

## Biological Activity of Phenylpropionic Acid Isolated from a Terrestrial *Streptomyces*

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### Abstract

The strain ANU 6277 was isolated from laterite soil and identified as *Streptomyces* sp. closely related to *Streptomyces albidoflavus* cluster by 16S rRNA analysis. The cultural, morphological and physiological characters of the strain were recorded. The strain exhibited resistance to chloramphenicol, penicillin and streptomycin. It had the ability to produce enzymes such as amylase and chitinase. A bioactive compound was isolated from the strain at stationary phase of culture and identified as 3-phenylpropionic acid (3-PPA) by FT-IR, EI-MS, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectral studies. It exhibited antimicrobial activity against different bacteria like *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Staphylococcus aureus* and some fungi including *Aspergillus flavus*, *A. niger*, *Candida albicans*, *Fusarium oxysporum*, *F. udum* and *Penicillium citrinum*. The antifungal activity of 3-PPA of the strain was evaluated in *in vivo* and *in vitro* conditions against *Fusarium udum* causing wilt disease in pigeon pea. The compound 3-PPA is an effective antifungal agent when compared to tricyclozole (fungicide) to control wilt caused by *F. udum*, but it exhibited less antifungal activity than carbendazim.

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Key words: *Streptomyces* strain ANU 6277, taxonomic studies, 3-phenylpropionic acid, biological activity of 3-PPA

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### Introduction

Actinomycetes are Gram-positive bacteria that are wide spread in nature and play a significant role in the production of bioactive metabolites mainly antimicrobial compounds (Sangler *et al.*, 1993). At least 90% actinomycetes isolated from soil have been reported to be *Streptomyces* spp. (Anderson and Wellington, 2001). Most of these are potentially useful as pharmacologically and agriculturally active agents (Berdy, 2005). Pathogens found to be drug resistant revealed the importance of novel bioactive compounds (Bonjan *et al.*, 2004). Microbial metabolites cause increasing attention as potential plant protection agents because they are expected to overcome the pollution problems caused by the synthetic chemical pesticides. Several novel metabolites of actinomycetes were widely useful for the control of plant diseases, insects and weeds (Li *et al.*, 2003). Phenylpropionic acid is a member of the phenylpropanoid family, com-

prising a wide variety of C<sub>6</sub>-C<sub>3</sub> compounds synthesized by plants from phenylalanine and important in plant physiology and defense mechanisms for the synthesis of flavonoids, insect repellents, UV protectants and signal molecules (Hahlbrock and Scheel, 1989). Phenylpropionic acid is rarely encountered as microbial metabolite. Cremin *et al.* (1994) reported that 3-phenylpropionic acid (3-PPA) is found in ruminal fluid as product of chemical reduction of dietary phenolic monomers by ruminal microorganisms. During the screening of actinomycetes for bioactive compounds, an actinomycete strain was found to be predominant in the random sampling of laterite soils present in different locations of Acharya Nagarjuna University (ANU) campus. The isolate was identified as *Streptomyces* and designated as strain ANU 6277. The strain was deposited at Microbial Type Culture Collection Centre (MTCC), IMTECH, Chandigarh (India) with accession number 6277. Very little is known about the biological activity of 3-PPA and no

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reports were found on its production by actinomycetes. Hence an attempt was made to evaluate biological properties of the bioactive compound from the strain. The present paper define the taxonomy position of the new isolated strain, presents the procedure of the extraction and elucidation of the biological activity of a compound obtained from the strain.

## Experimental

### Materials and Methods

**Strain used.** *Streptomyces* strain ANU 6277 was isolated from laterite soil sample collected at Acharya Nagarjuna University (ANU) campus by dilution plate technique using asparagine-glycerol-salts agar medium supplemented with streptomycin sulphate (10 µg/ml) and amphotericin-B (50 µg/ml). The strain was maintained on yeast extract-malt extract-dextrose (YMD) agar medium at 4°C (Williams and Cross, 1971).

