Scientia Horticulturae 193 (2015) 114–120

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of bio-organic fertilizer on pepper growth and *Fusarium* wilt biocontrol

Yue Wu [a], Chunyan Zhao [b], John Farmer [a], Junde Sun [a,*]

[a] College of Land and Environment, Shenyang Agricultural University, Shenyang 110866, China
[b] Food Science College, Shenyang Agricultural University, Shenyang 110866, China

ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form 21 June 2015
Accepted 24 June 2015
Available online 24 July 2015

Keywords:
Fusarium wilt
Bio-organic fertilizer
Defense enzyme
Growth promotion
Biological control

ABSTRACT

Fusarium wilt of pepper outbreaks in the region where pepper crop has been grown for many years and it has already become a major phytopathogen all over the world. The goal of this study is to check bio-organic fertilizer capability on controlling pepper *Fusarium* wilt and promoting plant growth in greenhouse and field. Pot experiments were conducted in greenhouse which showed that isolates Ljx101 and Lja002, identified as *Bacillus amyloliquefaciens* and *Bacillus subtilis*, had satisfactory biocontrol effects on *Fusarium* wilt and growth promoting abilities on pepper plant. In addition, the bio-organic fertilizer (BOF), mixed with Lja002 and Ljx101, not only controlled *Fusarium* wilt disease but also significantly promoted plant growth in pot. In the treatment of BOF1, pepper plant survival rate was increased by 58.42% in greenhouse condition and defense enzymes including polyphenol oxidase, peroxidase and superoxide dismutase activity were largely enhanced by 45.54, 68.78 and 46.21% in roots as compared with pathogen control. Field experiments indicated that BOF had stable characteristics on controlling pepper *Fusarium* wilt and increasing pepper yield. These results demonstrate that BOF is a promising way to control soil-borne pathogen and promote crops growth in field production.

1. Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable cash crop worldwide. *Fusarium* wilt of pepper caused by *Fusarium oxysporum*, is one of the most damaging diseases that is difficult to eradicate. The pathogen could survive in soil for a long period of time through the production of chlamydospores (Beckman, 1987). Once it enters into plant, fungi mycelium colonizes in the vascular tissue and causes wilting. Soil disinfection using various chemicals (Cebolla et al., 2000) or pesticides (Fravel et al., 2005) is a traditional and cheap practice which has been widely used all over the world. However, these treatments also have been proved to be temporary on pathogen, toxic on microbial communities and have the ability of rebounding pathogen pesticide-resistance (Huang et al., 2011). Therefore, environment-friendly pesticide alternatives such as organic amendments (Céline et al., 2007) and beneficial microorganisms (Wang et al., 2012) have been developed as new part of conventional methods for biological control of soil-borne disease.

Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in rhizosphere (Ahmad et al., 2008). They not only associate with root to exert beneficial effects directly or indirectly on plant development (Kloepper et al., 1980) but also have positive effects on controlling phytopathogenic microorganisms (Son et al., 2014). Several reports have shown that a special bio-organic fertilizer (BOF) combining PGPR with manure composts could enhance biocontrol and growth-promoting effects of PGPR (Chen et al., 2011). Many PGPR have been proved to be effective biocontrol agents in laboratory or greenhouse conditions, such as *Bacillus* spp. (Gong et al., 2006) and *Pseudomonas* spp. (Leonardo et al., 2006). Among these, *Bacillus subtilis*, *Bacillus cereus* and *Bacillus amyloliquefaciens* are the most effective species in controlling plant diseases through various mechanisms (Francis et al., 2010). The ability of forming endospores allows them to survive in a wide range of environmental conditions and facilitates the formulation of BOF (Perez-Garcia et al., 2011).

BOF are biological preparations with sufficient densities of potent microorganisms which have a tangible beneficial role in fitting a proper rhizosphere for plant growth (Saber, 2001). Furthermore, these contained microorganisms also have the ability of converting nutritionally important elements from unavailable to available through biological process (Vessey, 2003) as well as inhibiting pathogens growth. Organic fertilizer, an important part

* Corresponding author at: Shenyang Agricultural University, Dongling Road 120#, Shenyang 110866, Liaoning, China.
E-mail address: syndwsw@163.com (J. Sun).

http://dx.doi.org/10.1016/j.scienta.2015.06.039
0304-4238/

of BOF, is essential for the development of plants, vegetables, flowers and fruits as it offers varieties of nutrients to plants and microorganisms (Zayed et al., 2013). The suitability and usefulness of organic fertilizer have been attributed to high availability of N, P, and K contents (Waddington, 1998), which are capable of enhancing soil fertility, extending microorganisms survival rates in soil and improving antagonistic isolates biocontrol effects (Yang et al., 2011). Trillas et al. (2006) reported that *Rhizoctonia* damping-off in cucumber plants was reduced using compost and biological control agent *T. asperellum* strain T-34. Ciampi-Panno et al. (1989) also showed that potato bacterial wilt incidence was significantly reduced in a naturally infested soil by the addition of a special amendment, an organic soil mixed with $CaCO_3$ at the ratio of 9:1 (w/w), containing high population of antagonistic isolate BC8.

Ljx101 and Lja002 isolates, obtained from health pepper plant rhizosphere, were identified as *B. amyloliquefaciens* and *Bacillus subtilis*. They have the abilities to inhibit *F. oxysporum* mycelium and promote pepper plant growth. The main objective of this study was to evaluate effects of BOF mixing with Lja002 and Ljx101 in controlling pepper *Fusarium* wilt and promoting pepper plant growth in greenhouse and field conditions.

