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ScienceDirect
Crop Protection 27 (2008) 369–376
Crop Protection
www.elsevier.com/locate/cropro

# Vermicompost enhances performance of plant growth-promoting rhizobacteria in *Cicer arietinum* rhizosphere against *Sclerotium rolfsii*

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Received 30 October 2006; received in revised form 28 June 2007; accepted 4 July 2007

**Abstract**

Collar rot of chickpea (*Cicer arietinum*) is caused by the soil-borne pathogen *Sclerotium rolfsii* and management of this ubiquitous pathogen is not possible through a single approach. An integrated approach was adopted by using vermicompost and an antagonistic strain of *Pseudomonas syringae* (PUR46) possessing plant growth-promoting characteristics. Treatments with vermicompost (10%, 25%, and 50% v/v) and PUR46 alone and in combination reduced seedling mortality in chickpea under glasshouse conditions. The combined effect of 25% vermicompost substitution along with seed bacterization with PUR46 was the most effective treatment, which not only increased the availability and uptake of minerals like P, Mn, and Fe in chickpea seedlings, resulting in an increase in plant growth, but also reduced plant mortality. These effects are correlated with improvement in soil physical conditions and enhanced nutritional factors due to vermicompost substitution as well as plant growth promotion and the antagonistic activity of PUR46 against the pathogen. Dual cultures of PUR46 with the *S. rolfsii* isolate revealed a high degree of antagonism by PUR46 against the pathogen. Performance of PUR46 was enhanced in the presence of 25% vermicompost compared with its application alone and therefore this combination may be a useful tool to manage *S. rolfsii* under field conditions.


*Keywords:* Chickpea; Collar rot; *Pseudomonas syringae*; *Sclerotium rolfsii*; Vermicompost

## 1. Introduction

Collar rot of chickpea (*Cicer arietinum*) is caused by the ubiquitous soil-borne pathogen *Sclerotium rolfsii*. The pathogen is difficult to control because of the production of hardy resistant survival structures called sclerotia (Elad, 1995). Management of this pathogen is not possible by adopting a single approach like cultural practices, fungitoxicants, host plant resistance, or bio-agents. In recent years, efforts were made to manage the pathogen more effectively through integration of disease management practices by a combination of appropriate techniques. Application of soil amendments or specific biocontrol agents can suppress soil-borne pathogens through manipulation of the physicochemical and microbiological environment (Funck Jensen and Lumsden, 1999). Biological control using plant growth-promoting rhizobacteria (PGPR) especially antagonistic *Pseudomonas* spp. appears to be a potential management tool for reducing the severity of several soil-borne plant pathogens. PGPR can affect plant growth directly or indirectly resulting from a sum of partial, favourable effects such as induction of resistance to pathogens (Sarma et al., 2002; Singh et al., 2003), inhibition of pathogens by antimicrobial compounds (Thomashow and Weller, 1995), mineralization of organic matter (Brito Alvarez et al., 1995), and improved nutrient availability for plants (Amara and Dahdoh, 1997). However, biocontrol agents alone may not completely and effectively manage a disease and therefore they should be used as one of the components of the integrated disease management (IDM) strategies. So, use of broad-spectrum antifungal isolates with effective mechanisms of disease suppression for IDM may provide a better alternative to existing control strategies.

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doi:10.1016/j.cropro.2007.07.001

Similarly, organic amendment in soil is recognized as one of the effective methods in the management of soil-borne phytopathogens by changing the soil and rhizosphere environment (Chen et al., 1987). It adversely affects the life cycle of pathogens and enables plants to resist their attack by achieving better vigour and/or altering root physiology. Vermicompost (VC) is a sustainable source of macro- and micro-nutrients, which enlivens the soil through partial substitution of the horticultural container media (Atiyeh et al., 2000). Enhancement in plant growth after substitution of soils or greenhouse container media with conventional composts is attributed to modifications in soil structure, change in water availability, increased availability of macro- and micro-nutrients, stimulation of microbial activity, augmentation of the activities of critical enzymes, or production of plant growth-promoting substances by microorganisms through interactions with earthworms (Marinari et al., 2000). It is therefore possible that VC, in a similar way to compost, can affect plant growth and manage soil-borne plant pathogens by modifying the physicochemical and microbiological characteristics of the plant growth medium beneficially.