**Phenotypic studies.** Morphological, cultural and physiological characteristics of the strain were performed according to the methods described by Shirling and Gottlieb, (1966). The strain was cultivated on different media including those recommended by International Streptomyces Project (ISP) media and non-ISP media. The media such as tryptone-yeast extract agar (ISP-1), YMD agar (ISP-2), oat meal agar (ISP-3), starch-casein-salts agar (ISP-4), glycerol-asparagine-salts agar (ISP-5), peptone-yeast extract-iron agar (ISP-6), tyrosine agar (ISP-7), nutrient agar and Czapek-Dox agar media were employed for the study of growth characteristics of the strain (Dietz and Thayer, 1980). Utilization of different carbon sources was studied in minimal medium containing those at 1% concentration. The strain grown at 37°C for 5 days on ISP medium 2 was used to study the micro-morphology with scanning electron microscope (SEM) (Yassin *et al.*, 1997). The culture was fixed with glutaraldehyde, and dehydrated with ethanol. The dehydrated samples were dried, mounted on aluminium stubs, and sputter coated with gold-palladium. Finally, they were observed with digital SEM (model JEOL JSM-5600).

**Phylogenetic analysis.** The chromosomal DNA of the strain ANU 6277 was isolated according to the procedure described by Rainey *et al.* (1996). The 16S rRNA gene was amplified with primers 8-27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1500r (5'-AGAAAGGAGGTGATCCAGGC-3'). The amplified DNA fragment was separated on 1% agarose gel, eluted from the gel and purified using Qiaquick gel extraction kit (Qiagen, Germany). The purified PCR product was sequenced with four forward and three reverse primers namely 8-27f (5'-AGAGTTT

GATCCTGGCTCAG-3'), 357f (5'-CTCCTACGGGAGGCAGCAG-'), 704f (5'-TAGCGGTGAAATGCGTAGA-3'), 1114f (5'-GCAACGAGCGCAACC-3'), 685r (5'-TCTACGCATTTACCGCTAC-3'), 1110r (5'-GGGTTGCGCTCGTTG-3') and 1500r (5'-GAAAGGAGGTGATCCAGGC-3'), respectively (*Escherichia coli* numbering system). The rDNA sequence was determined by the dideoxy chain-termination method using the Big-Dye terminator kit using ABI 310 Genetic Analyzer (Applied Biosystems, USA).

The 16S rDNA sequence of the strain ANU 6277 generated in this reaction (1478 bases) was aligned with the 16S rDNA sequence of other closely related *Streptomyces* species retrieved from the GenBank data base. A sequence similarity search was done using GenBank BLASTN (Altschul *et al.*, 1997). Sequences of closely related taxa were retrieved, aligned using Cluster X programme (Thompson *et al.*, 1997) and the alignment was manually corrected. For the neighbour-joining analysis (Saitou and Nei, 1987), the distances between the sequences were calculated using Kimura's two-parameter model (Kimura, 1980). Bootstrap analysis was performed to assess the confidence limits of the branching (Felsenstein, 1985).

**Cultivation of the strain for secondary metabolites production.** Actively growing pure culture of the strain was inoculated into 250 ml Erlenmeyer flasks, each containing 50 ml of seed medium consisting of 0.4% dextrose, 0.4% yeast extract, 1% malt extract and 0.2% calcium carbonate (pH 7.2). The culture was incubated on a rotary shaker (250 rpm) at 28°C for two days. The seed culture (10%) of the strain was transferred into a culture medium (4% dextrose, 0.9% proteose peptone, 0.1% yeast extract, 0.6% calcium carbonate, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.1% MgSO<sub>4</sub>×7H<sub>2</sub>O, 0.01% MnSO<sub>4</sub>×4H<sub>2</sub>O, 0.005% FeSO<sub>4</sub>×7H<sub>2</sub>O (pH 7.2) and incubated at 28°C for 5 days.

**Extraction, purification and identification of active metabolite.** The culture filtrate was collected at the end of five day incubation period and extracted twice with equal volume of ethyl acetate. The solvent extract was evaporated *in vacuo* to dryness. The dark brown residue was obtained and partially purified on silica gel column chromatography (22×5 cm, Silica gel 60, Merck) and eluted with gradient solvent system consisting of ethylacetate: hexane. Active fraction was collected and concentrated. Further purification was carried out in HPLC preparative column (10 mm ×250 mm, 5 µ using hexane: 2-propanol (8:2 v/v). Structure elucidation of pure bioactive compound from the strain was carried out by FT-IR, EI-MS, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectral studies.

