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Paromomycin Derived from *Streptomyces* sp. AG-P 1441 Induces Resistance against Two Major Pathogens of Chili Pepper

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This is the first report that paromomycin, an antibiotic derived from *Streptomyces* sp. AG-P 1441 (AG-P 1441), controlled *Phytophthora* blight and soft rot diseases caused by *Phytophthora capsici* and *Pectobacterium carotovorum*, respectively, in chili pepper (*Capsicum annum* L.). Chili pepper plants treated with paromomycin by foliar spray or soil drenching 7 days prior to inoculation with *P. capsici* zoospores showed significant ($p < 0.05$) reduction in disease severity (%) when compared with untreated control plants. The disease severity of *Phytophthora* blight was recorded as 8% and 50% for foliar spray and soil drench, respectively, at 1.0 ppm of paromomycin, compared with untreated control, where disease severity was 83% and 100% by foliar spray and soil drench, respectively. A greater reduction of soft rot lesion areas per leaf disk was observed in treated plants using paromomycin (1.0 μg/ml) by infiltration or soil drench in comparison with untreated control plants. Paromomycin treatment did not negatively affect the growth of chili pepper. Furthermore, the treatment slightly promoted growth; this growth was supported by increased chlorophyll content in paromomycin-treated chili pepper plants. Additionally, paromomycin likely induced resistance as confirmed by the expression of pathogenesis-related (*PR*) genes: *PR-1*, β-1,3-glucanase, chitinase, *PR-4*, peroxidase, and *PR-10*, which enhanced plant defense against *P. capsici* in chili pepper. This finding indicates that AG-P 1441 plays a role in pathogen resistance upon the activation of defense genes, by secretion of the plant resistance elicitor, paromomycin.

Keywords: Chili pepper, induced resistance, paromomycin, *Phytophthora* blight, soft rot, *Streptomyces* sp.

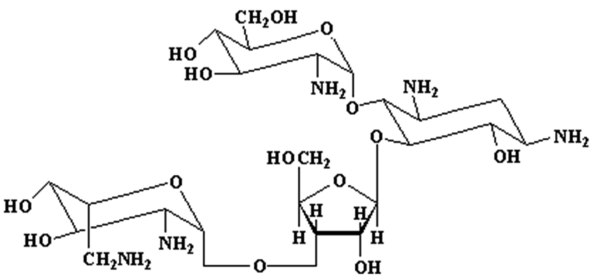
Introduction

Phytophthora capsici is a soil-borne pathogen that causes *Phytophthora* blight in nearly all cultivars of chili pepper (*Capsicum annum* L.). *Phytophthora* blight is one of the most economically destructive diseases in chili pepper production, causing annual losses in regions growing this species worldwide [9, 12]. In general, it is difficult to manage diseases caused by *Phytophthora* spp. because of their aggressiveness and increasing resistance to chemical compounds [8]. *Pectobacterium carotovorum* subsp. *carotovorum*, formerly known as *Erwinia carotovora* subsp. *carotovorum*, mainly affects crops in subtropical and temperate regions [13]. It is the causal agent of bacterial soft rot, a severe disease of many economically important food crops such as potato, tomato, chili pepper, eggplant, and Chinese cabbage [5, 7].

Various chemical fungicides have been used to control root diseases, and the extensive use of chemicals has led to the development of resistant strains of pathogens [24, 34]. The need to reduce pesticide application on food crops and

the concern for environmental pollution require alternative methods for disease control, such as biocontrol agents. The aminoglycoside paromomycin (Fig. 1) was first isolated from the actinobacterium *Streptomyces rimosus* subsp. *paromomycinus* and some of its biological properties have been documented [29]. The aminoglycosides, commonly known as a group of bactericidal antibiotics derived from *Streptomyces* spp., are the most promising biocontrol agents of plant diseases. They are effective owing to secondary metabolite production, and they are also ubiquitous in the rhizosphere. Their ability to exude a variety of fungal cell-wall- and insect exoskeleton-degrading enzymes has been well documented [20, 33]. The antibiotics produced by *Streptomyces* spp. may protect the host plants against phytopathogens [28]. In support of this finding, Xiao *et al*. [34] reported that *Streptomyces* isolates substantially reduced the root rot severity in alfalfa and soybean caused by *Phytophthora* spp. The tissue-cultured seedlings of rhododendron treated with non-antagonistic *Streptomyces* spp. showed minor wilting due to *Pestalotiopsis sydowiana*. The seedlings accumulated anthocyanin(s) and activated defense responses through the phenylpropanoid pathway rather than through antibiosis [25]. To date, most of the studies concerning the biological properties of aminoglycoside antibiotics have focused on the activities against bacteria, yeast, and protozoa [3, 11].

