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Induction of potato systemic resistance against the potato virus Y (PVY^{NTN}), using crude filtrates of *Streptomyces* spp. under greenhouse conditions

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Abstract

The potato virus Y (PVY) is considered one of the most important viruses in terms of both economical and biological impact. Potential of using three different *Streptomyces* spp. crude filtrates (SCF) to induce of resistance against tuber necrotic strain of potato virus Y (PVY^{NTN}) in four potato cultivars namely, Spunta, Nicola, Selatar, and Diamant, were evaluated. Foliar spraying of *Streptomyces netropsis* DSM 40093 (SCF7), *S. ambofaciens* (SCF11) and *S. actuosus* (SCF20), applied separately on potato cultivated in pots, showed various inhibitory activities against PVY^{NTN} infection in all the potato cultivars. SCF20 was the most effective one, as expressed by (100%) reduction of apical necrosis symptoms on potato cv. Spunta, Nicola, and Diamant leaves under greenhouse conditions. Application was done 7 days before virus inoculation with PVY^{NTN} (DBVI) and 7 days after inoculation (DAVI). The concentration of PVY^{NTN} in treatments was significantly reduced than in the untreated PVY^{NTN} infected plants as detected by DAS-ELISA (expressed by decreasing in OD value). Expression of PR-1b gene was detected 21 (DAVI) in all SCF11-treated cultivars but was not expressed in healthy and infected control plants. Gas chromatography-mass spectrometry (GC-MS) analysis of *S. actuosus* (SCF20) ethyl acetate extract revealed the majority of fatty acids (FAs) and their derivatives, Citroflex A, Hexanoic acid, anhydride, Hexadecanoic acid, Hexadecanoic acid, ethyl ester, and Diisooctyl phthalate which suggested to act as elicitor molecules for induction of systemic acquired resistance (SAR). Overall, the application of culture filtrates of *Streptomyces* has the potentiality to be used as inducer against PVY^{NTN} infection.

Keywords: Potato, Systemic acquired resistance, PVY^{NTN}, *Streptomyces* spp., PR-1b gene

Background

Potato (*Solanum tuberosum* L.) is known as the fourth largest food crop in terms of fresh production after rice, wheat, and corn, and the world's most widely grown tuber crop. The total potato production in Egypt reached about 5 million tons per year. However, the total exported amount reached about 755 thousands of tons that represent about 13.5% of the total local production (FAO STAT 2018). The potato virus Y (PVY) is an important potato pathogen worldwide, belonging to the family Potyviridae (Singh et al. 2008). PVY^{NTN} is currently considered the most aggressive PVY strains because of its epidemic spread within the potato crop in Europe and other continents from the 1980s onwards (Singh et al. 2008). PVY^{NTN} infection was previously isolated in Egypt by (Mahfouze et al. 2012 and Abdalla et al. 2018). In susceptible potato cultivars, PVY^{NTN} triggers the development of potato tuber necrotic ringspot symptoms, causing a decrease in the quality and quantity of potato production. The virus exists as a complex form of different strains, which induces variety of symptoms in potato leaves and tubers depending on the potato cultivar, the infecting PVY strain(s), the time of infection occurrence, and environmental factors. Most potato cultivars are susceptible to PVY^{NTN} infection and induce a

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diverse of symptoms including severe necrotic ringspots on tubers and necrotic spots (local lesions), wrinkles and mosaic chlorosis on leaves, followed by newly deformed leaves, and finally the plant death (Warren et al. 2005).

The lack of an active defense response could be the main reason of virus spreading to neighboring cells causing disease symptoms, expressed by structural and physiological changes. A variety of passive and active defense mechanisms against plant pathogens including viruses were previously detected. Physiological, histological, and molecular analyses could be used for detection of such plant-pathogen interaction (Whitham et al. 2006 and Nasr-Eldin et al. 2018). Plant-pathogen interaction can set up a complex defense response, but sometimes could be neither rapid nor intensive enough to manage the viral spread replication. Various reactions occurred in induced defense of susceptible and resistant plants indicate that effective resistance is depending on the speed of pathogenesis process (O'Donnell et al. 2003). Plant's natural defense mechanisms, e.g., systemic acquired resistance (SAR), could be induced using biotic or a biotic elicitors in order to control viral disease (Falcioni et al. 2014).

Soil-borne *Streptomyces* spp. had been known as the main producer of antibiotics in the world (El-Naggar et al. 2006). The use of Streptomycetes as potential biological control agent has been explained by the ability of these microorganisms to produce a wide diversity of useful bioactive metabolites (Anderson and Wellington 2001). The culture filtrates of *Streptomyces* spp. were found to contain substances with antiviral activities against tobacco mosaic virus (TMV) and potato virus Y (PVY) (Mohamed and Galal 2005). TMV symptoms in the leaves of *Datura metel* was inhibited by using of cell-free suspension of *Streptomyces rochei* (Mansour et al. 1988). Ningnanmycin extracted from *Strepcomces noursei* var xichangensisn induce plants resistance against TMV (Han et al. 2014). Zhang et al. (2015) isolated a new glycoprotein GP-1 from *Streptomyces* sp. (ZX01), with TMV inhibition rate more than 80% at the concentration of 1 mg ml^{-1}, which exhibited potential applications. Systemic resistance for cucumber mosaic virus (CMV) infection could be induced in plants inoculated with certain *Streptomyces* strains (Shafie et al. 2016). Pathogenesis-related protein 1 (*PR1*) gene expression is a useful molecular marker for the systemic acquired resistance (SAR) response against fungal, bacterial, viral, and viroid diseases, as well as to some chemicals elicitors. The broad occurrence of type PR-1 proteins both in monocotyledons and dicotyledons confirms their important role in plant responses to stress (Dzhavakhiya et al. 2007). The production of PR proteins in the uninfected parts of affected plants can cease further virus infection (Musidlak et al. 2017), and induces resistance to viral replication and movement (Hull 2014). Identification of bioactive compounds in plants and microorganisms using gas chromatography–mass spectrometry (GC–MS) analysis is widely used in biology (Casuga et al. 2016).