**Biological activity testing.** Minimum inhibitory concentrations (MIC) of 3-PPA obtained from the strain against different microorganisms including bacteria and fungi were determined by conventional agar

dilution method (Cappuccino and Sherman, 1999) using nutrient agar for bacteria and Sabouroud's agar medium for fungi. Different concentrations of 3-PPA (0 to 1000 µg/ml) were prepared in dimethyl sulphoxide (DMSO) and assayed against tested organisms. The organisms used in this assay are *Bacillus cereus* MTCC 430, *B. subtilis* MTCC 441, *Escherichia coli* MTCC 40, *Klebsiella pneumonia* MTCC 109, *Proteus vulgaris* MTCC 742, *Pseudomonas aeruginosa* MTCC 424, *P. fluorescens* MTCC 103, *Staphylococcus aureus* MTCC 96, *Aspergillus flavus*, *A. niger*, *Candida albicans* MTCC 183, *Fusarium oxysporum*, *F. udum* MTCC 2204 and *Penicillium citrinum*. The antimicrobial activity was observed after 24–48 h incubation at 37°C for bacteria and 48–72 h incubation at 28°C for fungi. Each experiment was performed in triplicates and proper controls were done. Lowest concentration of compound that showed antimicrobial activity against test organisms was recorded as MIC value (Hwang *et al.*, 2001).

The cytotoxic activity of 3-PPA was tested on U-937 (Human leukemic monocyte lymphoma cell line) cells using MTT assay (Plumb *et al.*, 1989). U-937 cells were obtained from National Centre for Cell Science, Pune (India) and were cultured at 37°C with 5% CO<sub>2</sub> using RPMI-1640 (Himedia<sup>®</sup>, India) media containing fetal bovine serum. U-937 (2 × 10<sup>4</sup> cells per well) were seeded in a 96-well plate containing 100 µl of RPMI medium and incubated for 24 h. The cells were then treated with different concentration of 3-PPA (0–150 µg/ml). After 48 h incubation, 100 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (Sigma Chemicals, USA) was added to each well, and the plates were incubated in a CO<sub>2</sub> incubator at 37°C for 4 h. Thereafter, the supernatant was removed from each well. Then 100 µl DMSO was added to dissolve the colored formazan crystals produced by the MTT. Subsequently, the optical density was measured at 570 nm using an ELISA reader (Molecular Devices Corp., USA).

*In vitro* and *in vivo* antifungal activity of 3-PPA from the strain was studied against *Fusarium udum* MTCC 2204, the causal agent of *Fusarium* wilt in *Cajanus cajan* L. The antifungal efficacy of 3-PPA was also compared with the activity of commercial fungicides such as carbendazim and tricyclozole. Conidial suspension of *F. udum* was prepared using the culture grown on potato dextrose agar for 10 days at 30°C (Hwang *et al.*, 2001). The conidial suspensions were mixed with 3-PPA, carbendazim and tricyclozole to give the concentration of 0, 1, 10, 50, 100, 500 and 1000 µg/ml. After incubation for 4 h at 28°C, conidial germination was microscopically examined in three replicates.

*In vivo* antifungal activity of 3-PPA was evaluated for its ability to suppress *Fusarium* wilt on red gram

plants in a growth chamber. Antifungal substances including 3-PPA, carbendazim and tricyclozole dissolved in water + methanol (95:5) were diluted to give different concentration of 0, 10, 100, 500 and 1000 µg/ml. Seeds of red gram (*Cajanus cajan* L.) were sown in glass beaker (18–14 cm) containing steam sterilized soil drenched with antifungal solution (30 ml). The soil was drenched with conidial suspension (10<sup>5</sup> spores/ml) when the seedlings were three day old (Hwang *et al.*, 2001). Disease severity on plants was rated 15 days after inoculation based on a scale from 0 to 5 as follows: 0 for no visible disease symptoms, 1 for slightly wilted leaves, 2 for 30 to 50% of the entire plant diseased, 3 for 50 to 70% of the entire plant diseased, 4 for 70 to 90% of the entire plant diseased and 5 for a dead plant. Data are the mean of 10 plants per treatment and result of two trials.

