Vol.2, No.4, 491-497 (2011)
doi:10.4236/as.2011.24063

Evaluation of the toxicity of *Streptomyces aburaviensis* (R9) extract towards various agricultural pests

Ismail Saadoun[1*], Sereen Bataineh[2], Qutaiba Ababneh[2], Khalid Hameed[2], Kevin Schrader[3], Charles Cantrell[3], Franck Dayan[3], David Wedge[3]

[1]Department of Applied Biology, College of Sciences, University of Sharjah, Sharjah, Sharjah-United Arab Emirates;
[*]Corresponding Author: isaadoun@sharjah.ac.ae
[2]Department of Applied Biological Sciences, Faculty of Science and Arts, Jordan University of Science and Technology, Irbid, Jordan;
[3]USDA, ARS, Natural Products Utilization Research Unit, Thad Cochran Research Center, University Avenue, Minneapolis, USA.

Received 7 September 2011; revised 19 October 2011; accepted 27 October 2011.

ABSTRACT

The dichloromethane extract of culture filtrate from *Streptomyces aburaviensis* R9 was evaluated using various rapid bioassays to determine potential inhibitory effects towards phytopathogenic fungi (*Colletotrichum acutatum*, *C. fragariae*, *C. gloeosoprioids*, *Botrytis cinerea*, *Fusarium oxysporum*, *Phomopsis viticola* and *P. obscurans*), fish bacterial pathogens (*Edwardsiella ictaluri* and *Flavobacterium columnare),* a green alga (*Selenastrum capricornutum*), plant seeds [Bent grass (*Agrostis* sp.) monocot and lettuce (*Lactuca sativa*) dicot] and 2-methylisoborneol (MIB)-producing cyanobacteria (*Planktothrix perornata* and *Pseudanabaena* sp.). The dichloromethane extract showed selective inhibition against the cyanobacterium *P. perornata*, with a lowest-complete-inhibition concentration (LCIC) of 10 mg/L and lowest-observed-effect concentration (LOEC) of 10 mg/L while LCIC and LOEC values were 100 mg/L when tested against *S. capricornutum*. This extract also showed slight meristematic cytogenic necrosis at 200 mg/L towards germinated seeds of both test plants. The compounds were not very toxic towards the channel catfish (*Ictalurus punctatus*) pathogenic bacteria *E. ictaluri* and *F. columnare.* Preliminary evaluation of the extract toward *C. acutatum*, *C. fragariae* and *C. gloeosoprioids* using TLC bioautography revealed moderate activity. However, further evaluation of the extract using a microtiter plate bioassay determined that inhibition was strongest against *C. acutatum* and *C. fragariae*, though this inhibitory activity diminished at 72 hours and was moderately less active than the commercial fungicides azoxystrobin and captan when comparing 1 - 100 mg/L levels at 48 hours.

Keywords: Algae; Catfish; Cyanobacteria; Fungi; Pathogens; Streptomyces

1. INTRODUCTION

Biological methods and technologies in coordination with agricultural production [1,2] were established to solve problems of the deleterious effects of chemical fertilizers and pesticides on the environment and the appearance of organisms with resistance resulting from the high application rates of currently used agrochemicals [3-5]. Such chemicals are widely used despite the growing public concerns of several undesirable consequences of using them such as accumulation in the environment, biomagnification and excessive persistence [6].

Microorganisms are ubiquitous in nature and well-known to produce bioactive materials of particular practical value. Among these beneficial microorganisms are the actinomycetes, a group of filamentous bacteria which include many species that are characterized by the production of important extracellular bioactive compounds including antibiotics [7,8]. The majority of those isolates producing bioactive compounds belong to species within the genus *Streptomyces*, and several *Streptomyces* spp. have been advocated as promising biocontrol agents against several phytopathogenic fungi and bacteria [9-14]. In addition, members of the genus *Streptomyces* are well known for their potential to produce herbicides [5,15]. Natural compounds, such as the secondary metabolites of actinomycetes and particularly streptomycetes, have been demonstrated to be bioherbicides and include anisomycin, bialaphos, herbicidans A and B [5]. Therefore, attempts have been made to isolate streptomycetes with bioherbicidal properties.