Improving plant vigour and maintaining optimal growth conditions can reduce host susceptibility to pathogen attack and the best way to maintain plant health is to manage its nutrient availability. By affecting the growth pattern, anatomy, morphology, and chemical composition in particular, nutritional availability to plants may contribute either to an increase or to a decrease of resistance and/or tolerance to pests and diseases (Perrenoud, 1990; Marschner, 1995). Several earlier studies have shown that micronutrient deficiencies predispose plants to infection by pathogens (Thongbai et al., 1993; Yamazaki and Hoshina, 1995). Phosphorus (P) and zinc (Zn) deficiencies, in particular in the external environment, promote leaking of cell contents such as sugars, amides, and amino acids, which serve as chemotaxis stimuli to pathogenic organisms. Graham and Webb (1991) described resistance in the host–pathogen relationship as the ability of plants to limit the penetration, development, and/or reproduction of invading pathogens. Although both factors are genetically controlled, the environment and thus nutrition of the host plant can modify its expression to a certain extent, especially in moderately susceptible genotypes/cultivars. However, unlike in human nutrition where the effect of nutrition on "health" has gained considerable importance, the implementation of "healthy" nutrition to improve resistance and tolerance of plants is lagging behind its potential. The relationship between nutrient uptake and resistance of chickpea towards *S. rolfsii* has not been elucidated thoroughly. Based on the above facts, the objective of the present investigation was to see the effect of food waste VC substitution in a greenhouse potting mixture and seed bacterization with a PGPR on growth and nutrient uptake by chickpea plants as well as its effect in reducing collar rot of chickpea under greenhouse conditions.

## 2. Materials and methods

### 2.1. Soil, vermicompost (VC), and potting mixture analysis

Sandy loam soils (pH 7.2) were sampled from a depth of 5–15 cm from the agricultural research farm of Banaras Hindu University. The soil was manually treated to remove gravel and stubble debris. After sterilization in an autoclave, the soils were used for the greenhouse experiment. VC was provided by Surbhi Research Center, Varanasi and consisted of vegetable peels and leaf litter processed by earthworms *Eisenia foetida* in indoor beds. VC was thoroughly mixed with sterilized soils at different ratios (10%, 25%, and 50% v/v). Soils without VC served as control. Soils, VC, and each mixture of both were analysed for available nitrogen (N) by the Kjeldahl method (Subbiah and Asija, 1956), P by the ascorbic acid reductant method (Watanabe and Olsen, 1965), potassium (K) with a flame photometer, and sulphur (S) with a spectrophotometer (Chesnin and Yien, 1950). Available diethylene triamine penta-acetic acid (DTPA) extractable Zn, manganese (Mn), and iron (Fe) were determined by atomic absorption spectrophotometry (Lindsay and Norvell, 1978).

### 2.2. Seed bacterization

*Pseudomonas syringae*, strain PUR46, was isolated from the rhizosphere soil of chickpea and selected for this experiment based on its in vitro performance. The strain PUR46 was selected from among 30 bacterial strains for this study, because of its unique ability to inhibit (82%) the growth followed by complete lysis of mycelia of *S. rolfsii* under in vitro conditions. PUR46 also solubilizes P, and produces indole-acetic acid and $NH_3$ (Sahni and Sarma, unpublished data).

The method of Weller and Cook (1983) was followed for seed bacterization; chickpea seeds (cv. Avrodhi) were surface sterilized with 1% NaOCl for 3–5 min, washed in sterilized distilled water (SDW) 3–4 times and air dried. Cells of PUR46 were grown in King's B broth (protease peptone 20 g + $K_2HPO_4 \cdot 3H_2O$ 1.908 g + $MgSO_4 \cdot 7H_2O$ 1.5 g + glycerol 15 mL + distilled water 985 mL) for 24 h at $28 \pm 1$ °C under shaking conditions and finally cells in the exponential phase were centrifuged at 7000 rpm for 15 min at 4 °C. The supernatant was discarded and pellets were washed with SDW and resuspended to obtain a population of $10^7$ cfu $mL^{-1}$. This suspension was mixed with 1% caboxymethyl cellulose (CMC). Surface-sterilized chickpea seeds of uniform size were then bacterized by dipping for 2 h into the bacterial suspension followed by air drying at room temperature under aseptic conditions. Care was taken to avoid clumping of seeds. Seeds coated with only a slurry of CMC without bacteria served as control.

### 2.3. Preparation of inoculum of Sclerotium rolfsii

*S. rolfsii* (isolate DL2) was isolated by picking off sclerotia produced on infected chickpea plants in the