) bioformulation
Talc-Ta.f-1	Seeds treated withTalc-Talaromyces flavus (isolate 1) bioformulation
Talc-Ta.f-2	Seeds treated withTalc-Talaromyces flavus (isolate 2) bioformulation

Table 3. Effects of developed bioformulations on the growth characteristics of garlic plants in the greenhouse experiment

Treatment	Plant Height (cm)	Bulb fresh weight (g)	Bulb dry weight (g)
RBTh1	39.75 a	10.49 a	6.14 a
RBTh2	36.75 ab	9.33 a	5.18 a
RBTa2	35.25 ab	9.56 a	5.21 a
RBTa1	34.00 abc	9.11 ab	4.76 ab
RBTf1	30.00 bcd	7.95 ab	3.60 b
RBTf2	29.25 cde	7.25 b	2.71c
TalcTh1	25.00 efg	5.99 bc	1.64 cd
TalcTh2	24.75 efg	5.92bc	1.57 cd
TalcTa2	22.75 fgh	5.70 bc	1.35 cd
TalcTa1	22 fgh	5.55 bc	1.2 cd
TalcTf1	20.75 ghi	5.40 c	1.05 d
TalcTf2	20.00 ghi	5.38 c	1.03 d
Cont.	6.25 j	2.90 d	0.42 e

Figures marked with the same letters are not statistically different according to the Duncan multiple range test (P>0.05).

DISCUSSION

The overall results of this study indicate that antagonistic fungi including Trichoderma harzianum, T. asperellum and Talaromyces flavus in combination with organic and inorganic carriers can be used for the development of effective powdery bioformulations which may be applied as seed (bulb) treatment may have the potential of enhancing and increasing the growth of garlic plants in the greenhouse conditions.

In this study we chose isolates of Trichoderma and Talaromyces fungal antagonists because these fungi have been used as potential candidates as both disease suppressing biocontrol agents (BCA) as well as the plant growth promotion fungi (PGPF) in several previous studies (.Naraghi et al. 2006, 2010, 2013; Kakvan et al.2013). The selection of rice bran and talc carriers was also based on their use along with various microbial antagonists in the development of new powdery bioformulations for biological promotion of the health and the growth of different plants in the numerous previous studies (Naraghi et al..2006, 2010, 2013; Jorjani et al.2011; Kakvan et al. 2013; Samavat et al. 2014).

Garlic was our test plant in this study because it is an important crop in many countries around the world including Iran and has been well known for its different use as a food source as well as a medicinal plant (Clarkson et al. 2002; Mahdizadehnaraghi et al. 2007; Bakonyiet al. 2011). In Iran garlic is considered as a major cash crop and is cultivated in several provinces for both national use and international exportation (Mahdizadehnaraghi et al. 2007). In this study biological promotion of its health and the growth was studied for the reduction of harmful chemicals residues and to make it safer and healthier for its use as a food source.

According to the results of our study, although almost all developed and prepared bioformulations enhanced and promoted the growth characteristics of the garlic, but, the effectiveness of rice bran-based bioformulations was more evident. The higher effectiveness of these bioformulations could be related to the organic nature of rice bran in comparison with talc which is an inorganic carrier and compound. Different performances of organic and inorganic based bioformulations in biological promotion of the health and the growth of various plants including cotton, potato and sugar beet have been reported in the previous studies (Naraghi et al.. 2006, 2010, 2013; Jorjani et al.2011; Kakvan et al. 2013; Samavatet al. 2014).

Antagonistic fungi including Trichoderma ssp. and Talaromyces flavus used in this study are primarily known for their disease suppressing activities and have previously used in the biological control of several plant diseases (Metcalf et al. 2004; Heydari and Pessarakli 2010; El-Hassan et al.2013; Kakvan et al. 2013; Naeimi and Zare 2013). In addition to the antagonism and biocontrol activities of the abov-mentioned fungi, their plant growth promotion potential have also been proved and reported in some recent studies. For example in a recent study Naraghi et al. (2012) used several isolates of Trichoderma and Talaromyces for the enhancement and promotion of growth characteristics of cotton and potato. In the present study bioformulations containing Trichoderma performed more effectively than Talaromyces fungal isolates that can probably be due to the differences in the mechanisms and genetics of these fungi which has been reported previously (Naraghi and others 2012; Kakvan and others 2013).

The results of the present study in the development of some new bioformulations are promising and may have practical application in the biological promotion of the growth of garlic in the field conditions. Obtaining positive results in the field conditions may replace or reduce the application of harmful chemical fertilizers and lead the garlic growers to increase the yield and production of this important crop and protect the agricultural environment and biological resources.

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