Several studies have previously been conducted with soil streptomycetes isolated in Jordan for their potential to produce antibiotics [16-18]. In a recent study by

Bataineh *et al*. [19], the herbicidal activity of streptomycetes isolated from different habitats in Jordan were evaluated for their phytotoxic potential against common broad leaf and grass. In addition, the optimal medium suitable for higher phytotoxic activity in the culture filtrate of isolates and the method for extraction of potential phytotoxic compounds were investigated.

The present study was conducted to extend that investigation by determining the activity of the cell-free culture extract of one streptomycete isolate (*Streptomyces aburaviensis* R9) against various agricultural pests. In addition to potential herbicidal activity, the extract was evaluated using rapid bioassays for activity against off-flavor compound-producing species of cyanobacteria (blue-green algae), bacterial pathogens of catfish, and fungal phytopathogens in order to determine if other useful bioactive compounds (toxins) were produced by *S. aburaviensis* R9.

2. MATERIALS AND METHODS

2.1. Extract Preparation

Extracts from *S. aburaviensis* R9 were obtained according to Mallik [15]. Isolate R9 (7-day growth) from an agar plate was used to inoculate a flask containing 25 mL of GPM broth (10 g/L glucose, 5.0 g/L peptone and 20.0 g/L molasses). This primary culture was incubated at 28˚C with shaking at 110 rpm for 5 days inside an orbital shaker incubator (TEQ, Portugal), after which the primary culture was used to inoculate a 500-mL mass culture of GPM broth. The mass culture was incubated for 7 days under the same conditions used for the primary culture. Mass culture cell-free filtrate was extracted with 100% dichloromethane (1:3 v/v) (Acros Organics, USA). The solvent was evaporated using a rotary evaporator (Heidolph, Germany) at 29˚C. The residues were then dissolved in 4 mL 100% dichloromethane and transferred into sterilized test tubes. To determine the total solid content of the extract, the dissolved residue was dried in a jet stream of N_2 gas.

2.2. Herbicide Bioassay

A standardized 24-well microtiter plate assay [20] was used to determine the R9 extract activity towards monocot (*Agrostis stolonifera* L. bentgrass) and dicot (*Lactuca sativa* L. lettuce) representative plant types. The wells in the microplate were prepared by adding 20 µL of each of the test solutions of the extract to filter paper, allowing the filter paper to dry and then adding 200 µL of water. Final exposure concentrations were 0.2, 2.0, 20.0, and 200.0 mg/L.

2.3. Algaecide Bioassay

The methods outlined in Schrader *et al*. [21] were used to evaluate the crude extract for selective toxicity towards the 2-methylisoborneol (MIB)-producing-cyanobacteria [*Planktothrix perornata* [Skuja] Anagnostidis and Komárek (syn. *Oscillatoria perornata*)], isolated from a Mississippi, USA, catfish pond, and *Pseudanabaena* sp. (strain LW397) [Skuja] Anagnostidis and Komárek, isolated from a municipal drinking water reservoir in Virginia, USA. The green alga (*Selenastrum capricornutum*) [Printz] was included as a representative of division Chlorophyta and to determine selective toxicity of the test extract.

The crude extract of *S. aburaviensis* R9 and the extract of the media (control) were dissolved separately in 100% dichloromethane (HPLC grade) to provide stock solutions of 2.0, 20.0, 200.0, and 2000.0 mg/L. Final test concentrations in microplate wells were 0.1, 1.0, 10.0, and 100.0 mg/L. Absorbance measurements of microplate wells were made every 24 h for 4 days using a Packard model SpectraCount microplate photometer (Packard Instrument Co., Downers Grove, Illinois, USA) at 650 nm. Three replications were used for each extract and control concentration and experiments were repeated. Mean absorbance measurements were calculated and graphed to determine the lowest-observed-effect concentration (LOEC) and lowest-complete-inhibition concentration (LCIC).