## Results and Discussion

Cultural and physiological characteristics of the strain are presented in Table I. The strain showed good growth on ISP-1, ISP-2, ISP-4 and ISP-5 media. Moderate growth was observed on ISP-3, ISP-6, ISP-7 and nutrient agar media. Pigment production by the strain varied with the culture media employed. Dark brown pigment was produced by the strain when grown on ISP-1,2 and 3, while yellowish brown to yellow pigments were found with ISP-4 and 5 and nutrient agar media. Diffused melanoid pigments were observed when grown on ISP-6 and ISP-7. Micromorphology of the strain was examined by SEM (Fig. 1). The culture showed extensively branched aerial mycelium and bear short chains of spores. As the sporogenous hyphae (sporophores) were straight to flexuous in nature bearing the spores with smooth surface, the strain may be placed in the *rectus-flexibilis* group of *Streptomyces* species (Pridham *et al.*, 1958).

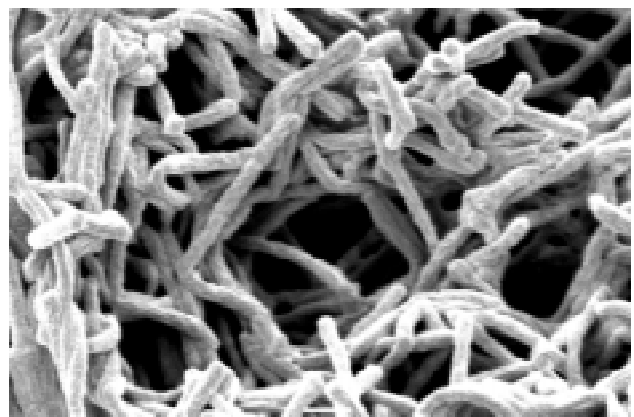


Fig. 1. Scanning electron microscopic photograph of strain ANU 6277 (magnification × 10,000, Bar 1 µm →)

Table I  
Characteristics of strain ANU6277

<b>Hydrolysis of</b>		<b>Pigment production in</b>	
casein	+	ISP-1	+, DB
esculin	+	ISP-2	+, DB
gelatin	+	ISP-3	+, DB
hypoxanthine	–	ISP-4	+, YB
starch	+	ISP-5	+, YB
tyrosine	+	ISP-6	+, M
xanthine	+	ISP-7	+, M
<b>Tolerance to</b>		Nutrient agar medium	+, Y
lysozyme (0.05%)	+	Czapek-Dox	–
NaCl (7%)	+	<b>Utilization of carbon sources (1%)</b>	
phenol (0.1%)	–	D-arabinose	+
<b>Growth at 45°C</b>	–	cellulose	–
<b>Production of</b>		D-glucose	+
melanoid pigments	+	D-fructose	+
H <sub>2</sub> S	+	dextrin	–
amylase	+	D-galactose	+
chitinase	+	glycerol	+
<b>Resistance to antibiotics (µg/disc)</b>		lactose	+
ampicillin (50)	–	mannitol	+
chloramphenicol (50)	+	D-mannose	+
penicillin- G (50)	+	raffinose	+
rifampicin (50)	–	rhamnose	+
streptomycin (100)	+	sucrose	–
tetracycline (100)	–	trehalose	+
		xylose	+

+, Positive result; –, Negative result; DB, Dark Brown; YB, Yellowish Brown; M, Melanin; Y, Yellow

The strain had the ability to hydrolyze casein, esculin, gelatin, starch, tyrosine and xanthine, but not hypoxanthine. The culture could tolerate NaCl levels up to 7%. It produced enzymes such as amylase and chitinase. It exhibited resistance to chloramphenicol, penicillin-G and streptomycin and showed sensitivity to ampicillin, rifampicin and tetracycline. The utilization of various carbon sources by the strain indicated its wide pattern of carbon assimilation potential. D-arabinose, D-fructose, D-glucose, D-galactose, glycerol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, trehalose and xylose supported growth of the strain, whereas cellulose, dextrin and sucrose did not support its growth.