The present study aimed to evaluate the efficacy of different *Streptomyces* spp. crude filtrates (SCF) as bio-elicitors against PVY^{NTN} infection under greenhouse conditions. Furthermore, the study aimed to verify SAR marker gene *PR-1b* in four different potato cultivars.

Materials and methods

Plant materials

Virus-free potato tubers cvs Spunta, Nicola, Selatar, and Diamant, used in this study, were kindly provided by the Potato Brown Rot Project, Ministry of Agriculture and Land Reclamation, Egypt.

Source of the viral isolate

Potato virus Y (PVY^{NTN}) isolate, used in this study, was previously isolated from infected potato plants (Mahfouze et al. 2012) and kindly provided by the Virology Laboratory, Agric. Microbiol. Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt. It was propagated and maintained on *Datura metel* L. plants. The PVY^{NTN} inoculum was prepared from infected potato plant leaves by extraction of infectious sap in the presence of 0.1 M potassium phosphate buffer pH 7 containing 0.02 M sodium sulfite (Nasr-Eldin et al. 2018).

Source of *Streptomyces* isolates

Streptomyces netropsis (DSM 40093) (SCF7), *S. ambofaciens* (SCF11), and *S. actuosus* (SCF20) were kindly obtained from Agric. Microbiol. Dept., Fac. of Agric., Ain shams Univ. (Cairo, Egypt) and subcultured and maintained on starch nitrate (SN) agar medium (Waksman and Lechevalier 1961). *Streptomyces* spp. were grown in a liquid medium and incubated on a shaking incubator at the optimum growth conditions (30 °C and 200 rpm for 5 days). The liquid cultures were filtered through two layers of Whatman No. 2 filter paper (0.5 pore size) and the filtrates were also sterilized through the Millipore filters (0.45 μm) (Megahed et al. 2013).

Greenhouse experimental design

Greenhouse experiment was carried out during the winter growing season of 2017 under conditions (25 °C ± 5) at the Faculty of Agriculture, Ain shams Univ. (Cairo, Egypt) for studying the effect of different CSFs on the induction of resistance in potato cultivars against PVY^{NTN}. Potato cvs. Spunta, Nicola, Selatar, and Diamant were cultivated in plastic pots (20 cm in diameter) containing 3 kg of clay soil as one potato tuber/each pot. The experiment was conducted in five treatments (12

replicates/potato cultivar) as follow: (foliar spraying of plants with SCF7 + PVY^{NTN}); (foliar spraying with SCF11 + PVY^{NTN}); (foliar spraying with SCF20 + PVY^{NTN}); (foliar spraying of plants with SN broth medium only + inoculation of viral extraction buffer, healthy control); and (foliar spraying of plants with SN broth medium only +PVY^{NTN}, infected control). The foliar spraying of SCF was applied two times; the first application with SCF was done individually 2 weeks after cultivation, using 5 ml of each SCF (7 days pre virus inoculation, DPVI). After 7 days, potato leaves of the different cultivars were mechanically inoculated by PVY^{NTN} and 7 days after virus inoculation (DAVI), the plants were foliar sprayed for the second time by 5 ml of SCF. All plants were daily observed and development of the symptoms was recorded. All experiment was repeated twice.

Evaluation of SAR

Recording of % of infection and disease severity

The progress of PVY^{NTN} disease was evaluated visually and % of infected plants was recorded relative to time of external symptoms appeared. Disease index was recorded based on a scale proposed by Nasr-Eldin et al. (2018) of 0–10 as follows: 0 = no symptoms; 2 = mild mosaic; 4 = mild necrosis; 6 = mild mosaic with mild necrosis; 8 = severe mosaic with necrosis; 10 = severe apical necrosis. The disease severity (DS) values were calculated 7, 14, and 21 days after viral inoculation DAVI using the following formula according to Yang et al. (1996). Area under disease progress curve (AUDPC) was calculated.

$$\mathrm{DS} = \frac{\sum(\text{disease grade X number of plants in each grade})}{(\text{Total number of plants} \times \text{Xhighest disease grade})}$$

Quantitative detection of PVY^{NTN} concentration by DAS-ELISA

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to detect the PVY^{NTN} infection and to check the virus concentration in potato leaves after 7 and 21 DAVI (Clark and Adams 1977) at the Virology and Phytoplasma Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. DAS-ELISA was repeated in triplicate/each treatment specific for cultivar.

Detection of PR-1b gene by real-time RT-PCR

For studying the gene expression of *PR-1b* gene in potato plants at 7 and 21 DAVI, total RNA was extracted from the collected fresh potato cvs leaves specific for each treatment. Leaves were grinded thoroughly under a liquid nitrogen using a mortar. The formed powder was placed into a separate micro-centrifuge tube and the total RNA extraction was performed by using TRIzol™ Reagent, Invitrogen, USA. The extracted RNA was stored at − 30 °C for further study.

Complementary DNAs (cDNAs) were carried out, using the high capacity cDNA reverse transcription Kits (Applied Biosystems, USA) according to the manufacturer's protocol. All reagents and supplies used in this step, were nuclease-free. Reverse transcription master mix (2×) was prepared. To create a 1× mix reaction, 2 μg of total RNA per 20-μl reaction was added individually to the 2× RT master mix and the reaction volume was set to 20 μl. The reactions were loaded into the thermal cycler (Biomtera, T personal, USA) and thermos cycling condition was programmed as following: step 1 (25 °C for 10 min), step 2 (37 °C for 120 min), step 3 (85 °C for 5 min), and step 4 (4 °C on hold).