2.4. Bactericide Bioassay

The same procedures used by Schrader and Harries [22] were used to evaluate the crude extract of *S. aburaviensis* R9 for antibacterial activity towards the catfish pathogenic bacteria *Edwardsiella ictaluri* and *Flavobacterium columnare* isolate 1016, except that a 96-well quartz microplate (Hellma Cells, Inc., Forest Hills, New York, USA) was used to perform the bioassay because the dichloromethane loading solvent is incompatible with polystyrene microplates. Stock solutions and final concentrations of the crude extract and media extract (control) were the same as those used in the algaecide bioassay.

2.5. Fungicide Bioassay

Isolates of *Colletotrichum acutatum* Simmonds, *Colletotrichum fragariae* Brooks, and *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. were obtained from Barabara J. Smith, USDA, ARS, Small Fruit Research Station, Poplarville, Mississippi, USA. *Colletotrichum fragariae* (isolate CF63), *C. acutatum* (isolate CAGoff), and *C. gloeosporioides* (isolate CG162) were used for the pathogen and bioautography studies. Isolate

CF63 is one of the most virulent isolates that infects strawberry plants and induces both crown and fruit rot [23]. CF63, CAGoff, and CG162 were used as standard test isolates because of our extensive knowledge of these isolates and their known fungicide sensitivity profiles in both bioautography and microtiter formats. The three *Colletotrichum* species were isolated from strawberry (*Fragaria* × *ananassa* Duchesne). *Botrytis cinerea* Pers.:Fr, was isolated from commercial grape (*Vitis vinifera* L.) and *Fusarium oxysporum* Schlechtend:Fr from orchid (*Cynoches* sp.) by D. E. Wedge, USDA ARS Natural Products Utilization Research Unit, University, Mississippi, USA. *Phomopsis viticola* (Sacc.) and *P. obscurans* (Ellis and Everh) Sutton were obtained from Mike A. Ellis, Ohio State University, Wooster, Ohio, USA. Fungi were grown on potato-dextrose agar (PDA, Difco, Detroit MI) in 9 cm petri dishes and incubated in a growth chamber at 24˚C ± 2˚C and under cool-white fluorescent lights (55 ± 5 µmols·m^{-2}·sec^{-1} light) with 12 h photoperiod.

Conidia were harvested from 7 - 10 day-old cultures by flooding plates with 5 mL of sterile distilled water and dislodging conidia by softly brushing the colonies with an L-shaped glass rod. Conidial suspensions were filtered through sterile miracloth (Calbiochem-Novabiochem Corp., La Jolla, California, USA) to remove mycelia. Conidia concentrations were determined photometrically, from a standard curve based on the absorbance at 625 nm and suspensions were then adjusted with sterile distilled water to a concentration of 1.0 × 10^6 conidia/mL.

Bioautography procedures of Meeaza *et al*. [24] and Tabanca *et al.* [25] for detection of naturally occurring antifungal agents were used to evaluate antifungal activity of the dried dichloromethane cell-free culture extract of R9 and GPM broth. Samples were prepared in dichloromethane at a concentration of 2000 mg/L and 4 and 8 µL were used in a dose-response to evaluate each sample. Conidia of *Colletotrichum fragariae*, *C. acutatum and C. gloeosporioides* suspensions were adjusted to 3.0 × 10^5 conidia/mL with liquid potato-dextrose broth (PDB, Difco, Detroit, Michigan, USA) and 0.1% Tween-80. Using a sterile 50-mL chromatographic sprayer, each glass silica-gel thin-layer chromatography (TLC) plate with fluorescent indicator (250 mm, silica gel GF Uniplate, Analtech, Inc., Newark, Delaware, USA) was sprayed lightly (to dampness) three times with the conidial suspension. Inoculated plates were placed in a 30 × 13 × 7.5 cm moisture chamber (100% RH, 398-C, Pioneer Plastics, Inc., Dixon, Kentucky, USA) and incubated in a growth chamber at 24˚C ± 1˚C and 12 h photoperiod under 60 ± 5 µmols·m^{-2}·sec^{-1} light. Inhibition of fungal growth was measured 4 d after treatment. Sensitivity of each fungal species to each test compound was determined by comparing size of inhibitory zones. Means and standard deviations of inhibitory zone size were used to evaluate antifungal activity of essential oils, solvent fractions, and pure compounds. Bioautography experiments were performed multiple times using both dose- and non-dose-response formats. Fungicide technical grade standards benomyl, cyprodinil, azoxystrobin, and captan (Chem Service, Inc., West Chester, Pennsylvania, USA) were used as controls at 2.0 mM in 2.0 µL of ethanol.

