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In-Vivo Antagonistic Potential Of Natural Extract And Rhizobacterial Isolates Against Root Rot Of Chili Incited By Pythium Aphanidermatum

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Abstract

In vivo experiments evaluated the effect of two rhizobacterial isolates and Ganoderma lucidum extract were tested against Pythium aphanidermatum.Bacillus subtilis, Pseudomonas fluorescence and G. lucidum extract were used as biocontrol agents under field conditions (Pots) to control Pythium aphanidermatum and growth promotion of chili plant. P. fluorescence showed more growth promotion including the shoot and root length, leaf size, dry root and shoot weight, fresh root and shoot length of chili, and also inhibit the infection of P. aphanidermatum. B. subtilis also inhibit the P. aphanidermatum, and showed more growth promotion but less than P. fluorescence. The growth promotion of chili and P. aphanidermatum inhibition ability of G. lucidum extract under field conditions (pots) was not so much satisfactory.

Key words: Capsicum annuum, Bacillus subtilis, Pseudomonas fluorescence and G. lucidum extract,growth inhibition, secondary metabolites, Plant growth promotion.

Introduction

Chili is an important cash crop of Pakistanbelonging to family Solanaceae and genus Capsicum which is popularly known as “Red Pepper”. The most cultivated species of this genus are C. pubescens,C. frutescens L, C. baccatum L, C. annuum L and C. chinense L (Bosland and Votava, 2000; Costa et al., 2009).“Capsanthin” andthe pungency are attributed to “Capsaicin”. Chilies are consumed as a spice and become an ingredient in medicines and beverages (Daundkar & Bairagi, 2015; Damodaran, & Velayutham, 2015). Green chilies provide proteins, minerals, vitamin A and C while dry chilies are known

as a source of vitamin A and D (Patel, 2014). Across the world, the capsicum is used in fresh, dried or powder form (El-Ghorab et al., 2013). It is rich in nutrients like mineral salts, lipids, proteins, carbohydrates, (Ca, Fe) fibers and in vitamins A, D3, E, C, K, B2 and B12 (El-Ghorab et al., 2013).

Though chilli plays a vital role in increasing the national economy, still the productivity and foreign exchange realized through chilli can be increased by the management of various diseases caused by pathogens of fungal, bacterial and viral origin. Among the fungal diseases, damping-off caused by Pythiumspecies cause more than 60 per cent mortality of seedlings both in nursery and main field (Manoranjitham et al., 2000). Management of Pythium is very difficult due to its wide host range, soil-borne nature and prolonged survival of propagules in the soil. Traditionally, this diseaseis controlled by the application of synthetic fungicides. But the indiscriminate use of fungicides resulted in the accumulation of residual toxicity, environmental pollution and altered the biological balance in the soil by over killing the non-targeted microorganisms. Besides development of resistance to fungicides in the pathogen Pythium spp (Muthukumar et al., 2008).

It is therefore essential to develop an effective, cheap and environmentally safe non-chemical method for the management of damping-off disease. Hence, Biological control has been developed as an alternative to synthetic fungicides and considerable success has been achieved by utilizing antagonistic microorganisms for controlling soil borne pathogens. The need for alternative control strategies, particularly those involving biological control, has increased greatly in the past two decades. Currently, numerous Antagonistic bacteria such as Bacillus spp, P. fluorescens, are used as biological control agent against Pythium damping-off(Gravel et al., 2005).

The objectives of the present study were (1) Determination of the effect of PGPR on P. aphanidermatum infection in chili crop. (2) Evalution of the effect of G. lucidum extract on P. aphanidermatum infection in chili crop. (3) Comparative analysis of PGPR and G. lucidum extract on inhibiting the effect of root rot disease in chili crop.(4) Impact of PGPR and G. lucidum on different growth parameters of chili plant.

Material and Methods

Isolation and Identification of pathogenic fungi

Disease causing pathogens were isolated from the infected roots of chili plants by baiting method. Soil samples were moistened enough to make a paste like uniformity in petri plates and then flooded with distilled water. Grass blades were cut into small pieces of 5 cm and placed in such a way that one end of the grass blade was in contact with the soil paste and the other end was away and incubated at 20^{o}C for seven days. Faded threads of fungal mycelium were observed after eight days of incubation and the

colonized grass blades were then inoculated on corn meal agar Corn meal agar (corn meal agar 17g/liter, PCNB 50mg/litre, 10mg/liter benomyl added before autoclaving, rifampicin 10mg/liter, ampicillin 250mg/litre and pimaricin 5mg/liter were added and blended with media before it solidified).Pythium cultures were identified and classified with the aid of keys of (Dick, 1990).