The yield concentration of cDNAs were measured in Nanodrop spectrophotometer (Thermo scientific, USA) and stored at − 30 °C for further study. PCR amplifications were performed using real-time PCR (7500, Applied Biosystems) in total volume 25 μl using the above cDNA as templates. In addition to the samples to be analyzed, a negative control reaction without cDNA was included, Reference gene (Cox^{a}) was included for each sample for potato plant (cox gene) (designed with the potato cytochrome oxidase gene sequence) with the following primer sequence: forward, 5-CGT CGCATT CCA GAT TAT CAA-3 and reverse, 5-AA CTACGG ATA TAT AAG AGC CAA AAC TG-3 according to Wilmer et al. (2006). Primers for PR-1b were designed as described by Baebler et al. (2009) were 5-GTATGA ATAATTCCACGTACCATATGTTC-3 (forward primer) and 5-GTGGAAACAAGAAGATGCAATACTTA GT-3 (reverse primer). The reaction mixture components of different genes expression consist of: 12.5 μl of SYBR Green (Applied Biosystems, USA) RT-PCR Master Mix (1×), 1.0 μl of forward primer and reverse primer (10 pM), 8.0 μl of RNAse-free H_2O, and 2.5 μl of cDNA templates. Thermo-cycler program was performed according to Oufir et al. (2008) as follow: 5 min at 94 °C, 40 cycles of (60 s at 95 °C, 60 s at 58 °C, 60 s at 72 °C). The fluorescence data was acquired during the 72 °C extension and the specificity and identity of the RT-PCR products were verified by performing a melt curve analysis: A melting step (dissociation stage) was added that ramps from 72 to 99 °C, raised by 1 °C in each step, and waited for 30 s on the first step and 5 s each step afterward. The standard curve method was used for relative gene expression quantification, and the transcript accumulation of each gene was normalized to Cox Reference gene. Expression level of PR1-b gene was performed at two time interval, 7 and 21 DAVI. The experiment was repeated twice. The results were the mean of three replicate/treatment. Relative expression levels were calculated

as the ratio of expression of each gene against that of the Cox gene. The normalized ΔCT data were used to calculate the relative gene expression fold change using a selected calibrator (reference sample) according to Livak and Schmittgen (2001).

Fold Change (RQ) = $2^{-\Delta\Delta CT}$

Where, ΔΔCt = Δ CT (test sample) – Δ CT (calibrator sample)

Δ CT (test sample) = CT (target of interest) – CT (reference gene in sample) and

Δ CT (calibrator sample) = CT (target in control) – CT (reference gene in control).

During calculation of the fold change in gene expression, the undetectable or low expression values of PR-1b gene in different treatments (Ct values were above 30 or undetermined) were excluded from analysis. As the gene was never expressed in PVY^{NTN} infected plants, therefore the Δ CT value of the PVY^{NTN} infected potato plants was equal to the value of ΔΔCt during calculation of fold change.

GC-MS fractionation of SCF20 ethyl acetate extract

The components of *S. actuosus* (SCF20) crude filtrate were extracted, using ethyl acetate as a solvent. The solvent was added to the filtrate in the ratio of 1:1 (*v*/*v*) and was shaken vigorously for 20 min. The ethyl acetate phase that contained the secondary metabolites was separated from the aqueous phase using separating funnel. Ethyl acetate layer was concentrated by evaporating to dryness at 50 °C using a rotary evaporator and the residue was injected through gas chromatography-mass spectroscopy (GC-MS) analysis (Ahmed 2007). GC-MS analysis was performed to identify the chemical compounds found in the crude extract of *S. actuosus.* Thermo scientific technologies Trace 1310 with capillary column TG-5 (30 m × 250 μm × 0.25 μm) system were used and the test was performed at the Regional Center For Mycology and Biotechnology, AL Azhar University, Cairo, Egypt. Mass detector used in split mode, and helium gas with flow rate of 1.5 ml/min was used as a carrier. Injector was operated at 230 °C and oven temperature for initial setup was 60 °C for 2 min, ramp 10/min to 300 °C for 8 min. Mass spectra were taken at 70 eV, during running time 45 min.

Statistical analysis

Post-hoc analysis (Dunnett's test) for one-way independent ANOVA (SPSS 23) was employed to test the significant difference in AUDPC between PVY infected control and each of the different treatments for separately. Post-hoc analysis (Bonferroni test) was conducted to test the significant difference in AUDPC among different potato verities. Linear contrast (one-way ANOVA) analysis was conducted to test the difference in virus concentration for different treatments after 7 and 21 DAVI for each potato cultivar separately.

Results and Discussion

In the present study, the potential activity of three different crude filtrates of *Streptomyces* spp. as bio-inducers (SCF) was assayed against PVY^{NTN} infection for their ability to enhance the SAR in four potato cultivars against the virus infection under greenhouse conditions. The evaluation of their activity was based on the disease severity, virus incidence in potato leaves, and the expression of pathogenesis related protein encoded for proteases (PR 1 gene).