A standardized 96-well microtiter plate assay developed for discovery of natural product fungicidal agents [26,27] was used to determine sensitivity of *B. cinerea*, *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, *F. oxysporum*, *Phomopsis viticola*, *and P. obscurans* to the various antifungal agents in comparison with known fungicidal standards. Captan and azoxystrobin were used as standards in this experiment. Each fungus was challenged in a 6-point dose-response format using test compounds where the final treatment concentrations were 0.01, 0.1, 1.0, 10.0 and 100.0 mg/L. Microtiter 96-well quartz plates (Hellma Cells, Inc., Forest Hills, New York, USA) were covered with a plastic lid and incubated in a growth chamber as described previously for fungal growth. Growth was then evaluated by measuring absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count).

Chemical sensitivity of each fungus was evaluated using 96-well plate microbioassay format. Each chemical was evaluated in duplicate at each dose (0.01, 0.1, 1.0, 10.0 and 100.0 mg/L). Mean absorbance values and standard errors were used to evaluate fungal growth at 48 h and 72 h, except for *P. obscurans* and *P. viticola* the data were recorded at 120 h. Analysis of variance of means for percent inhibition of each fungus at each dose of test compound (n = 4) relative to the untreated positive growth controls (n = 32) were used to evaluate fungal growth inhibition. Each test fungicide was run in duplicate at each concentration, and the experiment was repeated three times.

3. RESULTS AND DISCUSSION

3.1. Extract Yields from Cultures of *Streptomyces aburaviensis* R9

Table 1 shows the yield of the concentrated dichloromethane cell-free extracted broth (mg) of *S. aburaviensis* R9 culture from different batches. An average of 138 mg of dried cell-free dichloromethane crude extract was obtained per 500 mL of GPM culture broth. However, the media extract (control) yielded an average 120 mg of dried extract per the same volume. This difference in the

dried weight yields is likely due to the growth and metabolites (e.g., phytotoxin) production by *S. aburaviensis* R9 when grown in GPM broth [19].

3.2. Herbicide Bioassay

In the study by Bataineh *et al*. [19], a total of 231 different soil *Streptomyces* isolates were assessed for their phytotoxic activity on seeds of cucumber and ryegrass on the basis of suppressed seed germination, discoloration of the root tip, reduced root and shoot growth and eventual death of the root. The phytotoxicity symptoms observed in the study by Bataineh *et al*. [19] were represented by discoloration and death of the root tips, and these symptoms were profoundly evident when the same isolate used in the current study, *S. aburaviensis* R9, was evaluated for activity. The results suggested that the phytotoxic effect is cytotoxic and affects the meristematic cells due to production of an extracellular agent(s) or toxin(s) by the bacterium.

The results of the current investigation determined that there was phytotoxic activity of the dichloromethane culture filtrate crude extract at 200 mg/L towards the germinated seeds of both test-plant types [*A. stolonifera* (monocot) and *L. sativa* (dicot)] (**Table 2**). None of the lower test concentrations of the *S. aburaviensis* R9 extract were phytotoxic. In accordance with the bioassay protocol, test results were reported in tenfold dilutions of the test material (e.g., extract). Overall, the results from this bioassay indicate that the *S. aburaviensis* R9 extract was weakly phytotoxic activity towards both the monocot and dicot plants tested because only the highest test concentration inhibited growth.