Isolation of Bacterial Isolates and Inoculum Production.

The soil suspensions were prepared by diluting 1g soil with 10 ml water. Then 1 ml of this dilution was transferred to 9 ml distilled water and so on. The serial dilutions were made up to 10^{-4} times. Soil suspension of about 100µl from each dilution was pipetted out and spreaded on LBA media. Plates were incubated at 37˚C for 24 hours in inverted position.

Organic and aqueous extraction of Ganoderma lucidum

For organic extractionG. lucidum were chopped into very small pieces, 20g of that was soaked in 100ml of acetone and kept in shaker at 100rpm for 6 hours. The extract was filtered through a whattman's No.4 filter paper. The acetone filtrate was concentrated by evaporating excess solvent at room temperature and aqueous filtrate was evaporated in a hot water at 40˚C. Final extract was stored in refrigerator at 4˚C (Jonathan and Awotona, 2013; Jaya Singh et al., 2014).

In vivo activity of G. lucidum extract and Bacterial isolates

The potentialrhizobacterial isolates were evaluated for efficacy under greenhouse conditions. The soil was fumigated and filled in pots @ 400 gram per pot. Chili seedlings of JALWA (susceptible variety) were purchased from Fruit and Vegetable Market, Multan Road, Lahore. The seedlings were sown in pots and placed at 25˚C in greenhouse. Seedlings without any fungus, Soil treated with P. aphanidermatum inocululm + G. lucidum extract, Soil treated with P. fluorescence inoculum + P. aphanidermatum, Soil treated with B. subtilis inoculum + P. aphanidermatum, Soil treated with P. aphanidermatum inoculum + G. lucidum extract+ B. subtilis inoculum + P. fluorescence inoculums, Soil treated with G. lucidum extract, Soil treated with P. fluorescence inoculums, Soil treated with B. subtilis inoculums and Soil treated with B. subtilis inoculum + P. aphanidermatum inoculum + P. fluorescence were different treatments applied.

Glass house experiment: Inoculation of Pathogenic fungus

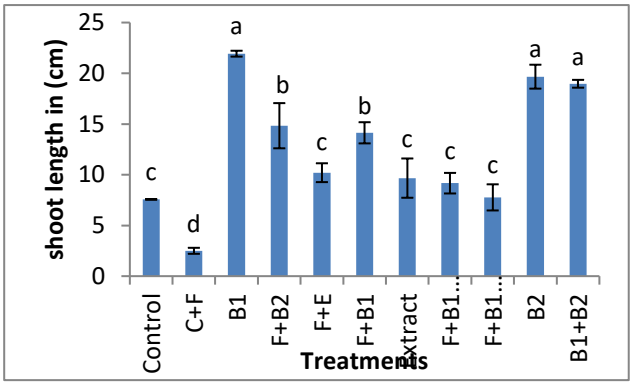
For inoculation of pathogenic fungus 4X 10^6 spore suspension was prepared using hemocytometer.. For inoculation purpose two holes at the depth of 5 inches were made at the side of each plant and 10ml of spore suspension was injected. Test plant was grown in un-inoculated soil with none amendment served as control. The number of seedlings emerged in each pot, seedling emergence,length of roots and

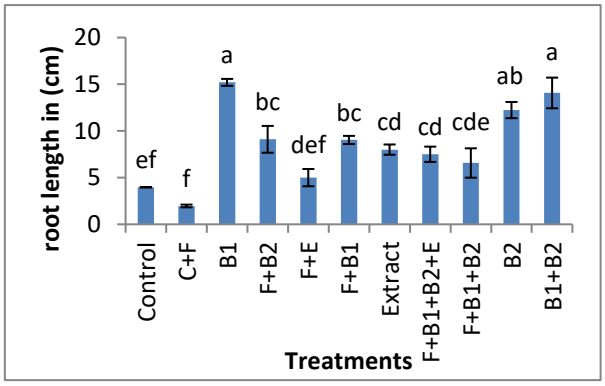
shoots, fresh and dry weight of roots and shoots, size of leaves were the parameters observed after treatment and data was analyzed by using Statistix 8.1 software.