Effect of different SCF on PVY^{NTN} infection and disease severity

The foliar-treated potato plants of the different cultivars with SCF exhibited a significant reduction in disease symptoms compared to non-treated PVY^{NTN}-infected control plants. Plants were observed for symptom development and severity after 7, 14, and 21 DAVI. The results demonstrated in Fig. 1 showed that foliar spraying of SCF20 was the most effective treatment, expressed by (100%) reduction of apical necrosis symptoms on potato plants cvs. Spunta, Nicola, and Diamant leaves when applied twice at 7 days before PVY^{NTN} inoculation and 7 DAVI. Spunta, Nicola, and Diamant were asymptomatic 21 DAVI, compared to PVY-infected control plants which showed severe symptoms of mosaic with deformed leaves and apical necrosis. Obtained results are in accordance with the results of Hewedy et al. (2008) who used *S. chibaensis* with Banana bunchy top virus infection and had a significant reduction effect when applied 10 days prior viral inoculation. In other case, culture filtrate of *S. albovianaceaus* and *S. sparasogenes* reduced the symptoms of Zucchini yellow mosaic virus in *Cucumis sativus* as 95–100% (Ghaly et al. 2005). This was also clear for other different viruses and crops such as application of the Streptomyces spp. on cucumber plants before CMV inoculation (Shafie et al. 2016). In the present study, the application of SCF11 showed also a significant reduction of 78.4% ($P < 0.01$) in DS for potato cvs. Spunta and Nicola, while it reduced the disease severity by 65 and 45% ($P < 0.01$) for potato cvs. Selatar and Diamant, respectively for the same treatment compared to PVY-infected control plants. The foliar spraying with SCF7 resulted in (100%) reduction in DS only in potato cv. Nicola-treated plants ($P < 0.01$) (no apical necrosis symptoms were detected), but it reduced the disease severity by 70% ($P < 0.01$), 13.34% ($P < 0.01$), and 7% (insignificant) in potato cvs. Selatar, Diamant, and Spunta respectively. The foliar applications with different SCF on potato cvs. exhibited more virus inhibition

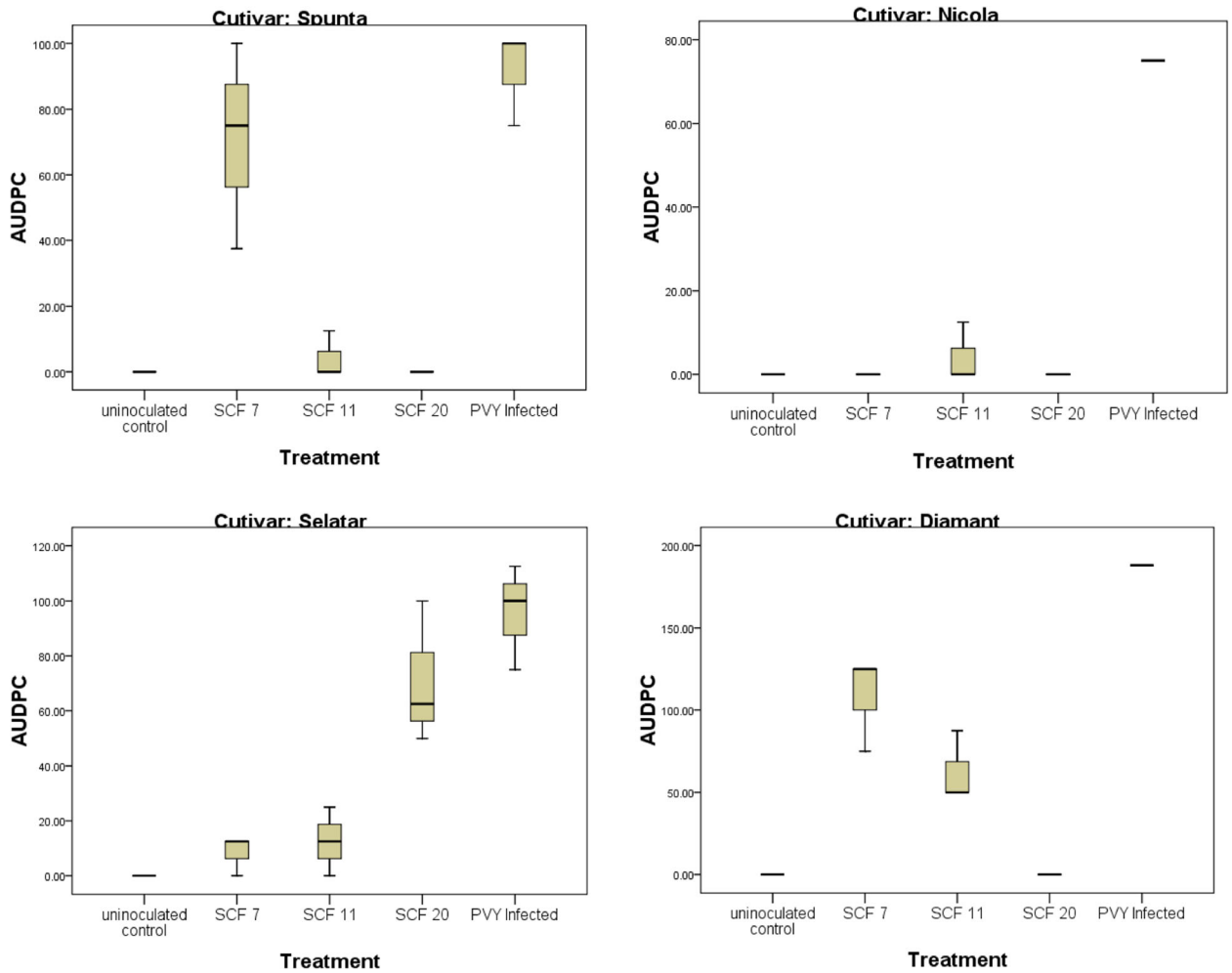

Fig. 1 Influence of foliar spraying of SCF7, SCF11, and SCF20 on AUDPC of PVY^{NTN} in four potato cvs. The actinomycetes' filtrates were applied before and post virus inoculation. Variation in data shown by boxplot histogram (SPSS17). *Streptomyces netropsis* DSM 40093 (SCF7), *Streptomyces ambofaciens* (SCF11), and *Streptomyces actuosus* (SCF20). The disease was most severe for Diamant, less severe for Selatar, Spunta, and least for Nicola. *AUDPC* area under disease progress curve

activity when applied two-times (once before virus inoculation and the other one was after virus inoculation).