3.3. Algaecide Bioassay

The dichloromethane extract showed selective toxicity against *P. perornata* with a lowest-complete-inhibition concentration (LCIC) of 10 mg/L and lowest-observed-effect concentration (LOEC) of 10 mg/L (**Table 3**). For *S. capricornutum*, the LCIC and LOEC values for the R9 extract were 100 mg/L, an order of magnitude less toxic which exemplifies the selective toxicity of the extract. It is interesting to note that the *Pseudanabaena* sp. was inhibited by 10 mg/L of the GPM broth medium extract (without *S. aburaviensis*-(R9) culture). Therefore, an ingredient in this media appears to be toxic towards this species of cyanobacteria. However, such effect against the green alga *S. capricornutum* was only at 100 mg/L as indicated by the LCIC and LOEC results. In accordance with the bioassay protocol, test results were reported in tenfold dilutions of the test material (e.g., extract).

Table 1. Yield of the concentrated dichloromethane cell-free extracted broth (mg) of *S. aburaviensis* R9 culture from different batches.

Batch No.	Weight of concentrated dichloromethane cell free broth (mg)				Control[a]
	Test 1	Test 2	Test 3	Average weight[b]	
1	126	154	141	140 ± 14	110
2	187	114	107	136 ± 44	130

[a]The control and tests volumes were 500 mL; [b]The average ± standard deviation were calculated from the tests of the same batch.

Table 2. Growth observation of monocot and dicot plants at different concentrations of the concentrated dichloromethane cell-free extracted broth of *S. aburaviensis* R9 culture.

Concentration (mg/L)	R9 culture filtrate extract		GPM broth medium extract	
	Lactuca sativa L (lettuce, dicot)	*Agrostis stolonifera* L (bentgrass, monocot)	*Lactuca sativa* L (lettuce, dicot)	*Agrostis stolonifera* L (bentgrass, monocot)
0.2	0[a]	0	0	0
2.0	0	0	0	0
20.0	0	0	0	0
200.0	1	1	0	0

[a]0: No effect, 1: Complete inhibition of growth; Growth was observed after 7 days.

Table 3. Activity of *S. aburaviensis* R9 culture filtrate extract on cyanobacteria and green alga as compared to the broth medium extract.

	Planktothrix perornata		*Selenastrum capricornutum*		*Pseudanabaena* LW397	
	LOEC[a]	LCIC[b]	LOEC	LCIC	LOEC	LCIC
Compound	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**
R9 culture filtrate extract	10	10	100	100	10	10
GPM broth medium extract	>100	>100	>100	>100	10	10

[a]LOEC: lowest-observed-effect concentration; [b]LCIC: lowest-complete-inhibition concentration.

3.4. Bactericide Bioassay

The extracts were not very toxic at the concentrations tested towards the catfish pathogenic bacteria *Edwardsiella ictaluri* and *Flavobacterium columnare* isolate 1016 (data not shown). For this bioassay, only activity below 100 mg/L for IC50 and MIC values is considered significant.

3.5. Fungicide Bioassay

Results of the preliminary evaluation of the *S. aburaviensis* R9 extract using TLC plates on *Colletrotichum acutatum* (CaGoff); *C. fragariae* (Cf63) and *C. gloeosporioides* (CG162) showed strong activity against these fungal isolates. However, results from the microtiter-plate bioassay found the most significant activity was towards *C. acutatum* and *C. fragariae* (**Table 4**). Generally, as the test concentration of culture extract increased, the percentage of growth inhibition of *C. acutatum* and *C. fragariae* increased. Also, the culture extract was moderately less active than the commercial fungicides azoxystrobin and captan when comparing 1, 10 and 100 mg/L treatments at 48 h. There was less inhibition of *C. acutatum* and *C. fragariae* by the culture extract at 1, 10, and 100 mg/L at 72 h compared to 48 h results (**Table 4**). This loss of activity could be due to hydrolyzation of the active compound by the fungal species, thereby making it less active. This can occur with many commercial fungicides which actually make them more fungistatic than fungicidal at the concentrations that they are applied. However, it is somewhat difficult to infer a direct comparison since we are comparing a crude extract to pure compounds. The variations of percent growth inhibition observed for the different dilutions of the control broth were attributed to incomplete solubilization of the control broth extract that was encountered when conducting the fungicide bioassay. It is interesting that the broth medium extract also inhibited the cyanobacterium *Pseudanabaena* sp. used in this study.