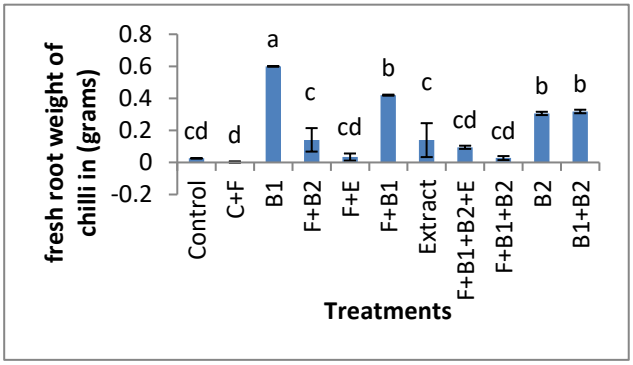
Results

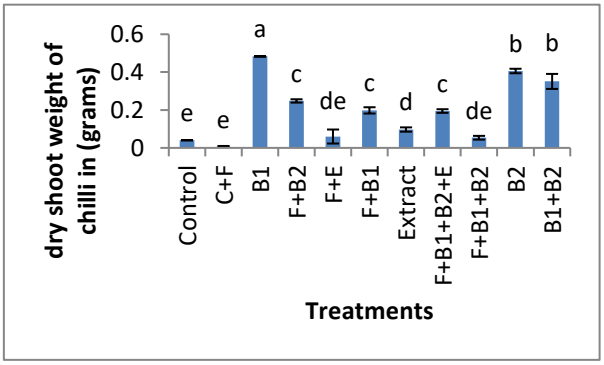
In vivo activity of G. lucidum extract and Bacterial isolates

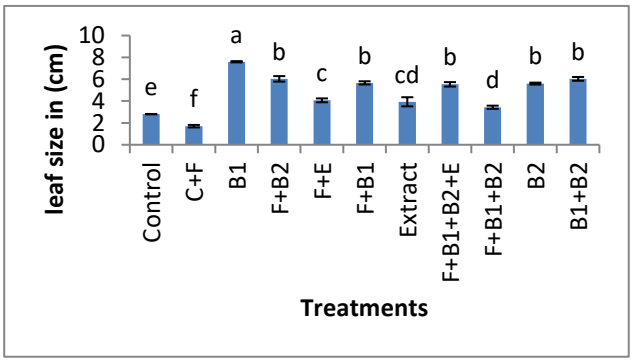
Different plant parameters were studied in order to check out the antifungal potential of rhizobacterial isolates and G. lucidum extract in field conditions to control the root rotdisease of Chili. According to results B1 showed maximum shoot length (21.93cm), root length (15.2cm), leaf size (7.6cm) and remained dominating over rest of the treatments. It was also observed as a result that fresh and dry weight of shoot and root has also increased when treated with B1. Least values of shoot length (2.5cm), root length (1.96), leaf size (1.7 cm) was observed when treated with C+F. This treatment has also shown least performance in case of fresh and dry shoot as well as root weight. Other treatments B2, B1+B2, F+B2, F+B1, F+E, Extract, F+B1+B2+E, and F+B1+B2 showed shoot length of 19.66cm, 18.96cm, 14.83cm, 14.13cm, 10.2cm, 9.66cm, 9.16cm and 7.76cm respectively.Other results B1+B2, B2, F+B2, F+B1, Extract, F+B1+B2+E, F+B1+B2, and F+E showed root length of 14.06cm, 12.33cm, 9.1cm, 9.03cm, 8cm, 7.5cm, 6.56cm, and 5cm from maximum to minimum respectively. Other results B1+B2, F+B2, B2, F+B1, F+B1+B2+E, F+E, Extract, and F+B1+B2 showed leaf size of 6.03cm, 6.03cm, 5.6cm, 5.66cm, 5.53cm, 4.06cm, 3.93cm and 3.43cm from maximum to minimum respectively. Other results B2, B1+B2, F+B2, F+B1, F+B1+B2+E, Extract, F+E, and F+B1+B2 showed fresh shoot weight of 1.83g, 1.74g, 1.32g, 1.08g, 0.48g, 0.36g, 0.36g, 0.22g from maximum to minimum respectively. Other results F+B1, B1+B2, B2, F+B2, Extract, F+B1+B2+E, F+E, and F+B1+B2 showed fresh root weight of 0.42g, 0.31g, 0.30g, 0.14g, 0.13g, 0.09g, 0.03g, and 0.027g from maximum to minimum respectively. Other results B2, B1+B2, F+B2, F+B1, F+B1+B2+E, Extract, F+E, and F+B1+B2 showed dry shoot weight of 0.406g, 0.351g, 0.24g, 0.198g, 0.194g, 0.09g, 0.06g, and 0.053g from maximum to minimum respectively. Other results B1+B2, B2, F+B2, F+B1, F+B1+B2+E, Extract, F+E, and F+B1+B2 showed dry root weight of 0.194g, 0.156g, 0.071g, 0.056g, 0.029g, 0.026g, 0.016g, and 0.015g from maximum to minimum respectively figure.