Effect of SCF on PVY^{NTN} concentration

The detection of the virus concentration in potato leaves for different cvs. by DAS-ELISA indicated a significant suppressive effect of all SCF against PVY^{NTN} as indicated by decreasing viral concentrations in the newly grown potato leaves as compared to untreated control. Twenty-one DAVI, the PVY^{NTN} systemic concentration in potato leaves was significantly reduced in all potato cultivars; foliar sprayed with SCF than in the control plants (Fig. 2). In general, all asymptomatic potato plants treated with different crude filtrates showed no PVY incidence in potato leaves detected by DAS-ELISA assay. A significant difference was detected between the treated and untreated potato plants (control). The presence of antiviral substances in the extract of *Streptomyces* spp. hypothesizes to play a major role in SAR. In this context, the metabolite of *S. olivaceus* 21 was found effective in controlling the CMV infection using spraying application method (Petrov et al. 2015; Latake and Borkar 2017).

Expression of pathogenesis-related gene (PR-1b) after SCF application

Induction of PRs has been found in many plant species belonging to various families, suggestive of a key role for these proteins in adaptation to biotic stress conditions and responsible for the state of SAR (Kombrink and Somssich 1997). Some of PR genes, such as PR1, PR2, and PR5, serve as robust markers for this systemic immune response (Elhalag et al. 2016). The expression of pathogenesis-related protein encoded for proteases (PR

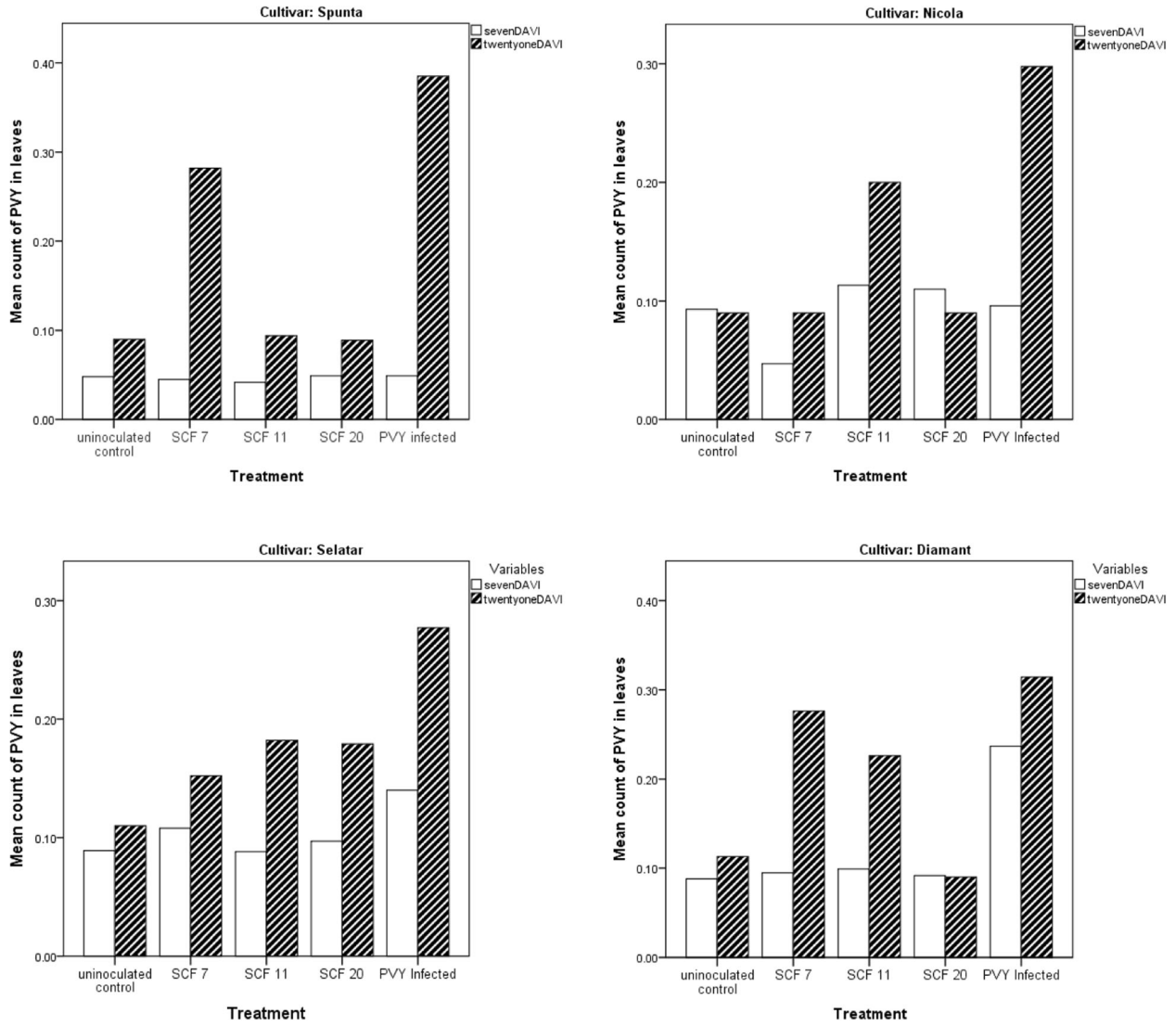

Fig. 2 The effect of different SCF on PVY^{NTN} concentrations in leaves of four potato cultivars using DAS- ELISA treated with *SCF* (SCF7, SCF11, and SCF20) at 7 and 21 days after virus inoculation (DAVI). SCF7 = *Streptomyces netropsis* DSM 40093, SCF11 = *Streptomyces ambofaciens*, and SCF20 = *Streptomyces actuosus*. Columns represent mean of OD values at 405 nm of PVY^{NTN} level using ELISA

