Scientia Horticulturae 168 (2014) 124–131

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Potential use of anaerobically digested manure slurry to suppress Phytophthora root rot of chilli pepper

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ARTICLE INFO

Article history:
Received 8 January 2013
Received in revised form 3 July 2013
Accepted 6 November 2013

Keywords:
Anaerobically digested slurry
Animal manure
Disease suppression
Phytophthora capsici
Mechanism

ABSTRACT

Anaerobic digestion is a promising way to treat the large amounts of animal manures produced from animal husbandry in terms of efficient recovery of energy by the anaerobic digestion and of more sustained use of the slurries. Side effects such as suppression of soil borne diseases are not yet well investigated and understood. The objective of the study was to evaluate the potential use of anaerobically digested slurry (ADS) to suppress *Phytophthora capsici*, the causative agent of Phytophthora root rot of chilli pepper. Mycelial growth and zoospore germination of *P. capsici* were inhibited by ADS and application of ADS significantly reduced the disease incidence under bioassay conditions. The percentage of reduction in zoospore germination of *P. capsici* and disease incidence by anaerobically digested pig slurry (ADP) were significantly greater than anaerobically digested dairy slurry (ADD). Filter-sterilization reduced disease suppression only to a certain degree. Exogenous applied ammonium and humic substances (HS) isolated from ADS suppressed *P. capsici* and the percentages of inhibition of hyphal growth and zoospore germination by HS extracted from ADP were greater than those derived from ADD. It is hypothesized that that the better control of Phytophthora root rot by ADP was attributed to the higher concentration of ammonium and the particular structure of HS.

1. Introduction

With the intensive and concentrated development of animal husbandry, the amount of livestock and poultry manure reached about 3.9 billion tons in China in 2007 (Jiang et al., 2011). Inappropriate management of animal manure is a serious threat to the environment and the livestock industry. Biogas generation from anaerobic digestion is a promising technology that can reduce environmental risks associated with manure management provided that is utilized in a sustainable manner (Zheng et al., 2012). In fact, more than 1 billion tons of anaerobically digested slurry (ADS), the main by-product of biogas, was produced from 3800 large-scale biogas plants (digestion reactor volume >500 m^3) in China annually (Jin and Chang, 2011). Application of ADS to soil could enhance the recycling of substrates and reducing environmental pollution (Siddiqui et al., 2009). In addition, benefits of organic amendments in suppression of soil borne diseases are evident from previous studies (Hoitink et al., 1997; Scheuerell and Mahaffe, 2002; Siddiqui et al., 2009). Many reports attributed the control efficacy of plant diseases to microbial populations in amendments (Hadar et al., 2013; Hoitink and Boehm, 1999; Hoitink and Fahy, 1986). Other research demonstrated that the physico-chemical properties and specific components such as organic matter, ammonium, volatile fatty acid, humic and phenolic compounds may protect plants against diseases through direct toxicity toward the pathogen, improved nutritional status or induced systemic resistance (Hoitink et al., 1997; Siddiqui et al., 2009; Zhang et al., 1998). Nevertheless, the beneficial effects are variable and depend on the type of organic substrates and their physico-chemical and biological properties, also on the type of pathosystem and the level of organic matter decomposition (Bonanomi et al., 2010). Therefore, the choice of the method for controlling plant pathogens should be done on a case-by-case basis, taking into account the pathogen physiology and the specific characteristics of source materials (Zmora-Nahum et al., 2008)

ADS contain rich nutrients and diverse microorganisms and were applied either as drench or directly to plants to promote plant growth and control plant-parasitic nematodes (Jothi et al., 2003; Min et al., 2007; Valocka et al., 2000). Although anaerobically digested manure slurry showed a strong correlation to the physico-chemical characteristics of previously-studied suppressive organic amendments (high C/N ratio and pH) (Bernal-Vicente et al., 2008;

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http://dx.doi.org/10.1016/j.scienta.2013.11.004

Table 1
Physico-chemical characteristic of the feedstocks and anaerobically digested slurry ($n = 3$).

	Pig manure	Dairy manure	ADP	ADD
pH	7.1b	7.6b	8.17a	8.10a
Total solid (TS) (%)	4a	3.4a	1.0c	3.3b
Chemical oxygen demand (COD) (mg/L)	38,304a	35,884a	12,160c	21,487b
Total N (mM)	94.5a	79.2b	75.3b	72.7b
NH_4^+-N (mM)	15.6c	18.1c	36.8a	20.3b
NO_3^--N (mM)	0.35c	0.66b	0.53b	0.77a
Total P (mg/L)	328b	489a	71.8c	477a
Total K (mg/L)	187d	344bc	271c	597a
Total volatile fatty acid (VFA) (mM)	173.4b	220.5a	2.6d	4.4c
Acetic acid (mM)	121.8b	165a	2.5c	3.9c
Propanoic acid (mM)	26.9a	30.5a	0.06c	0.2b
n-Butyric acid (mM)	3.4a	3.5a	ND	0.07b
Isobutyric acid (mM)	14.2a	15.2a	ND	0.1b
n-Valeric acid (mM)	4.5a	3.4a	ND	0.05b
Isovaleric acid (mM)	2.6a	2.9a	ND	0.06b
Humic substance (HS) (g/kg)	170.9b	165.5b	197.6a	176.0b

ADP, anaerobically digested pig slurry; ADD, anaerobically digested dairy slurry. "ND" refers to not detectable; the values are the average of the three mixed subsamples, and values with the same letters are not significantly different according to least significant difference (LSD) test ($P < 0.05$).