2. Materials and methods

2.1. Cultivation of pathogen and isolation of antagonistic microbes

Pathogen, *F. oxysporum* f. sp. *capsicum*, was isolated from diseased pepper plant and cultured in potato dextrose agar (PDA) medium. For the isolation of antagonistic isolates, rhizosphere soil was sampled from healthy pepper plant roots, after 10-fold serial dilutions, microorganism suspension was spread onto nutrient broth agar (NA) media and cultured at 28 °C for 4 days. Bacterial colonies with different morphological characteristic were purified by streaking and anti- *F. oxysporum* activity was determined by dual culture (Ju et al., 2014) and Oxford cup (Wang et al., 2012) tests. Isolates with inhibition zone were selected and purified for 4 times. Two strains, Lja002 and Ljx101 with strong antagonistic ability against *F. oxysporum* were selected as experimental strains.

2.2. Identification of bacteria strain

Bacterial isolates Lja002 and Ljx101 were partially identified by analyzing 16S rDNA sequences. Genomic DNA from isolate was extracted using a Wizard Genomic DNA Purification Kit (Promega, USA). 16S rDNA gene was amplified by PCR using the universal sequencing primer 7F as forward primer (5′-CAG AGT TTG ATC CTG GCT-3′) and 1540R as reverse primer (5′-AGG AGG TGA TCC AGC CGC-3′). The PCR mixture (25 μl) contained 10.5 μl of distilled water, 12.5 μl of TaqMix, 0.5 μl of each primer (10 μM) and 1 μl of DNA template. Thermal cycling conditions were as follows: 4 min denaturation at 95 °C, followed by 32 cycles of denaturation for 1 min at 94 °C, annealing for 30 s at 55 °C, extension for 2 min at 72 °C, and a final extension for 8 min at 72 °C. PCR products were sequenced and compared to existing sequences in the GenBank database using NCBI Blast server.

2.3. Preparation of pathogen conidia and antagonistic isolate suspensions

Four pieces of *F. oxysporum* colony margin (8 mm diameter each) were inoculated in liquid potato dextrose broth (PDB) and cultured in 28 °C on a rotary shaker (120 rpm) in dark for 7 days. Conidia spore suspension was collected by filtering the culture through a two-layer cheese cloth and adjusted to 1×10^6 conidia/ml. Two loops of Lja002 and Ljx101 were each inoculated in liquid nutrient broth (NB) medium and cultured at 30 °C on a rotary shaker (120 rpm) in dark for 2 days. The fermented culture was centrifuged in 10.000 rpm/min for 30 min and final concentration of the bacterium suspension was 1×10^8 cfu/ml.

Table 1
Basic soil properties of experiment plots at 0–15 cm.

Soil properties	Plot 1	Plot 2
Organic matter ($g\,kg^{-1}$)	20.63 ± 2.15	21.70 ± 1.46
Avaliable *N* ($mg\,kg^{-1}$)	134.51 ± 3.48	141.92 ± 1.22
Avaliable *P* ($mg\,kg^{-1}$)	23.14 ± 3.69	25.68 ± 1.37
Avaliable *K* ($mg\,kg^{-1}$)	69.27 ± 5.81	73.43 ± 2.09

2.4. Experimental site

Pot experiments in greenhouse and field trial were conducted in Shenyang Agricultural University Experimental station, Shenyang, Liaoning province, China. The region is temperate continental, monsoon affected and is characterized by high temperature, rainy summers, and cold drying winters. Two pathogen booming field plots, planted with pepper for two years were used in the field experiment. The soil from experimental fields tested was brown soil, which is rich in organic matter. Soil samples were collected from the top soil layer (0–15 cm) of each plot and chemical properties were detected (Table 1). In pot experiments, pathogen booming soil was collected from field plot 1 and healthy soil was collected according to the chemical properties of pathogen booming soil.

2.5. Growth promotion and anti-pathogen characteristics of isolates

The application of Ljx101 or Lja002 on plant was conducted over 45-day period in pots in greenhouse (25 ± 5 °C, 60% RH, 10–12 h daylight) with 5 replications of 8 plants per treatment and the test was conducted for two times. Pepper seed, Dayu NJJ which was known to be pathogen susceptible was used in this test. Healthy and disease booming soil were passed through 10-mm sieve before putting into pots. Pathogen infected soil was made by mixing disease booming soil with pathogen conidia suspension at the rate of 10 ml/kg and the final pathogen density was nearly 1×10^7 cfu/ml. Pathogen-soil was made by mixing sterile soil (disease booming soil was sterilized at 121 °C for three times) and pathogen conidia suspension at the rate of 10 ml/kg and pathogen density was the same as pathogen infected soil. Each pot ($11 \times 30 \times 30$ cm) was filled with 2 kg of soil and transplanted with 8 two-leaf stage pepper seedlings, per seedling was irrigated with 3 ml of Ljb001 or Lja002 suspension (1.0×10^8 cfu/ml). All pots were arranged randomly and cultured in greenhouse. Eight treatments were used: (1) Ljx101 and pathogen infected soil; (2) Ljx101 and healthy soil; (3) Ljx101 and pathogen-soil; (4) sterile water and pathogen infected soil as negative control; (5) sterile water and healthy soil as positive control; (6) Lja002 and pathogen infected soil; (7) Lja002 and healthy soil; (8) Lja002 and pathogen-soil. After growing in greenhouse, pepper plant survival rate of each treatment was recorded. Total height, root length and dry weight were recorded according to the mean value of 5 plants per treatment, collected randomly.

2.6. Growth promotion traits of isolates

To assay the growth-promoting characteristic of isolates, indole-3-acetic acid (IAA) and siderophore production abilities of these two isolates were tested. Salkowski reagent was used to test IAA content in isolate fermentation according to Glickmann and Dessaux (1995). Briefly, isolate was cultured in Luria–Bertani (LB)