Induced resistance in plants can be developed by the application of a variety of biotic and abiotic agents [32]. Chemical activators, including acibenzolar-*S*-methyl (ASM) and β-aminobutyric acid, are widely reported to induce resistance against a broad spectrum of pathogens in many plant species [32]. The resistance may be systemic-acquired resistance (SAR) or induced systemic resistance (ISR) based on the signaling pathway. For example, ASM was reported to induce SAR against rust in faba beans [26], and resistance



Paromomycin ($C_{23}H_{45}N_5O_{14}$)

Fig. 1. Chemical structure of paromomycin.

to *P. infestans* in squash [14]. Some aminoglycoside antibiotics from *Actinomyces* spp. were found to be selectively active against oomycetes such as *Phytophthora* and *Pythium* species, and paromomycin exhibited the highest activity both in vitro and in vivo [18]. The ability of the aminoglycoside antibiotics to induce systemic resistance against pathogens in chili pepper has not yet been reported. This research gap encouraged us to investigate the efficacy of *Streptomyces* sp. AG-P 1441-derived paromomycin for the disease control of *P. capsici* and *P. carotovorum* in chili pepper through induced resistance.

Materials and Methods

Aminoglycoside Paromomycin from *Streptomyces* sp. AG-P 1441

The aminoglycoside paromomycin, derived from *Streptomyces* sp. AG-P 1441 (AG-P 1441), was kindly provided by Dr. Chang-Jin Kim of the Korean Research Institute of Bioscience and Biotechnology (KRIBB, Daejeon, South Korea). Isolation and purification of paromomycin from AG-P 1441 were performed at KRIBB. The purified paromomycin was stored at room temperature. Paromomycin was dissolved in 1.0 N NaOH before dilution with distilled water at various concentrations (μg/ml).

Isolation and Purification of Paromomycin Compound from *Streptomyces* sp. AMG-P1

Purification of paromomycin from *Streptomyces* sp. AMG-P1 is shown in Fig. S1. The extract of *Streptomyces* sp. was obtained from TSA with 80% methanol using a rotary vacuum evaporator. The residue was subjected to column chromatography (Amberite IRC50, Sigma-Aldrich Co., USA) to obtain 0.5 N NH_4OH and 1 N NH_4OH gradient fractions. The fraction from 1 N NH_4OH was subjected to Amberite CG50 column chromatography (IRC50) to obtain three different fractions on the basis of gradient, of which the active fraction was subjected to Sephadex LH20 column chromatography, eluted with methanol and 1 N NH_4OH at 1:1 ratio to obtain different fractions. On the basis of antibiosis assay, LH41 was found to be an active fraction, and thus the obtained fraction was subjected to TLC for purification. The ^1H NMR spectra of authentic (Sigma Co., USA) and isolated samples of paromomycin were recorded using a Bruker 500 MHz NMR instrument in D_2O (Fig. S2).

Preparation of Spore Suspensions of *P. capsici* and Bacterial Pathogen *P. carotovorum* Inocula

The fungal pathogen *P. capsici* and the bacterial pathogen *P. carotovorum* were obtained from the Korean Agriculture Cultural Collection, National Academy of Agricultural Sciences, Suwon, South Korea. *P. capsici* inoculum was prepared as described by Ploetz *et al*. [21]. In brief, a 5-mm diameter mycelial plug of the isolate was transferred to a V8 agar plate. After 1 week incubation at 25°C, V8 agar plugs with mycelia were placed onto a Petri dish

containing V8 broth and allowed to grow for another week under the same conditions. The V8 broth was then drained, and each plate was washed twice with sterile distilled water (SDW). SDW was added to cover the mycelia on each plate; afterwards, the plates were placed under a wide-spectrum light at room temperature for 24–48 h to induce sporangial development. The sporangia were chilled at 4°C for 45 min to induce the release of zoospores. The zoospore suspensions were adjusted to a final concentration of 1×10^5 zoospores/ml using a hemocytometer before the challenge inoculation. For the preparation of *P. carotovorum* inoculum, the bacterial cell suspensions were prepared from 24-h-old culture at 28°C. Ten milliliters of SDW was poured on a tryptic soy agar culture plate and scraped with a sterile plastic loop and adjusted to a final concentration of 1×10^8 CFU/ml (OD_{600} = 0.8) before application.