1 gene) was employed in this study as a molecular marker for SAR. The gene expression of PR-1b encoded plant pathogenesis-related protein was undetected in all treated potato cultivars after 14 days of first application of different SCF including, *S. netropsis* (DSM 40093, SCF7), *S. ambofaciens* (SCF11), and *S. actuosus* (SCF20) separately (7 DAVI). While, after 14 days from the second application with all strains of *Streptomyces* (21 DAVI), all potato cultivar showed different gene expression of PR-1b gene compared to untreated potato plants (PVY^{NTN}-infected control) (Fig. 3 and Table 1). The gene was never expressed in all untreated potato plants (PVY^{NTN}-infected control) and also in all non-PVY^{NTN} infected (healthy control). The induction of such gene expression has protected potato plants from viral systemic infection as previously recorded by Elmorsi et al. (2015). In the present study, foliar application of SCF7 in Spunta and Diamant cultivars did not showed expression of PR-1b, while the gene was expressed by the other two cultivars (Nicola and Selatar). For SCF11, foliar spraying induced the expression of PR-1b gene in all the treated potato cultivars than in the control. While, SCF20 enhanced the expression of PR-1b gene in all the treated potato cultivars except for the Selatar cultivar. Induction of pathogenesis-related gene 1 (PR-1) by acibenzolar-s-methyl application in pineapple showed that PR-1 induction was initiated as early as 1 day after acibenzolar application and continued through 3 weeks thereafter (Chinnasri et al. 2016). In this investigation, the highest fold change in the expression of PR-1b gene,

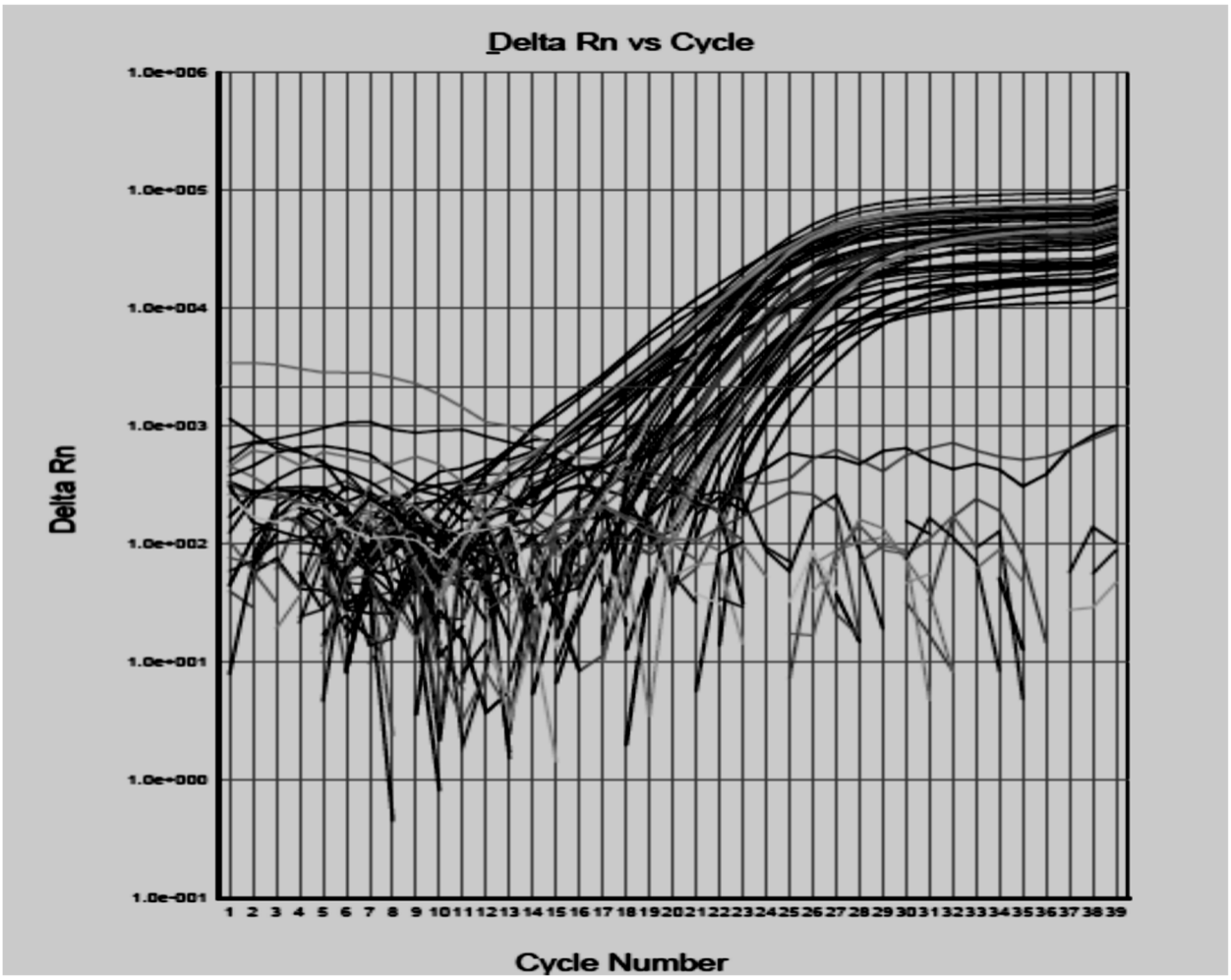

Fig. 3 Real-time PCR output, showing different expression level of PR-1b gene after application of different SCF at 21 Days after virus inoculation (DAVI) against PVY^{NTN} infection. Data is the amplification plot detected by real-time PCR output 7500(Applied Biosystems). The PCR was run for 40 cycles. CT value is the PCR cycle number at which the curve intersects the threshold (horizontal line), where fluorescence meets the threshold in the amplification plot. Undetected was considered negative result, cycle threshold Ct > 30 considered negative while, Ct < 30 considered positive result. Data were collected from the mean values of Ct of two replicated run by real time PCR and the whole experiment was conducted twice

Table 1 Expression of PR1-b gene in different potato cultivars after application of different SCF under greenhouse conditions.