Phylogenetic study of the strain was performed by 16S rRNA analysis. An almost complete 16S rDNA gene nucleotide sequence (1478 bp) of the strain was identified by BLASTN programme and submitted to Genbank with accession number EF 142856. The strain showed high homology (96% identity) with *Streptomyces albidoflavus*. The evolutionary distance was calculated by the Kimura's two parameter model and a phylogenetic tree was constructed using neighbour-joining method (Fig. 2). Based on observations such as white spore mass, yellow to brown pigment production, rectus-flexibilis sporophore, spore with smooth surface, antibiosis against fungi, resistance to penicillin-G and 7% NaCl, hydrolysis of starch and

xanthine and absence of growth at 45°C, the strain seemed to closely resemble *Streptomyces albidoflavus*. These findings are in confirmity with reports of Williams *et al.* (1989), Gurtler *et al.* (1994) and Augustine *et al.* (2004).

The structure of white crystalline compound obtained from the crude extract after purification was elucidated by FT-IR, EI-MS, <sup>1</sup>H NMR and <sup>13</sup>C NMR studies. In the FT-IR spectrum, *V*<sub>max</sub> was obtained at 697.96, 931.96/cm (aromatic, C-H), 1218.19/cm (aromatic, C = C), 1301.93/cm (C-O), 1699.90 (C = O), 2928.47, 3030.43/cm (CH<sub>3</sub>-C-H) and 3390.13 (OH-group broad peak). The compound gave molecular ions in positive mode at *m/z* are 150(50), 104(95), 91(100), 78(40) and 51(35) suggested a molecular weight of 150 from EI-MS analysis. NMR data indicated a hydrogen count of 10 and a carbon count of 9 in CD<sub>3</sub>OD at 300MHz. <sup>1</sup>H NMR showed protons at 2.70δ (t, 2H), 2.90δ (t, 2H), 7.10 to 7.20δ (dd, aromatic-protons) and 11.0δ (broad, s, O-H.). The <sup>13</sup>C NMR spectrum of bioactive compound exhibited peaks at 30.0 (s, C-2), 36.0 (s, C-3), 126.0, 128, 129 and 140 (aromatic carbons) and 180.0 (s, C-1). Based on above data, the bioactive compound was characterized as 3-phenylpropionic acid with molecular formula C<sub>9</sub>H<sub>10</sub>O<sub>2</sub> (Fig. 3).

The bioactive compound, 3-phenylpropionic acid (3-PPA) from strain showed antimicrobial activity

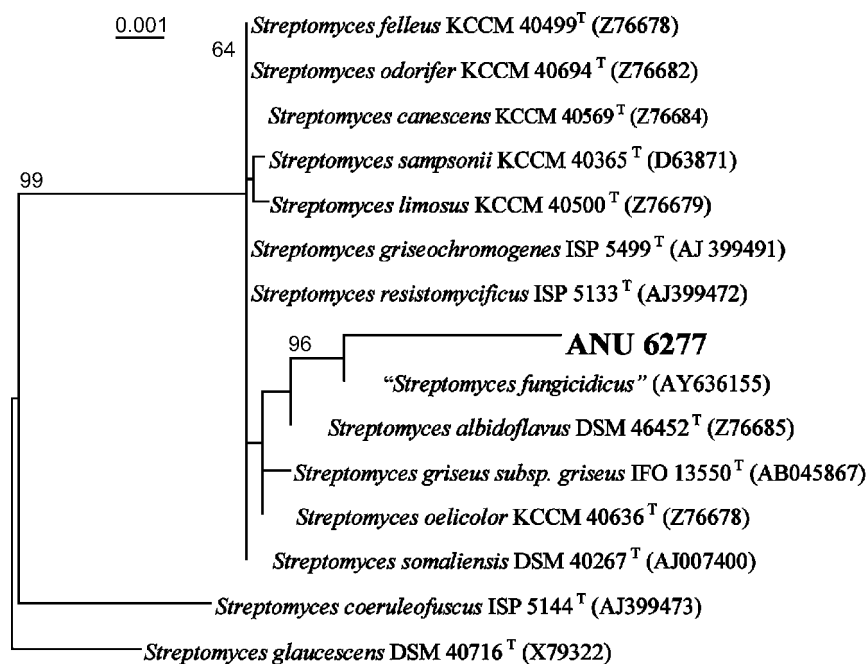


Fig. 2. Neighbour-joining tree based on 16S rDNA sequences showing the phylogenetic relationship between strain ANU 6277 and other closely related species of the genus *Streptomyces*, Bootstrap values (expressed as percentage of 1000 replications) greater than 50% are given at the nodes. The scale indicates 1% sequence variation.