Evaluation of Paromomycin for Induced Resistance against *P. capsici* in Chili Pepper under Greenhouse and Field Conditions

Chili pepper cv. Hanbyul seedlings at the first-branch stage were used in this study. The seeds were sown in a plastic tray (55 cm × 35 cm × 15 cm) containing a soilless potting mix (TKS2, Flora Gard Ltd., Germany). Seedlings at the two-leaf stage were transplanted to plastic pots (5 cm × 15 cm × 10 cm) containing the same soil mix. Complex fertilizer was applied to the plants once a week after transplanting. Chili pepper plants were raised in a growth room under light conditions of 16 h/day at 27°C ± 2°C. For the induction of protection from *P. capsici* in chili pepper, the paromomycin was applied by soil drench of 30 ml to each plant and foliar spray at different concentrations (0.1, 1.0, 10, 100, and 1,000 μg/ml) under greenhouse conditions. Each experiment used a randomized complete block design with six replications per treatment. Dimethomorph, a common systemic fungicide at 1.0 μg/ml, and SDW were used as positive and negative controls, respectively. A week later, the treated plants were challenged with 1.0 ml of zoospore suspensions of *P. capsici* (1×10^5 zoospores/ml) near the stem region with the help of a pipette. The plants were transferred to greenhouse conditions after incubation at 27°C for 24 h in a humidity chamber. The percent disease severity of *Phytophthora* blight was recorded at 7 days after the challenge inoculation according to the modified method by Sunwoo *et al*. [27] based on a 0–5 scale, where 0 = no visible disease symptoms; 1 = leaves slightly wilted with brownish lesions beginning to appear on stems; 2 = 30–50% of entire plant diseased; 3 = 50–70% of entire plant diseased; 4 = 70–90% of entire plant diseased; and 5 = completely wilted or plant dead. Another set of 3-week-old paromomycin-treated seedlings was transplanted under field conditions in naturally *P. capsici*-contaminated soil. Plants were treated by soil drench as above, in comparison with the SAR inducer benzo[1,2,3]thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) 0.1 mM or water-treated control. Three weeks later, the plants were observed for disease incidence, and the percent disease severity was recorded as above. The experiments were performed two times with 12 replications (plants) per treatment.

Evaluation of Paromomycin for Inducing Resistance against *P. carotovorum* in Chili Pepper under Greenhouse Conditions

Three-week-old chili pepper seedlings were treated with 100 μl of paromomycin at different concentrations (0.1, 1.0, 10, 100, and 1,000 μg/ml) applied to each of the two second bottom leaves by infiltration with a sterile syringe without a needle. BTH at 0.1 mM and SDW were used as positive and negative controls, respectively. Another set of plants (12) was treated with paromomycin at 1.0 μg/ml by soil drench. A week later, the third or fourth bottom leaves (untreated leaves) from the treated plants were harvested for disease assessment, and leaf disks (8 mm in diameter) were made using a sterile cork borer. The disks (12) were placed in sterile 24-well culture plates containing the *P. carotovorum* pathogen suspensions (1×10^8 CFU/ml). The percent disease lesion area per leaf disk was recorded 24 h after incubation of the plates at 28°C by visual observation. The experiment was performed two times, and each treatment (concentration) consisted of 12 replicates (plants). Twelve discs from 12 plants were used per treatment.

Effect of Paromomycin on Plant Growth Promotion and Chlorophyll Content

The effect of paromomycin treatment on growth promotion of chili pepper plants was evaluated. The purified compound was tested for its plant growth-promoting activity by the soil drench method at various concentrations (1.0, 10, and 100 μg/ml). Three-week-old seedlings were soil drenched with 30 ml of paromomycin per pot. BTH (0.1 mM) and SDW were used as positive and negative controls, respectively. The height of the plants was recorded 40 days after the treatments during two experiments. The estimation of chlorophyll content was determined by the method described by Graan and Ort [10]. The chlorophyll content was determined from the leaves of paromomycin-treated chili pepper plants under greenhouse conditions after the extraction of the pigment with 80% acetone. Fresh chili pepper leaves (1 g) obtained from the field conditions were ground in a small volume of acetone solution. The extract was diluted to a final volume of 4 cm^3. The absorbance was measured at 600 nm by spectrophotometer. Twelve plants were used in each treatment and the experiment was repeated twice.

Molecular Analysis for the Expression of Pathogenesis-Related (*PR*) Defense Genes

To analyze the expression of *PR* defense genes, the third leaves from treated plants were sampled at 12 and 24 h after the challenge inoculation with *P. capsici*, and the leaf tissues were frozen in liquid nitrogen until use. Leaf samples collected from BTH- and SDW-treated plants were used as positive and negative controls, respectively. Defense-related *PR-1*, β-1,3-glucanase, chitinase, *PR-4*, peroxidase, and *PR-10* genes were assessed for expression using the reverse transcriptase (RT)-PCR method. Total RNA was isolated using the easy-spin IIP Total RNA Extraction Kit (iNtRON Biotechnology, South Korea). RT-PCR was performed according to Kishimoto *et al*. [16] with Ex Taq polymerase (Takara Biomedicals,

Table 1. Sequences of gene-specific primers used for RT-PCR analysis.