Treatment	Spunta (CT value)				Nicola (CT value)				Selatar (CT value)				Diamant (CT value)			
	PR1	Cox	ΔΔCT	RQ	PR1	Cox	ΔΔCT	RQ	PR1	Cox	ΔΔCT	RQ	PR1	Cox	ΔΔCT	RQ
SCF7	Und.	20.0	Und.	Und.	16.5	20.2	− 3.7	12.9	18.0	22.0	− 4.0	16.0	Und.	19.9	Und.	Und.
SCF11	17.7	20.0	− 2.3	4.9	16.3	23.1	− 6.8	11.4	17.4	21.4	− 4.0	16.0	23.2	23.6	− 0.4	1.3
SCF20	18.0	20.0	− 2.0	4.0	19.1	22.0	− 2.9	7.4	Und.	21.8	Und.	Und.	20.0	21.4	− 1.4	2.6
Healthy (Non-PVY infected)	Und.	20.0	Und.	Und.	Und.	22.0	Und.	Und.	Und.	20.9	Und.	Und.	Und.	20.7	Und.	Und.
PVY Infected	Und.	21.0	Und.	Und.	Und.	19.6	Und.	Und.	Und.	22.0	Und.	Und.	Und.	22.0	Und.	Und.

Represent the expression level of PR1-b gene as detected at two time intervals; 7 days after virus inoculation (DAVI) (not expressed) and 21 DAVI (current table)
The foliar spraying of potato cultivars using different Streptomyces crude filtrates at 7 DBVI and also 7 DAVI
Represent culture filtrates of *Streptomyces netropsis* DSM 40093 (SCF7), *Streptomyces ambofaciens* (SCF11), and *Streptomyces actuosus* (SCF20)
CT value is the PCR cycle number at which the curve intersects the threshold (horizontal line), where fluorescence meets the threshold in the amplification plot. Undetected was considered negative result, cycle threshold Ct > 30 considered negative while, Ct < 30 considered positive result. PR1: gene encoding for pathogenesis related protein. Cox: encoding for cytochrome oxidase of potato (reference gene). Und: undetected. Data were collected from the mean values of Ct of two replicated run by real time PCR and the whole experiment was conducted twice.

detected in all the potato cultivars, was recorded by SCF11, followed by SCF20, and SCF7 at 21 DAVI. Where, SCF11 induced PR-1b gene in all potato cultivars, while SCF20 and SCF7 only induced PR-1b gene in three and two potato cultivars respectively (Table 1). These results explained the effect of different SCF to reduce the incidence and severity of virus infection PVY^{NTN}. The expression of PR proteins and activation of phenylpropanoid pathway leads to the synthesis of higher phenolic compounds (Behuvaneshwari et al. 2015). Phenolics also have anti-viral characteristics (Kamboj et al. 2012). Phenolic acids are involved in phytoalexin accumulation, biosynthesis of lignin, and formation of structural barriers, which play a major role in resistance against the viruses (Sudhakar et al. 2007). According to the obtained results of our study, the difference in viral inhibition percentages with different treatments applied in this study might be accounted for the difference in potato cultivars resistance against PVY^{NTN} and also to the inductive effect of different secondary metabolites present in *Streptomyces* culture filtrate (Ghaly et al. 2005; Shafie et al. 2016). The most effective crude filtrate of SCF20 against PVY^{NTN} infection was analyzed in this study for its metabolite components using GC-MS.

GC-MS fractionation of the crude extract of SCF20 component

According to the result of SAR against PVY ^{NTN} infection of potato cultivars, the SCF20 was found the most effective one for inhibition of PVY^{NTN}. These effects might be related to a specific component found in SCF20. Crude ethyl acetate extract of S. *actuosus*, the most effective strain for viral inhibition, was analyzed to identify the bioactive constituents. GC-MS fractionation revealed 25 chemical compounds with different molecular weight including; long-chain fatty compounds, the alcohols 1-eicosanol, 1-docosanol, and Behenic alcohol (Fig. 4). While in a previous study, 21 metabolites were extracted from *S. olivaceus* culture filtrates and were effective in controlling the CMV infection under glasshouse conditions using the spraying application method (Latake and Borkar 2017). In the present study, the most abundant compounds were Citroflex A (23.8%), 1-Docosanol (CAS) (14.96%), and Hexanoic acid, anhydride (9.9%) followed by 1-Eicosanol (CAS) (6.81%), Hexadecanoic acid (CAS) (6.2%), Diisooctyl phthalate (5.43%), and Hexadecanoic acid, ethyl ester (4.95%) (Table 2). The rest of the compounds showed neglected ratios (less than 5% each). Long-chain fatty compounds and unsaturated fatty acids such as linoleic and oleic acids, detected in this study, were suggested to participate in defense to modulate basal, effector-triggered, and systemic immunity in plants (Kachroo and Kachroo 2009). Other antiviral substances such as furan-2-yl acetate which suppress the replication of the viral were suggested by Suthindhiran et al. (2011) and Fattiviracin A1 which was found to inactivate the viral entry to the host cell (Yokomizo et al. 1998) as well as many antiviral substances (Han et al. 2015). Hexanoic acid was also detected in the filtrates of *Streptomyces actuosus* (SCF20) with a high abundance, and this acid is known to have a direct antifungal effect and later has been confirmed to also induce resistance against *Botrytis cinerea* and

