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ORIGINAL ARTICLE

Alleviation of fungicide-induced phytotoxicity in greengram [*Vigna radiata* (L.) Wilczek] using fungicide-tolerant and plant growth promoting *Pseudomonas* strain

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Abstract This study was designed to explore beneficial plant-associated rhizobacteria exhibiting substantial tolerance against fungicide tebuconazole vis-à-vis synthesizing plant growth regulators under fungicide stressed soils and to evaluate further these multifaceted rhizobacteria for protection and growth promotion of greengram [*Vigna radiata* (L.) Wilczek] plants against phytotoxicity of tebuconazole. Tebuconazole-tolerant and plant growth promoting bacterial strain PS1 was isolated from mustard (*Brassica compestris*) rhizosphere and identified as *Pseudomonas aeruginosa* following 16S rRNA gene sequencing. The *P. aeruginosa* strain PS1 solubilized phosphate significantly and produced indole acetic acid, siderophores, exo-polysaccharides, hydrogen cyanide and ammonia even under tebuconazole stress. Generally, tebuconazole at the recommended, two and three times the recommended field rate adversely affected the growth, symbiosis, grain yield and nutrient uptake in greengram in a concentration dependent manner. In contrast, the *P. aeruginosa* strain PS1 along with tebuconazole significantly, increased the growth parameters of the greengram plants. The inoculant strain PS1 increased appreciably root nitrogen, shoot nitrogen, root phosphorus, shoot phosphorus, and seed yield of greengram plants at all tested concentrations of tebuconazole when compared to the uninoculated plants treated with tebuconazole. The results suggested that the *P. aeruginosa* strain PS1, exhibiting novel plant growth regulating physiological features, can be applied as an eco-friendly and plant growth catalyzing bio-inoculant to ameliorate the performance of greengram in fungicide stressed soils.

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1. Introduction

Rhizosphere microorganisms play a key role in biogeochemical cycling of elements and supply plants with the vital nutrients (Ahemad and Khan, 2011a). Bacteria of rhizosphere origin

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improving plant growth are generally, referred to as plant growth promoting rhizobacteria (PGPR) (Zaidi et al., 2009). Among PGPR, phosphate-solubilizing bacteria (PSB) supply phosphorus (P) to plants by solubilizing insoluble P; principally through acidification process (Ahemad and Khan, 2012). In addition, PSB enhance the growth of plants by other mechanisms such as biological nitrogen fixation, providing trace elements (such as iron and zinc), synthesizing key plant growth promoting substances including siderophores and indole acetic acid (Tank and Saraf, 2003) and providing protection to plants against soil borne pathogens (El-Mehalawy, 2009). PSB without tolerance/resistance toward stress factors like fungicides in polluted soils would likely not facilitate the growth and yields of crops efficiently when used as bio-inoculants. To enhance the overall performance of crops in polluted environment including fungicide enriched soils, the bacterial cultures as inoculants must possess the potential to tolerate/detoxify pollutants including fungicide vis-à-vis the normal plant beneficial activities.

Prior to sowing, seed dressing with fungicides is regularly practiced in farming which sometimes fails to protect the seeds/plants against the intended pathogens or even suppress the production of secondary metabolites by plant-beneficial soil microbial communities (Yu et al., 2009; Ahemad and Khan, 2011a,b) besides impeding the overall productive efficiency of various crops including legumes (Fox et al., 2007). Tebuconazole [(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl) pentan-3-ol; CAS No. 107534-96] is a systemic and broad spectrum fungicide belonging to triazole group which is used widely as eradicant, and protectant to counterbalance phyto-pathogenic fungi (e.g. *Curvularia* spp., *Fusarium* spp., etc.) which cause powdery mildew, loose smut, and rust in both legume and non-legume crops (Kishorekumar et al., 2007; Singh and Dureja, 2009; Mohapatra et al., 2010). Mode of action of tebuconazole on fungal pathogens is to hamper the sterol biosynthesis leading to disruption in membrane formation (Tomlin, 1997). Despite its extensive use in pest control, the simultaneous effects of tebuconazole on PSB and greengram (*Vigna radiata* L. wilczek) are scarcely reported. The present study was therefore, directed to evaluate the possible impacts of tebuconazole on plant growth promoting (PGP) potentials of *Pseudomonas aeruginosa* strain PS1. The performance of the tebuconazole tolerant strain PS1 inoculated greengram [*V. radiata* (L.) Wilczek] plants was also assessed in tebuconazole treated alluvial soils.