Cotxarrera et al., 2002; Rose et al., 2003), few investigations were available on potential use of anaerobically digested manure slurry to control plant soil borne diseases.

Chilli pepper (*Capsicum frutescens* L.) is a commercial vegetable and is grown on more than 1.3 million hectares in China. *Phytophthora capsici* is a causative agent of Phytophthora root rot of greenhouse- and field-grown chilli pepper. Chemical control of Phytophthora root rot are either not very effective or imply negative effects on the environment. Biological control of Phytophthora root rot by composts or compost extracts has been developed in recent years (Kim et al., 1997; Ma et al., 2008; Sang et al., 2008). The objective of the study was to investigate efficacy of ADS to suppress Phytophthora root rot in chilli pepper plant. The potential mechanisms involved in biocontrol of plant diseases using ADS produced from pig and dairy manure were examined.

2. Materials and methods

2.1. Preparation of anaerobically digested slurry (ADS)

Pig manure and dairy manure were collected from a livestock farm near Jiangsu Agricultural Academy of Sciences in Nanjing, Jiangsu Province. Feedstocks were obtained in one batch and stored at 4 °C before use. The digested dairy manure taken from a lab-scale reactor operated at 37 °C was used as the inoculum. The content of total solids (TS) was 3% (w/w) and the content of volatile solid (VS) was 78% of TS (w/w). Anaerobic digestion of manure was tested in a 13.2 L continuous stirred tank reactor (CSTR) with a working volume of 10.5 L. The reactor temperature was kept at 37 ± 2 °C by water circulation in the water bath surrounding the reactor. After the biogas reactor ran stably for 100 days, the feedstocks and anaerobically digested slurries (ADS) were collected each day and stored at 0–4 °C. The sampling continued for 1 month. The daily collected samples were mixed every 10 days, resulting 3 mixed samples for physico-chemical and biological analysis. The characteristics of the feedstocks and ADS were listed in Table 1.

2.2. Physico-chemical characteristics of ADS

Total solid was determined by the dry matter method (105 °C for 24 h). A pH meter (PHS-2F, Shanghai Precision & Scientific Instrument Ltd., China) was used to determine the pH of each sample. Chemical oxygen demand (COD) was analyzed using potassium dichromate oxidation method. Total nitrogen (TN) and ammonia nitrogen (NH_4^+-N) were analyzed by the continuous flow analyzer (FIAstar™ 5000 Systems, FOSS, USA). Total phosphorous (TP) was measured by VIS Spectrophotometer (Spectrumlab 725S, Lingguang Ltd., China). Total potassium (TK) was analyzed by flame photometer (FP640, Xingyi Ltd., China). Volatile fatty acids (VFA) were measured using a gas chromatograph (GC-2014, Shimadzu, Japan). Three replicates for each mixed sample were maintained.

The contents of total humic substances (HS) were extracted from freeze dried samples. The freeze dried samples were mixed with 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH at a ratio of 1:25 (w/v). The mixture was kept in boiling water for 30 min and then filtered after cool. The total HS in the extract was determined using $K_2Cr_2O_7$ titration method (Bao, 2000). For the extraction of HS, freeze dried anaerobically digested slurry was mixed with 0.1 M NaOH at a ratio of 1:10 (w/v) under a nitrogen atmosphere, stirred for 24 h and then centrifuged at 7000 rpm for 20 min. The HS was separated from solution by settling at pH 1. The extraction was repeated three times until a yellow extract was obtained. The HS was purified by 0.1 M HCl plus 0.3 M HF, washed with distilled water and finally freeze-dried. The Fourier transform infrared (FT-IR) spectra of HS were recorded on a Nicolet Nexus 870 spectrophotometer on pellets obtained by pressing a mixture of 1 mg of HS sample and 400 mg of dried KBr. The spectra were recorded in the 4000–400 cm^{-1} range at a resolution of 2 cm^{-1}.

2.3. Biological characteristics of ADS

The populations of cultivable bacteria (total, *Bacillus* sp., and fluorescent pseudomonads), fungi and actinomycetes were estimated using a standard dilution-plating procedure on different selective culture media. Total bacteria, fungi and actinomycetes were grown on beef broth peptone medium, Gause's No. 1 medium (soluble starch 2 g, KNO_3 0.1 g, K_2HPO_4 0.05 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, NaCl 0.05 g, $FeSO_4 \cdot 7H_2O$ 1 mg) and Martin medium amended with 0.3 g/L streptomycin, respectively. *Bacillus* sp. was counted on *Bacillus*-selective medium (Turner and Backman, 1991). King's B medium amended with 75 μg/mL chloramphenicol, 75 μg/mL ampicillin and 100 μg/mL cycloheximide was used for estimation of fluorescent pseudomonads. Colonies on each selective medium were counted after an incubation period of 2–4 days at 28 °C. Three replicates for each ADS sample were performed to determine the densities of cultivable microorganisms. Based on morphology (color, shape, type of growth, etc.), each different type of bacterial and fungal colony was isolated and preserved on slant tryptone soya agar (TSA) and potato dextrose agar (PDA). The antifungal activity of each isolated bacteria and fungi was monitored on PDA plates. A mycelia plug of *P. capsci* was placed in the center of the medium and the tested isolated microorganism was inoculated on