Gene family	Specific class	Accession number[a]	5′ Primer	3′ Primer
PR-1	PR protein 1	AF053343	5′-TGCAACACTCTGGTGGCCCT-3′	5′-AAGGCCGGTTGGTCTTCGAG-3′
GLU	β-1,3-Glucanase	AF227953	5′-GCTGCCACCCTTCAATGCAA-3′	5′-TGTCACGCGGATTACCAGCA-3′
Chi	Class II chitinase	AF091235	5′-CATTCATAACTGCAGCCAATTC-3′	5′-GTCATCCAGAACCATATTGCTGT-3′
PR-4	PR protein 4	AF244122	5′-GGCGCAGAGTGCTACGAAC-3′	5′-AGTGTCCAATTGGTTAAACACG-3′
PO 1	Peroxidase	AF442386	5′-CTATGGTATTAGGCCAAGGG-3′	5′-CTCACAAGAACGGAATCACGG-3′
PR-10	PR protein 10	AF244121	5′-CTTTACTGACAAGTCCACAGCCT-3′	5′-GCAGAAGCTTCAAATTTGCC-3′
18s rRNA	18s rRNA	EF564281	5′- CGGTCCGCCTATGGTGAGCACCGGTCG-3′	5′-TTCTTGCATTTATGAAAGACGAACAACTGC-3′

[a]http://www.ncbi.nlm.nih.gov.

Japan). The sequences of the gene-specific primer pairs used in this study for chili pepper are listed in Table 1 and were described by Sang *et al*. [23]. The reaction mixture contained 0.1 µg of cDNA, 10 pmol each of the forward and reverse primers, 250 nM dNTPs, and 0.5 U of Ex *Taq* polymerase in 20 µl of buffer solution. PCR was conducted in a MJ Research thermal cycler (PTC-100, USA) under the following conditions: 94°C for 5 min, 25 cycles of 94°C for 1 min and 57°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were separated by electrophoresis on a 1.5% agarose gel in 0.5× Tris-acetate-EDTA buffer at 80 V for 60 min. The experiment was performed two times with three replicates (plants) per treatment.

Statistical Analysis

The data were subjected to analysis of variance using SAS JMP software, SAS Institute, USA. Significant differences in the treatment means were determined using LSD at $p = 0.05$. All of the experiments were performed two times. For each experiment, the data were analyzed separately. The results of one representative experiment are shown.

Results

Effect of Paromomycin Treatment on Chili Pepper Infection by *P. capsici*

The paromomycin treatments reduced *Phytophthora* leaf blight infection by *P. capsici* substantially, under greenhouse conditions, when compared with the water-treated control upon pathogen challenge infection. Among the different modes of applications, foliar spraying was found to be more effective than soil drenching. The greatest reduction of *Phytophthora* blight infection in chili pepper was obtained using foliar spray with paromomycin at 1.0 µg/ml, which resulted in a disease severity of 8% in comparison with water-treated control (83%), whereas the paromomycin treatment at higher dosages (100 and 1,000 µg/ml) resulted in a disease severity of 33% (Table 2 and Fig. 2). The foliar spray treatment with dimethomorph (1.0 µg/ml) suppressed the *P. capsici* infection completely. There were still 33% and 100% disease severities for soil drenching in the positive (dimethomorph) and negative (SDW) controls, respectively (Table 2). The highest reduction of *P. capsici* infection for soil drenching was found to be 50% at 1.0 µg/ml of paromomycin when compared with other concentrations, where there was a minimum level of disease suppression. However, the disease severity (83%) was at the same level at concentrations of 10 and 1,000 µg/ml. Under field conditions, the disease severity was significantly ($p < 0.05$) reduced to 25% compared with the water-treated control (66%) for the soil drench method, whereas the chemical control (BTH) at 0.1 mM also showed a greater disease

Table 2. Suppression of *Phytophthora* blight disease caused by *Phytophthora capsici* in paromomycin-treated red-pepper plants by foliar spray and soil drench under greenhouse conditions.

Treatment	% of disease severity[a]	
	Foliar spray	Soil drench
Control (water)	83.3[a]	100[a]
Dimethomorph 1.0 µg/ml	0.00[c]	33.3[c]
Paromomycin 0.1 µg/ml	33.3[b]	66.6[b]
Paromomycin 1.0 µg/ml	8.3[c]	50.0[b]
Paromomycin 10.0 µg/ml	38.3[b]	83.3[a]
Paromomycin 100.0 µg/ml	33.3[b]	100[a]
Paromomycin 1000.0 µg/ml	33.3[b]	83.3[a]
LSD ($p = 0.05$)	26.4	32.5

[a]Three-week-old plants treated with various concentrations of paromomycin or sterile distilled water (negative control) or 0.1 ppm dimethomorph (positive control) by foliar spray and soil drench were challenge inoculated with zoospore suspensions of *P. capsici* after 7 days of treatment by soil drench. Percent disease severity was recorded 7 days after inoculation. The experiment was repeated at least two times, with 12 replicates per treatment. The values presented in the column followed by the same letter(s) are not significantly different from each other according to least significant difference ($p < 0.05$).