Table 4. Evaluation of the crude extract of *S. aburaviensis* R9 culture fractions for fungicidal activity towards different fungal phytopathogens at different concentrations as compared to known fungicidal standards, azoxystrobin and captan.

Concentration (mg/L)	Sample	% Inhibition[a,b]	
		C. acutatum	*C. fragariae*
0.001	Culture extract	4.5 (0)	1.0 (1.3)
	Control broth	10.0 (7.2)	12.4 (8.0)
	Azoxystrobin[c]	5.3 (0)	0 (0)
	Captan[c]	2.7 (0)	4.5 (7.3)
0.01	Culture extract	17.4 (18.4)	0 (0)
	Control broth	0 (0)	22.3 (20.7)
	Azoxystrobin	37.6 (6.7)	47.5 (36.8)
	Captan	5.1 (0)	19.3 (14.8)
0.1	Culture extract	5.1 (0)	21.0 (15.4)
	Control broth	5.9 (14.3)	0 (3.2)
	Azoxystrobin	75.2 (26.9)	77.2 (58.8)
	Captan	12.2 (2.2)	26.6 (21.8)
1	Culture extract	13.8 (0.06)	53.9 (30.5)
	Control broth	0 (0)	25.3 (25.9)
	Azoxystrobin	77.9 (39)	76.98 (63.5)
	Captan	72.5 (43.9)	59.4 (9.5)
10	Culture extract	45.5 (0)	66.4 (14.0)
	Control broth	0 (0)	21.3 (22.0)
	Azoxystrobin	86.6 (64.7)	82.0 (68.2)
	Captan	100.0 (95.6)	100.0 (100.0)
100	Culture extract	78.7 (27.4)	91.6 (20.8)
	Control broth	12.3 (15.0)	17.0 (24.3)
	Azoxystrobin	88.7 (78.0)	85.9 (73.5)
	Captan	98.1 (98.0)	99.2 (99.8)

[a]Sample results only indicate inhibition. Zero (0) does not indicate the degree of stimulation, only that there was no inhibition. [b]Numbers not enclosed in parentheses = 48 h results; numbers enclosed in parentheses = 72 h results. [c]Internal standard compound(s) utilized in the 96-well assay.

The antifungal activity of the culture extract is likely due to the production of an antibiotic, though such a metabolite produced by *S. aburaviensis* R9 appears to not be a broad-spectrum antibiotic, but more specific towards certain species of fungi. While growth inhibition of some of the test species of fungi (e.g., *Colletrotichum acutatum* (CaGoff); *C. fragariae* (Cf63) and *C. gloeosporioides* (CG162) occurred in the presence of the culture extract, there was little to no activity towards the Gram-negative bacteria fish pathogens used in this study (*Edwardsiella ictaluri* and *Flavobacterium columnare* isolate 1016). A previous study by Raytapadar and Paul [28] determined that another isolate of *Streptomyces aburaviensis* from Indian soil produced an antifungal antibiotic, and they identified the isolate as *Streptomyces aburaviensis* var. *ablastmyceticus* (MTCC 2469). Another study by Thumar *et al*. [29] identified antibiotic production by a halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8 that inhibited the growth of the Gram-positive bacterium *Bacillus subtilis*. At present, it is unknown if the antibiotic production of *S. aburaviensis* R9 is similar or identical to those cited in the previous studies above. Future isolation and characterization of the active antifungal metabolite(s) would determine these properties. Additional studies would also aid in determining if the active antifungal metabolite(s) produced by *S. aburaviensis* R9 is also responsible for the phytotoxic activity observed towards the plants *A. stolonifera* and *L. sativa* and the cyanobacterium *P. perornata*.