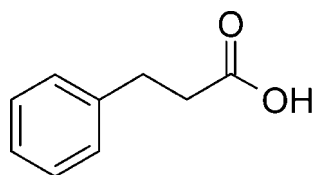


Fig. 3. Molecular structure of 3-phenylpropionic acid

against different test microorganisms including bacteria and fungi. The minimum inhibitory concentration (MIC) of 3-PPA ranged between 10 and 100 µg/ml (Table II). Among the test bacteria, *Pseudomonas*

Table II

Activity of 3-phenylpropionic acid from strain ANU 6277 against different test microorganisms

Tested microorganism	MIC (µg/ml)
<b>Bacteria:</b> <i>Bacillus cereus</i>	75
<i>B. subtilis</i>	50
<i>Escherichia coli</i>	50
<i>Klebsiella pneumoniae</i>	100
<i>Proteus vulgaris</i>	50
<i>Pseudomonas aeruginosa</i>	10
<i>P. fluorescens</i>	10
<i>Staphylococcus aureus</i>	100
<b>Fungi:</b> <i>Aspergillus flavus</i>	25
<i>A. niger</i>	50
<i>Fusarium oxysporum</i>	50
<i>F. udum</i>	10
<i>Penicillium citrinum</i>	25
<i>Candida albicans</i>	100

*aeruginosa* and *P. fluorescens* are highly susceptible to 3-PPA followed by *B. subtilis*, *Escherichia coli* and *Proteus vulgaris*. Among fungi, *F. udum* exhibited high sensitivity followed by *Aspergillus flavus*, *Penicillium citrinum* and *A. niger*. The bioactive compound (3-PPA) from *S. albidoflavus* strain ANU 6277 did not exhibit significant cytotoxicity on U-937 cells up to the concentration of 100 µg/ml. The compound 3-PPA showed inhibitory activity (IC<sub>50</sub>) on U-937 cell growth at 128.20 µg/ml, while the widely used anti-cancer drug, Etoposide (positive control) exhibited cytotoxicity activity (IC<sub>50</sub>) on U-937 cells at 10.26 µg/ml (Table III).

Table III

Cytotoxic activity of 3-phenylpropionic acid on U-937 growth *in vitro*

Compound	IC <sub>50</sub> (µg/ml)
3-PPA	128.20
* Etoposide	10.26

\* Positive control

In *in vitro* conditions, conidial germination of *F. udum* was totally inhibited with carbendazim at 50 µg/ml, with 3-PPA at 100 µg/ml and tricyclozole at 500 µg/ml (Fig. 4). *In vivo* efficacy of 3-PPA, carbendazim and tricyclozole for the control of *Fusarium* wilt was evaluated (Fig. 5). The symptoms of wilt began to appear on red gram plants one week after inoculation. Initial symptoms of the disease consist of

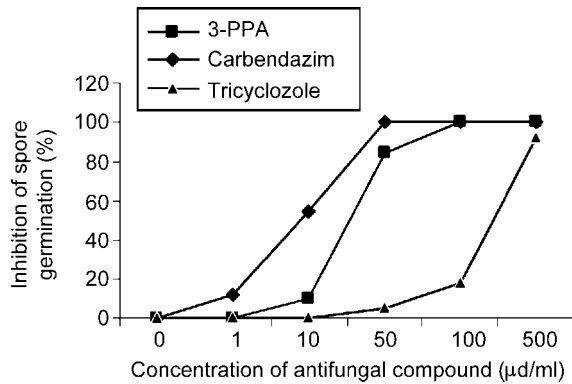


Fig. 4. *In vitro* antifungal activity of 3-PPA from strain ANU 6277 against *F. udum*