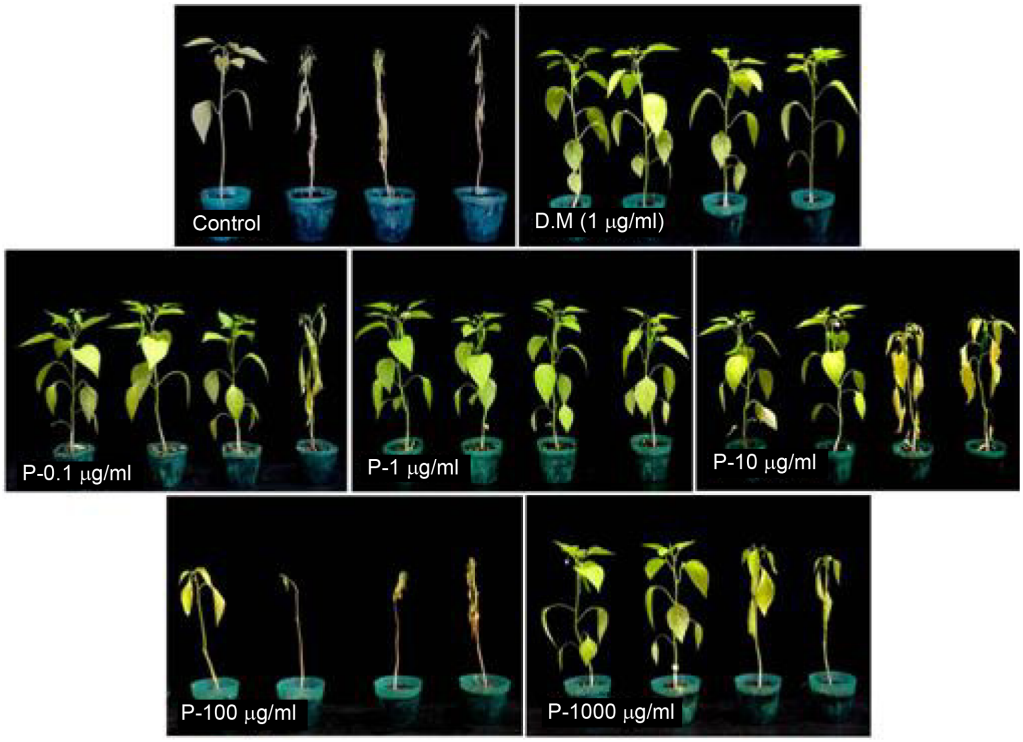


Fig. 2. Induced suppression of disease development in red-pepper plants against *Phytophthora capsici* by soil drench with paromomycin (P) at various concentrations under greenhouse conditions.

Disease severity (%) was recorded 7 days after pathogen challenge with *P. capsici* zoospore suspensions by soil drench. Paromomycin at lower concentration (1.0 μg/ml) induced the suppression of disease development, which is on par with chemical elicitor dimethomorph (D.M) and greater than the higher concentration of paromomycin. Water-treated control plants were completely affected by disease symptoms of *P. capsici.* The experiment was repeated at least two times with 12 replicates per treatment.

reduction (33%) when compared with the water-treated control, but was less effective than the paromomycin treatment (Fig. 3). Thus, our results demonstrate that the paromomycin treatment is beneficial in protecting chili peppers from *Phytophthora* leaf blight.

Effect of Paromomycin on Disease Suppression of *P. carotovorum* in Chili Pepper

Treatments with paromomycin and BTH (positive control) by leaf infiltration significantly ($p < 0.05$) reduced the soft rot incidence of chili pepper plants treated with paromomycin when compared with the water-treated control in a 24-well-plate assay (Fig. 4). There was a greater reduction of lesion area per leaf disk (3.3%) at 1.0 μg/ml of paromomycin when compared with the water-treated control (71.7%) and the chemical inducer BTH (16.6%). However, paromomycin at higher concentrations did not show any significant effect in the reduction of lesion area percentage per leaf disk. Paromomycin treatment by soil drench had a greater effect on the reduction of the lesion area percentage of soft rot per leaf disk (8%) when compared with the water-treated control (75%), whereas BTH (0.1 mM) treatment also caused a considerable reduction in lesion area per leaf disk (16%), and had a similar effect as the *P. capsici* reduction mentioned the above (Fig. 5).

Effect of Paromomycin on Plant Growth and Chlorophyll Content

No considerable effect was noted for the growth of chili pepper plants treated with paromomycin when compared with the water-treated control (Fig. 6). However, the SAR inducer, BTH at 0.1 mM, reduced the plant height. Increased chlorophyll content was observed in paromomycin-treated chili peppers when compared with the BTH- and water-treated controls grown under field conditions for two experiments (Fig. 7).