4. ACKNOWLEDGEMENTS

Mr. Dewayne Harries provided technical support for the fungicide assay. Appreciation is extended to University of Sharjah/UAE and to Jordan University of Science and Technology for administrative support.

REFERENCES

[1] Reganold, J.P., Papendick, R.I. and Parr, J.F. (1992) Sustainable agriculture. *Scientific American*, **262**, 112-120. doi:10.1038/scientificamerican0690-112

[2] Parr, J.F. and Hornick, S.B. (1992) Agricultural use of organic amendments: A historical perspective. *American Journal of Alternative Agriculture*, **7**, 181-189. doi:10.1017/S0889189300004781

[3] Cohen, Y. and Coffey, M.D. (1986) Systemic fungicides and the control of oomycetes. *Annual Review of Phytopathology*, **24**, 311-338. doi:10.1146/annurev.py.24.090186.001523

[4] Fruh, T., Chemla, P., Ehrler, J. and Farooq, S. (1996) Natural products as pesticides: Two examples of strereoselective synthesis. *Pesticide Science*, **46**, 37-47. doi:10.1002/(SICI)1096-9063(199601)46:1<37::AID-PS339>3.0.CO;2-K

[5] Mallik, M.A.B. (2001) Selective isolation and screening of soil microorganisms for metabolites with herbicidal potential. *Journal of Crop Production*, **4**, 219-236. doi:10.1300/J144v04n02_07

[6] Heisey, R.M. and Putnam, A.R. (1990) Herbicidal activeity of the antibiotics geldanamycin and nigericin. *Journal of Plant Growth Regulation*, **9**, 19-25. doi:10.1007/BF02041937

[7] Higa, T. (1991) Effective microorganisms: A biotechnology for mankind. In: Parr, J.F., Hornick, S.B. and Whitman, S.E., Eds., *Proceedings of the* 1*st International Conference on Kyusei Nature Farming*, USDA, Washington, DC, 8-14.

[8] Blatz, R.H. (1998) Genetic manipulation of antibiotic producing *Streptomyces*. *Trends in Microbiology*, **6**, 76-83. doi:10.1016/S0966-842X(97)01161-X

[9] El-Trabily, K.A., St. J. Hardy, G.E., Sivasithamparam, K., Hussein, A.M. and Kurtböke, D.I. (1997) The potential for the biological control of cavity-spot disease of carrots, caused by *Pythium coloratum*, by streptomycete and non-streptomycete actinomycetes. *New Phytologist*, **137**, 495-507. doi:10.1046/j.1469-8137.1997.00856.x

[10] El-Tarabily, K., Soliman, M., Nassar, A., Al-Hassani, H., Sivasithamparam, K., McKenna, F. and St. J. Hardy, G.E. (2000) Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. *Plant Pathology*, **49**, 573-583. doi:10.1046/j.1365-3059.2000.00494.x

[11] Saadoun, I. and Al-Momani, F. (1997) Streptomycetes from Jordan soils active against *Agrobacterium tumefaciens*. *Actinomycetes*, **8**, 29-36.

[12] Saadoun, I., Hameed, K., Al-Momani, F., Malkawi, H., Meqdam, M. and Mohammad, M.J. (2000) Characterization and analysis of antifungal activity of soil streptomycetes isolated from North Jordan. *Egyptian Journal of Microbiology*, **35**, 463-471.

[13] Seto, H., Fujioka, T., Furihatha, K., Kaneko, J. and Takahkashi, S. (1989) Structure of complestain a very strong inhibitor of protease activity of complement in the human complement system. *Tetrahedron Letters*, **37**, 4987-4990. doi:10.1016/S0040-4039(01)80562-1

[14] Tahtamouni, M.E.W., Hameed, K.M. and Saadoun, I. (2006) Biological control of *Sclerotinia sclerotiorum* using indigenous chitinolytic actinomyctes in Jordan. *The Plant Pathology Journal*, **22**, 107-114.