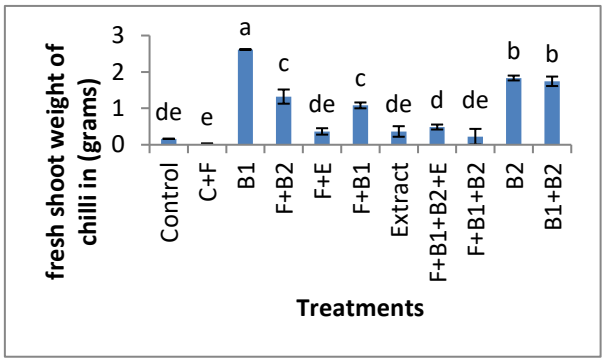

shoot length in (cm)
25
20
15
10
5
0
c d a b c b c c c a a
Control C+F B1 F+B2 F+E F+B1 Extract F+B1... F+B1... B2 B1+B2
Treatments


root length in (cm)
20
15
10
5
0
ef f a bc def bc cd cd cde ab a
Control C+F B1 F+B2 F+E F+B1 Extract F+B1+B2+E F+B1+B2 B2 B1+B2
Treatments


fresh root weight of chilli in (grams)
0.8
0.6
0.4
0.2
0
-0.2
cd d a c cd b c cd cd b b
Control C+F B1 F+B2 F+E F+B1 Extract F+B1+B2+E F+B1+B2 B2 B1+B2
Treatments


dry shoot weight of chilli in (grams)
0.6
0.4
0.2
0
e e a c de c d c de b b
Control C+F B1 F+B2 F+E F+B1 Extract F+B1+B2+E F+B1+B2 B2 B1+B2
Treatments


leaf size in (cm)
10
8
6
4
2
0
e f a b c b cd b d b b
Control C+F B1 F+B2 F+E F+B1 Extract F+B1+B2+E F+B1+B2 B2 B1+B2
Treatments


fresh shoot weight of chilli in (grams)
3
2
1
0
de e a c de c de d de b b
Control C+F B1 F+B2 F+E F+B1 Extract F+B1+B2+E F+B1+B2 B2 B1+B2
Treatments

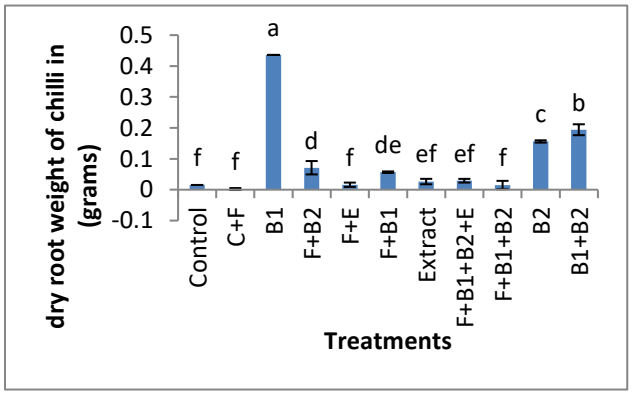

dry root weight of chilli in (grams)
0.5
0.4
0.3
0.2
0.1
0
-0.1
f f a d f de ef ef f c b
Control C+F B1 F+B2 F+E F+B1 Extract F+B1+B2+E F+B1+B2 B2 B1+B2
Treatments

Figure: Comparative study for chili plants after treating with different biological control agents and their combinations.

Discussion

Root rot caused by P. aphanidermatum is a major constraint to Chili (Capsicum annuum L) production throughout the world. 60% yield losses of chili have been detected by different researchers at nursery and field stage due to P. aphendiatum which cause root rotin chilies.(Jadhav and Ambadkar, 2007).