Effect of Paromomycin on Defense-Related Gene Expression in Chili Pepper Leaves through RT-PCR

To ascertain the changes in SAR-related genes by the

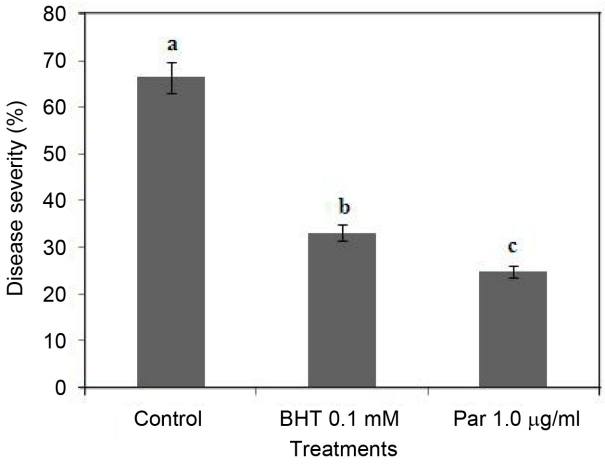


Fig. 3. Disease severity (%) caused by *Phytophthora capsici* in chili pepper seedlings after soil drench with paromomycin in comparison with positive (BTH) and negative (water) controls under field conditions.

The experiment was conducted at least two times with 12 plants per treatment. Bars with the same letters indicate statistically not significant between the treated and control according to the least significant difference test ($p < 0.05$).

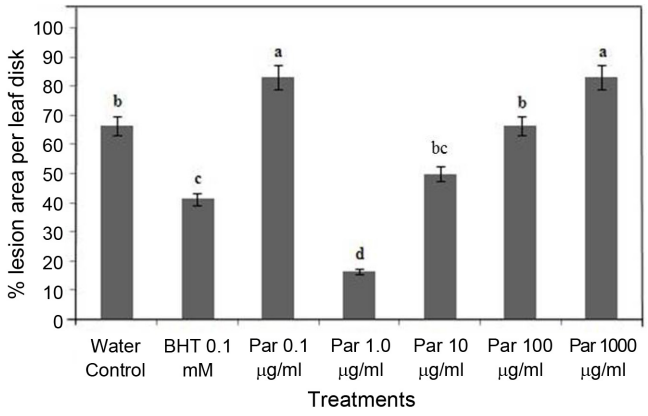


Fig. 4. Percent lesion area per leaf disk caused by *P. carotovorum* SCC1 in paromomycin-treated chili pepper plants by infiltration in comparison with positive (BTH) and negative (water) controls.

The experiment was repeated at least two times with 12 replicates per treatment producing similar results. Bars with the same letters indicate statistically not significant between the treated and control according to the least significant difference test ($p < 0.05$).

paromomycin derived from AG-P 1441, the expression of defense genes in chili pepper plants challenged with *P. capsici* was measured using RT-PCR analysis (Fig. 8). Compared with the treatments without the pathogen, treatments after pathogen challenge generally enhanced

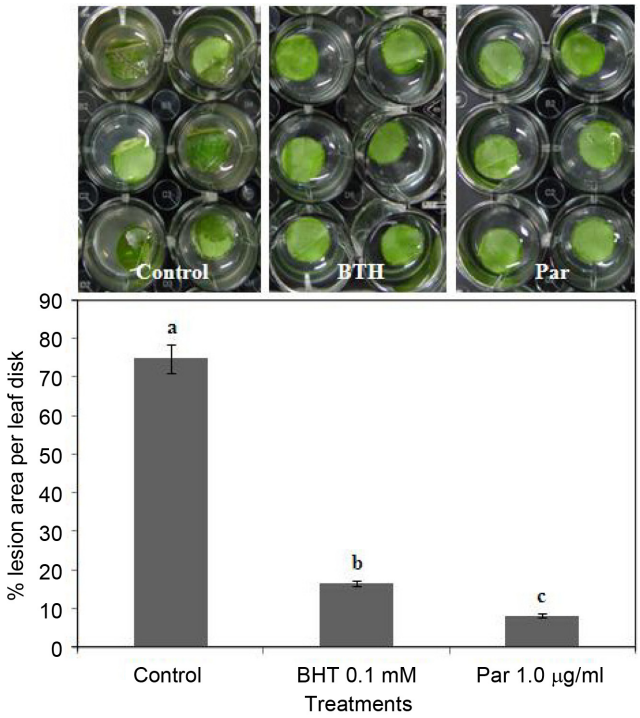


Fig. 5. Percent lesion area per leaf disk caused by *P. carotovorum* in paromomycin (Par)-treated chili pepper plants by soil drench in comparison with positive (BTH) and negative (water) controls.