[15] Mallik, M.A.B. (1997) Isolates of soil actinomycetes with potential for phytotoxin production. *Journal of Chemical Ecology*, **23**, 2683-2693. doi:10.1023/A:1022502724711

[16] Saadoun, I., Al-Momani, F., Malkawi, H. and Mohammad, M.J. (1999) Isolation, identification and analysis of antibacterial activity of soil streptomycetes isolates from North Jordan. *Microbios*, **100**, 41-46.

[17] Saadoun, I. and Gharaibeh, R. (2003) The *Streptomyces* flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. *Journal of Arid Environments*, **53**, 365-371. doi:10.1006/jare.2002.1043

[18] Saadoun, I., Wahiby, L., Ababneh, Q., Jaradat, Z., Massadeh, M. and Al-Momani, F. (2008) Recovery of soil streptomycetes from arid habitats in Jordan and their potential to inhibit multi-drug resistant *Pseudomonas aeruginosa* pathogens. *World Jorunal of Microbiology and Biotechnology*, **24**, 157-162.

doi:10.1007/s11274-007-9451-2

[19] Bataineh, S., Saadoun, I., Hameed, K.M. and Ababneh, Q. (2008) Screening for soil Streptomycetes from north Jordan that can produce herbicidal compounds. *Polish Journal of Microbiology*, **57**, 297-305.

[20] Dayan, F.E., Romagni, J.G. and Duke, S.O. (2000) Investigating the mode of action of natural phytotoxins. *Journal of Chemical Ecology*, **26**, 2079-2094. doi:10.1023/A:1005512331061

[21] Schrader, K.K., De Regt, M.Q., Tucker, C.S. and Duke, S.O. (1997) A rapid bioassay for selective algicides. *Weed Technology*, **11**, 767-774.

[22] Schrader, K.K. and Harries, M.D. (2006) A rapid bioassay for bactericides against the catfish pathogens *Edwardsiella ictaluri* and *Flavobacterium columnare*. *Aquaculture Research*, **37**, 928-937. doi:10.1111/j.1365-2109.2006.01514.x

[23] Smith, B.J. and Black, L.L. (1990) Morphological, cultural, and pathogenic variation among *Colletotrichum* species isolated from strawberry. *Plant Disease*, **74**, 69-76. doi:10.1094/PD-74-0069

[24] Meazza, G., Dayan, F.E. and Wedge, D.E. (2003) Activity of quinones on *Collectotrichum* species. *Journal of Agricultural Food and Chemistry*, **51**, 3824-3828. doi:10.1021/jf0343229

[25] Tabanca, N., Bedir, E., Ferraira, D., Slade, D., Wedge, D.E., Jacob, M.R., Khan, S.I., Khan, I.A., Kirimeri, N. and Baser, K.H.C. (2005) Bioactive constituents from Turkish *Pimpinella* species. *Chemistry and Biodiversity*, **2**, 221. doi:10.1002/cbdv.200590005

[26] Wedge, D.E. and Kuhajek, J.M. (1998) A microbioassay for fungicide discovery. *SAAS Bulletin*, *Biochemistry and Biotechnology*, **11**, 1-7.

[27] Wedge, D.E., Nagle, E. and Dale, G. (2002) A new 2D-TLC bioautography method for the discovery of novel antifungal agents to control plant pathogens. *Journal of Natural Products*, **63**, 1050-1054. doi:10.1021/np990628r

[28] Raytapadar, S. and Paul, A.K. (2001) Production of an antifungal antibiotic by *Streptomyces aburaviensis* 1DA-28. *Microbiological Research*, **155**, 315-323. doi:10.1016/S0944-5013(01)80010-0

[29] Thumar, J.T., Dhulia, K. and Singh, S.P. (2010) Isolation and partial purification of an antimicrobial agent from halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8. *World Journal of Microbiology and Biotechnology*, **26**, 2081-2087. doi:10.1007/s11274-010-0394-7