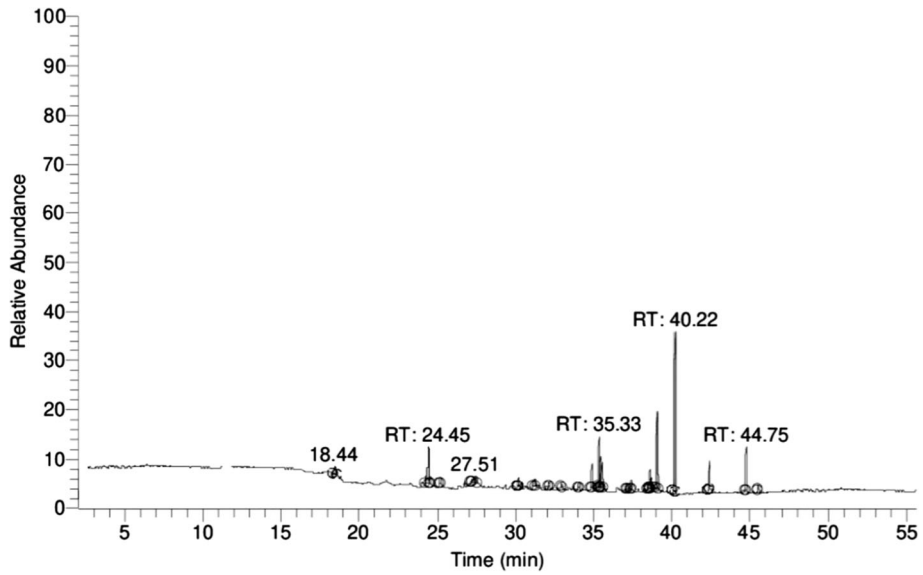

Fig. 4 Histogram showing GC–MS fractionation of ethyl acetate extract of *S. actuosus* (SCF20), (the most suppressive actinomycetes for PVY^{NTN} infection)

Table 2 Compounds identified by GC-MS fractionation for *S. actuosus* (SCF20)

Peak	Retention time (min)	Area %	Detected compounds	Chemical formula	Molecular weight (g/mol)
1	18.44	2.00	1-Deoxy-d-mannitol	$C_6H1_4O_5$	166
2	*24.45*	*9.90*	*Hexanoic acid, anhydride*	$C_{12}H_{22}O_3$	*214*
3	24.57	1.16	ë-Nonalactone	$C_9H_{16}O_2$	156
4	25.19	1.02	2-Acetylamino-3-hydroxy-propionic acid	$C_5H_9NO_4$	147
5	27.21	0.36	Deoxyspergualin	$C_{17}H_{37}N_7O_3$	387
6	27.51	3.17	L-Isoleucine, N-acetyl-	$C_8H_{15}NO_3$	173
7	30.19	1.21	Chlorozotocin	$C_9H_{16}ClN_3O_7$	313
8	31.24	4.86	Dodecanoic acid (CAS)	$C_{12}H_{24}O_2$	200
9	32.15	0.33	Perhydroindene-4- carboxylic acid	$C_{18}H_{22}O_8$	366
10	32.95	0.24	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376
11	34.05	0.38	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	$C_{19}H_{34}O_6$	358
12	34.88	2.71	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	$C_{22}H_{34}O4$	362
13	*35.33*	*6.81*	*1-Eicosanol (CAS)*	$C_{20}H_{42}O$	*298*
14	*35.40*	*4.95*	*Hexadecanoic acid, ethyl ester*	$C_{18}H_{36}O_2$	*284*
15	37.12	0.45	Epibuphanamine	$C_{17}H_{19}NO_4$	301
16	37.40	0.78	Methyl 9,9-dideutero-octadec anoate	$C_{19}H_{36}D_2O_2$	298
17	*37.56*	*6.20*	*Hexadecanoic acid (CAS)*	$C_{16}H_{32}O_2$	*256*
18	38.48	0.42	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308
19	38.59	2.94	Ethyl Oleate	$C_{20}H_{38}O_2$	310
20	38.70	2.06	Oleic Acid	$C_{18}H_{34}O_2$	282
21	*39.06*	*14.96*	*1-Docosanol (CAS)*	$C_{22}H_{46}O$	*326*
22	*40.22*	*23.80*	*Citroflex A*	$C_{20}H_{34}O_8$	*402*
23	42.41	3.41	Behenic alcohol	$C_{22}H_{46}O$	326
24	45.52	0.43	Lucenin 2	$C_{27}H_{30}O_{16}$	610
25	*44.75*	*5.43*	*Diisooctyl phthalate*	$C_{24}H_{38O4}$	*390*

Shaded rows with italic font represent compounds with high abundance among other detected compounds (high peaks)

Pseudomonas syringae in tomato plants (Scalschi et al. 2013; Alexandersson et al. 2016).

Several other antiviral substances were previously extracted and identified from *Streptomyces* strains such as borrelidin, clindamycin, fattiviracins, ningnanmycin, and cytosinpeptidemycin (Ghaly et al. 2005; Han et al. 2014; Zhao et al. 2017).

Conclusion

Data presented in this study concluded that different CSF (*Streptomyces netropsis*, DSM 40093- (SCF7), *S. ambofaciens* (SCF11), and *S. actuosus* (SCF20)) were potent to induce SAR in four potato cvs. (Spunta, Nicola, Selatar, and Diamant) for inhibition of PVY^{NTN} infection. The protection of theses potato plants was evinced by reduction in disease severity and viral concentration in the host leaves. A future overview for fractionation and separation of different chemicals, identified by GC-MS for SCF20 to be used individually as signaling molecules for SAR against plant virus infection is suggested.

Abbreviations

CMV: Cucumber mosaic virus; DAVI: Days after virus inoculation; DBVI: Days before virus inoculation; GC-MS: Gas chromatography-mass spectrometry; PVY: Potato virus Y; SAR: Systemic acquired resistance; SCF: *Streptomyces* spp. crude filtrates; SCF11: *Streptomyces ambofaciens*; SCF20: *Streptomyces actuosus*; SCF7: *Streptomyces netropsis* (DSM 40093); TMV: Tobacco mosaic virus

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Author's contributions

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Competing interests
The authors declare that they have no competing interests.

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