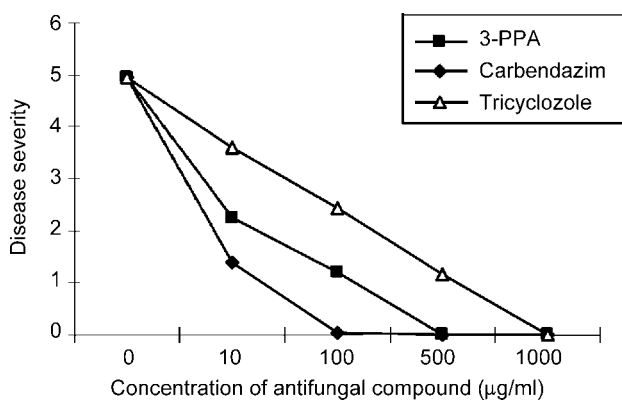


Fig. 5. *In vivo* antifungal activity of 3-PPA from strain ANU 6277 against *F. udum* causing wilt in pigeon pea plants

wilting of individual branches. The foliage symptoms are characterized by drooping of the leaves followed by upland curling. Treatment with the antifungal substances, 3-PPA and carbendazim greatly inhibited the wilt in red gram plants. The suppressive effect of 3-PPA against *Fusarium* was observed at 500 µg/ml. In contrast, the commercial fungicide carbendazim completely inhibited the development of *Fusarium* wilt at the concentration of 100 µg/ml, while the treatment with tricyclozole showed maximum antifungal activity at 1000 µg/ml. The efficacy of 3-PPA against *Fusarium* wilt was better than tricyclozole but less effective than carbendazim.

The bioactive compound, 3-PPA from the strain exhibited less cytotoxicity, while carbendazim the synthetic fungicide showed high toxicity to humans, animals and plants (Mantovani *et al.*, 1998). Soil bacteria such as *Achromobacter*, *Nocardia* and some *Pseudomonas* species could degrade 3-PPA (Fu and Oriol, 1999) indicating its susceptibility to the microbial degradation in soil what results in the lack its accumulatin in nature like synthetic fungicides. Hence 3-PPA can be preferred over carbendazim to control *Fusarium* wilt as an eco-friendly compound.

Among *Streptomyces* spp., *S. albidoflavus* is one of the potential species that elaborate number of industrially and agriculturally important metabolites. Enzymes like chitinase and serine proteinases are reported from *S. albidoflavus* (Broadway *et al.*, 1995; Bressollier *et al.*, 1999). An odoriferous actinomycete, *S. albidoflavus* strain DSM 5415 was reported to produce a new sesquiterpene, albaflavenone (Gurtler and Pedersen, 1994). Antimicrobial properties of a non-polyene antibiotic (poly-hydroxy-poly ether compound) have been reported from *S. albidoflavus* strain PU 23 (Augustine *et al.*, 2005). The bioactive compound dibutyl phthalate was reported from *S. albidoflavus* strain 321.2 (Roy *et al.*, 2006). In the present study, the strain ANU 6277 was found to elaborate a bioactive compound, 3-phenylpropionic acid (3-PPA). This is the first report of 3-PPA from actinomycetes especially *Streptomyces* spp.

Phenyl acids like phenylacetic acid from *Streptomyces humidus* are known to possess antimicrobial activity against several bacteria and fungi (Hwang *et al.*, 2001). Phenylpropionic acid was reported to be detected in culture filtrates from media after inoculation with isolated rumen bacteria or rumen fluid in the absence of added phenolic acids (Chesson *et al.*, 1982). *Clostridium bifermentans* strain TYR-6 reported from oil mill waste waters could convert cinnamic acid to 3-phenylpropionic acid (Chamkha *et al.*, 2001). Phenylpropionic acid derivatives are pharmaceutically important agents. Anti-inflammatory and analgesic drugs like isoprofen, ketoprofen, naproxen *etc.* are phenylpropionic acid based drugs (Saisho and Ishibashi, 1998). Nagano *et al.* (2001) reported pyloricidin, a novel anti-*Helicobacter pylori* antibiotic produced by *Bacillus* sp. Phenylpropionic acid moiety of pyloricidin is essential for anti-*H. pylori* activity. The present paper investigated the extraction, physico-chemical properties and biological activities of 3-PPA from *Streptomyces* strain ANU 6277. The bioactive compound, 3-PPA from strain ANU 6277 is a promising compound as it exhibited antimicrobial activity against gram-positive as well as gram-negative bacteria and fungi. It can also be useful as bio-control agent against *Fusarium* wilt.

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