The experiment was repeated at least two times with 12 replications per treatment. Bars with the same letters indicate statistically not significant between the treated and control, according to the least significant difference test ($p < 0.05$).

the expression of the *PR* genes studied. After 12 h of pathogen challenge, the BTH treatment increased the expression of chitinase, peroxidase, and *PR-10* genes compared with the water-treated control, whereas paromomycin treatment also enhanced the *PR-1*, β-1,3-glucanase, and *PR4* genes. In some cases for paromomycin, the defense gene expression began upon pathogen challenge; for BTH, gene expression was mostly enhanced 24 h following pathogen challenge.

Discussion

This study investigated the potential for paromomycin, an aminoglycoside derived from *Streptomyces* sp. AG-P 1441, in controlling *P. capsici* and *P. carotovorum* infections in chili pepper through induced resistance. The results demonstrated that the AG-P 1441-derived paromomycin tested in this study had effectively suppressed the development of both pathogens on the leaves of chili pepper plants. In support of our study, aminoglycoside

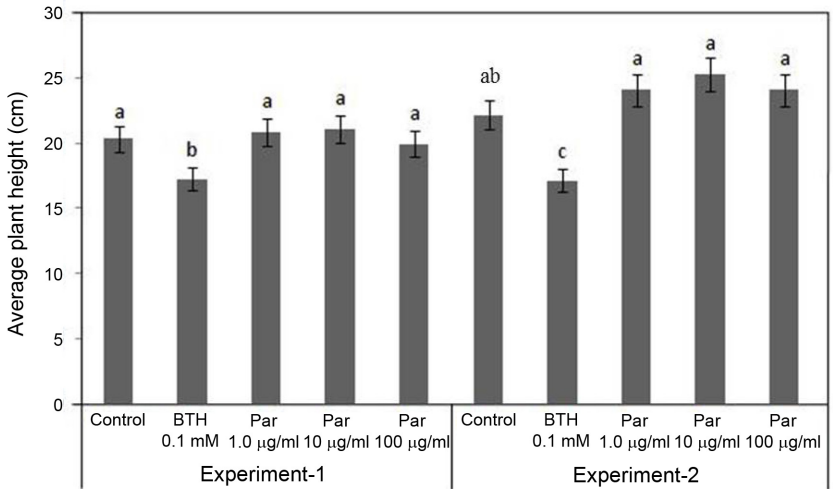


Fig. 6. Height of chili pepper plants 40 days after paromomycin treatment by soil drench in comparison with positive (BTH) and negative (water) controls during two experiments with 12 replicates per treatment.

Bars with the same letters indicate statistically not significant between the treated and control, according to the least significant difference test ($p < 0.05$).

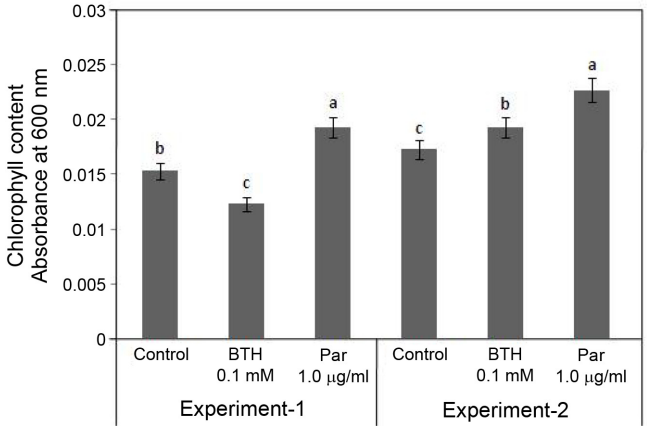


Fig. 7. Absorbance values of an acetone leaf extract of chili pepper seedlings 40 days after paromomycin treatment by soil drench in comparison with positive (BTH) and negative (water) controls during two experiments under greenhouse conditions with 12 plants per treatment.

Bars with the same letters indicate statistically not significant between the treated and control, according to the least significant difference test ($p < 0.05$).

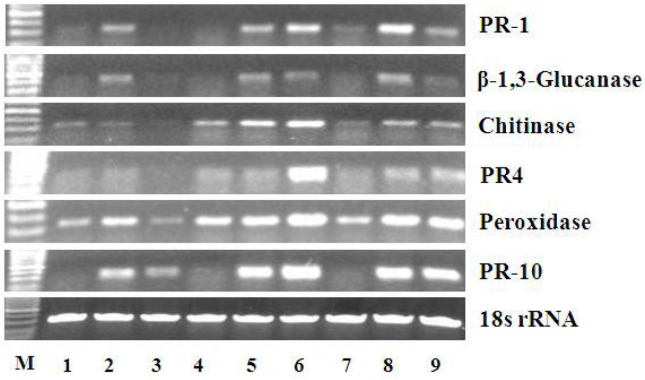


Fig. 8. Gene expression of pathogenesis-related (*PR*) proteins in the leaves of 30-day-old chili pepper plants after paromomycin treatment by soil drench, in comparison with positive (BTH) or negative (SDW) controls, 12 and 24 h after *P. capsici* challenge.