Current research was planned to find out the efficacy of G. lucidum extract and rhizobacteria to suppress the growth of P. aphanidermatum causing root rotdisease in chili. In this study total of ten bacterial strains were purified from the rhizospheric soil of the green chilies. These bacterial isolates were B. subtilis, P. fluorescence, P. aeruginosa, Weeksella virosa, Actinobacillus spp, P. stutzeri, P. putida, B. licheniformis, B. fortis, and B. lentus.These isolates were also reported by (Indiragandhi et al., 2008; Joseph et al., 2007; Canbolat et al., 2006; and Pandey et al., 2006) from rhizospheric soil. Biocontrol agents suppress disease by a number of mechanisms such as parasitism, competition, induction of resistance and antibiosis in plants (Whipps, 2001). The strategies involved in the present research work are solely artificial in comparison with natural infection. Thus, it is quite difficult to estimate the required amount of antagonistic inoculum under natural condition to suppress the disease.

During in vivo experiment B. subtilis,P. fluorescence and G. lucidum acetone extract were applied to check the antifungal activity against P. aphanidermatum. The bacteria and extract were also used as Plant Growth Promoters. These agents were found as best PGPR and antifungal agents. Among

these biocontrol agents P. fluorescence was best plant growth promoter and disease inhibitor. B. subtilis was also showing its best potential that was very near to the P. fluorescence.G. lucidum was also observed as good disease inhibitor and growth promoter but less than B. subtilis and P. fluorescence. In experiment various growth factors like shoot length, root length, leaf size, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight were measured. During observation of shoot length P. fluorescence showed maximum root length of 21.93cm and B. subtilis showed 19.66cm and G. lucidum extract showed 9.66cm while the control was 2.5cm. In case of root length measurement the control was 1.96cm, P. fluorescence showed maximum of 15.2cm, B. subtilis showed 11.23cm and G. lucidum extract showed 12.33cm of root length.

While in case of leaf size of chili after treating with bacterial isolates and G. lucidum extract, P. fluorescence showed maximum leaf size of 7.6cm and B. subtilis showed 5.6cm and G. lucidum extract showed 3.933cm while the control showed 1.7cm leaf size. In case of fresh shoot weight of chili P. fluorescence showed maximum fresh shoot weight of 2.61grams and B. subtilis showed 1.83g and G. lucidum extract showed 0.36g while the control showed 0.02g fresh shoot weight.

In case of fresh root weight of chili P. fluorescence showed maximum fresh root weight of 0.59g and B. subtilis showed 0.30g and G. lucidum extract showed 0.13g while the control showed 0.0025g fresh root weight. In case of dry shoot weight of chili P. fluorescence showed maximum dry shoot weight which was 0.482g and B. subtilis showed 0.406g and G.lucidum extract showed 0.09g while the control showed 0.006g dry shoot weight. In case of dry root weight of chili showed P. fluorescence maximum dry root weight which was 0.436g and B. subtilis showed 0.15g and G. lucidum extract showed 0.0263g while the control showed 0.0021g dry root weight.

In overall results of in vivo experiment revealed that inoculated plants grew faster with higher height and weight as compared to control. P. fluorescence showed maximum of its potential to control the P. aphanidermatum and as plant growth promoter. The B. subtilis also have very good potential but less than P. fluorescence. While G. lucidum extract also have a potential to control the P. aphanidermatum but it showed minimum potential in comparison with the bacterial agents. The potential of G. lucidum may be increased by changing the concentration of extract. (Hou et al., 2013) also revealed the P. fluorescence as best plant growth promoter and antifungal agent. It has been reported that P. fluorescens has the ability to promote the plant growth (Kloepper et al., 1988; Howie and Echandi, 1983; Kurek and Jaroszuk-Scisel, 2003), and to control many plant pathogens (Ramamoorthy et al., 2002; Paulitz et al., 1992).While no data was observed regarding G. lucidum

extract in vivo experiment. It is newely used as biocontrol agent in pot experiment. It can be concluded that bacteria B. subtilis and P. fluorescence and G. lucidum extract can be better bio-control agents against the P. aphanidermatum and this can be landmark in the field of plant pathology. Thus, in a nut shell present research has concluded that the biological means of controlling plant pathogenic fungi is more potential, effective as well as environment friendly approach. It is need of the hour to replace the chemical control strategies with biological ones. A future perspective of this research is to formulate and commercialize a bio-pesticide for sustainable agriculture.

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