2. Materials and methods

2.1. Soil samples and microbial diversity

The soil samples were collected from the rhizospheric soils of chickpea (*Cicer arietinum* L.), lentil (*Lens esculentus*), greengram, pea (*Pisum sativum*) and mustard (*Brassica compestris*) grown at the experimental fields of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh (27°29′ latitude and 72°29′ longitude), Uttar Pradesh, India. These agricultural fields were specifically selected to isolate the rhizobacteria because the selected sites were continuously exposed to a wide range of pesticides in crop production since 10 years. From each site, three soil samples were collected in sterilized polythene bags ($15 \times 12\ cm^2$). The samples were mixed well and were used to determine microbial diversity including total bacterial population, fungal population and phosphate solubilizing microorganisms (PSM) using standard microbiological methods (Holt et al., 1994). The soil samples were serially diluted in sterile normal saline solutions and 10 µl of diluted suspension was spread plated on nutrient agar, Martin's medium and Pikovskaya (Pikovskaya, 1948) medium for total bacterial count, fungal populations and phosphate solubilizers, respectively. Each sample was in three replicates and incubated at 28 ± 2 °C for 3, 5 and 7 days for total bacteria, fungi and phosphate solubilizing microorganisms, respectively.

2.2. Isolation of tebuconazole-tolerant and phosphate solubilizing bacteria

A total of 50 PSB were isolated from the rhizosphere of mustard (due to the maximum diversity of PSM) using soil dilution plate technique and tested for their phosphate-solubilizing activity (King, 1932). The sensitivity of bacterial strains against the increasing concentrations (100–3200 µg ml^{-1}; at a dilution interval of 100 µg ml^{-1}) of technical grade tebuconazole (a.i. 100% w/w; Parijat Agrochemicals, New Delhi, India) was evaluated by the plate assay using minimal salt agar medium (g/l: KH_2PO_4 1; K_2HPO_4 1; NH_4NO_3 1; $MgSO_4 \bullet 7H_2O$ 0.2; $CaCl_2 \bullet 2H_2O$ 0.02; $FeSO_4 \bullet 7H2O$ 0.01; agar 15; pH 6.5). Plates were incubated at 30 °C for 7 days. The maximum concentration of tebuconazole supporting bacterial growth was defined as the maximum tolerance level (MTL). Subsequently, 18 bacterial strains: PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS9, PS10, PS12, PS14, PS16, PS17, PS19, PS20, PS21, PS22 and PS23 endowed with the higher MTL (> 400 µg ml^{-1}) against tebuconazole were selected (Fig. 1).

Exponentially grown cultures of the test organisms were inoculated into minimal salt medium treated with 0 (control), 100, 200, and 300 µg l^{-1} of tebuconazole and incubated at 30 °C in rotary shaker (150 g). Growth was determined turbidometrically at different time intervals by measuring optical density (OD) at 540 nm.

2.3. Assay of plant growth promoting traits

Bacterial strains were evaluated for phosphate solubilization, indole-3-acetic acid (IAA), siderophores [salicylic acid (SA) and 2,3-dihydroxy benzoic acid (DHBA)], exo-polysaccharide (EPS), hydrogen cyanide (HCN) and ammonia both in the absence and the presence of tebuconazole 100 (recommended dose), 200 and 300 µg l^{-1}. The various concentrations of tebuconazole used in experiments were corresponding to the field doses. Phytohormone, IAA was quantitatively analyzed by the method of Gordon and Weber (1951), later modified by Brick et al. (1991). The siderophore production by bacterial isolates was determined qualitatively using Chrome azurol S (CAS) agar (Alexander and Zuberer, 1991) as well as quantitatively (Reeves et al., 1983). The EPS and HCN produced by the rhizobacterial strains were determined by the method of Mody et al. (1989) and Bakker and Schipper (1987), respectively. The synthesis of ammonia by the bacterial strains was assayed using peptone water (Dye, 1962). Each individual experiment was repeated three times at different time intervals.

Out of 18 rhizobacterial strains, four bacterial strains (PS1, PS2, PS9 and PS19) producing the PGP substances in the highest amount and concurrently possessing greater values