Lanes are identified as follows: M, 100 bp DNA ladder; 1, water; 2, Water + pathogen at 12 h (p12); 3, Water + pathogen at 24 h (p24); 4, BTH; 5, BTH + p12; 6, BTH + p24; 7, Paromomycin; 8, Paromomycin + p12; 9, Paromomycin + p24. 18s rRNA is the internal standard. No expression of *PR* genes was observed in the un-inoculated red pepper plants. The experiment was repeated two times, producing similar results.

antibiotic compounds have been reported to reduce diseases caused by oomycetes, including *Phytophthora infestans* [18, 34]. Commercial aminoglycoside antibiotics such as neomycins, ribostamycin, streptomycin, and paromomycin have been tested against *P. infestans*. It has been reported that paromomycin at 10 μg/ml was the most active against the pathogen under in vitro conditions to reduce mycelial growth [18]. However, in our study, we demonstrated that the antibiotic paromomycin could reduce *P. capsici* and *P. carotovorum* disease symptoms in chili pepper plants under greenhouse conditions. Paromomycin at 125 μg/ml was found to be effective in controlling tomato late blight;

however, at concentrations above 250 μg/ml, there were minor levels of phytotoxicity [18]. In our study, no phytotoxicity was found in paromomycin-treated chili pepper plants. Disease severity was reduced using the lowest concentration of paromomycin, and increased at high concentrations. Foliar spray of paromomycin at 1.0 μg/ml was the most effective in reducing disease incidence. Furthermore, paromomycin is reported to inhibit in vitro growth of oomycete plant pathogens of the genera *Phytophthora* and *Pythium*, and to exhibit potent in vivo activity against chili pepper and tomato late blight [17]. Moreover, it was reported that the minimal inhibitory concentration of paromomycin against *P. capsici* was 500–1,000 μg/ml. On the other hand, our study identified the application of paromomycin at lower concentration (1.0 μg/ml) is an ideal dosage for disease suppression through induced resistance than higher concentrations, suggesting that a dosage-dependency manner is followed.

This study also clearly demonstrated that paromomycin treatment significantly controlled *P. carotovorum*, and there was increased disease suppression in paromomycin-treated chili pepper plants upon pathogen challenge. Paromomycin treatment of the plants resulted in greater suppression of soft rot disease than BTH (positive control) and SDW (negative control). The antibiosis activity of paromomycin against a major group of plant pathogens has been documented in earlier reports [18, 34]. In addition, paromomycin had no effect on plant growth. Instead, the increased chlorophyll content suggests a change in plant metabolism.

Molecular evidence of *PR* gene expression demonstrated that paromomycin soil drenching induced resistance in chili pepper leaves. Specifically, paromomycin at 1.0 μg/ml enhanced the expression of the *PR* genes, *PR-1*, β-1,3-glucanase, peroxidase, and *PR-10* at 12 h after pathogen inoculation, which resulted in induced resistance against *P. capsici* infection in chili pepper plants. Previously, Lee and Hwang [19] demonstrated that the induction of defense-related genes such as *PR-1* was essential for establishing local and systemic acquired resistance in chili pepper plants. Expression of the *PR-1* gene is known to be triggered through a SA-dependent signaling pathway and to be related to SAR [4]. It has been well documented that disease-suppressing rhizobacteria enhance a plant's defense capacity by inducing defense genes against invading pathogens [1, 15]. Conrath *et al*. [6] described the priming mechanism in plant-microbe interactions in vitro that can help plants overcome biotic or abiotic stresses. Priming in beneficial plant-microbe associations has been studied through the interaction of plants with ISR. In most cases, plant-microbe associations induce the defense capacity of the plant against a broad spectrum of pathogens [22]. The elevated levels of *PR* gene expression were related to increased activities of β-1,3-glucanase, chitinase, and peroxidase, which are key enzymes in plant defense. These defensive responses of pepper plants may be more rapid and substantially induced compared with water-treated controls because of the induction of *PR* gene expression in leaves infected by *P. capsici*, as observed in *Arabidopsis* inoculated with *Pseudomonas* [31]. Previous studies [2, 30] have reported that the signaling pathways can be activated by various inducers, such as callose deposition, synthesis of defense enzymes, phytoalexins, accumulation of *PR* proteins, volatile organic compounds, and antimicrobial compounds.

In conclusion, our results suggested that paromomycin treatment enhanced systemic resistance against *P. capsici* by activating defense genes. The aminoglycoside paromomycin derived from *Streptomyces* sp. AG-P 1441 may play a role in protecting chili pepper plants against invading pathogens through induced resistance, and thus might serve as an alternative approach to chemical fungicides.

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