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Antifungal Activity of *Pseudomonas fluorescens* Metabolites against some Phytopathogenic Fungi

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ABSTRACT

The effects of *Pseudomonas fluorescens* culture, culture filtrate and the crude antibiotics extracted from culture filtrate were *in vitro* studied against six plant pathogenic fungi (*Fusarium oxysporium, Fusarium solani, Fusarium semitectium, Rhizoctonia solani, Sclerotium rolfsii* and *Botrytis cinerea*). *P. fluorescens* effectively inhibited the mycelial growth of all fungi in dual culture tests. Also, the culture filtrate at different concentrations reduced the mycelial growth except *S. rolfsii*. The culture filtrate at the EC_{50} concentration was effective in reducing the total contents of soluble sugars, free amino acids, total proteins and enzyme activities produced by the phytopathogenic fungi. The antifungal compounds were extracted with equal volume of ethyl acetate. The antifungal compounds from *P. fluorescens* at 100 mg ml^{-1} completely inhibited *F. oxysporium* and *S. rolfsii* and purified by column puriflash and re-tested for antifungal activity. The major compound in the crude antibiotics was characterized by TLC, mass spectrometry and FTIR. The molecular weight of this compound was 255.4 m/z. In FTIR analysis, antifungal compound extracted from *P. fluorescens* revealed absorption at 3318.13 per cm pyrrole ring and CH3 (stretch) (1450.2) and C=C aromatic weak intensity (1662.44), C-Cl_2 (624.81). This confirms that the antifungal compound in crude extract is pyrrolnitrin.

***Keywords*:** *P. fluorescens*, pyrrolnitrin, phytopathogenic fungi, secondary metabolites

Introduction

It is well known that fungal diseases are among the main constrains affecting production of crops both in terms of quality and quantity all over the world. Although chemical control usually achieves considerable results, thought it causes many problems to the environment and human health. Biological control is promising and safe in this respect. *P. fluorescens* encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. It secretes a soluble greenish fluorescent pigment called fluorescein, particularly under conditions of low iron availability. Secondary metabolites are small heterogenous organic molecules, that display prominent ecological benefits to the host organisms in providing defense against predators, parasites, diseases, interspecies nutritional competence, and competitive edge over interaction with the environment (Dixon, 2001). *P. fluorescens* isolates possess a variety of promising properties of antifungal activity due to its secondary metabolites which make it as a biocontrol agent (Kliebenstein, 2004).

The ability of *P. fluorescens* to suppress phytopathogenic fungi depends on its ability to produce some antibiotic metabolites such as pyoluteorin, pyrrolnitrin, phenazine 1- carboxylic acid, 2, 4 diacetylphloroglucinol, hydrogen cyanide, kanosamine, pyocyanin and viscosinamide (Prabhukarthikeyan and Raguchander, 2016). In this investigation, attempts were made to study the activity of *P. fluorescens* against some phytopathogenic fungi and identify some of its antifungal compounds and testing their antifungal activities.

Materials and Methods

Bacterial strain

The bacterial strain *P. fluorescens* used in the present study was obtained from Soils, Water and Environment Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

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Fungal pathogens

Naturally-diseased tomato plants showing root rot and wilt symptoms were collected from private fields from Benisewif and Quliubia governorates. Plant root and stem samples were washed carefully with tap water to remove the adhering soil particles, cutted into small pieces (1x1 cm) and surface-sterilized by immersion in 5 % sodium hypochlorite for 5 min according to the method of Burr *et al*. (1978). The segments were rinsed three times in sterilized distilled water, dried between two sterilized filter papers and transferred under aseptic conditions to sterilized Petri dishes containing potato dextrose agar medium (PDA). Plates were incubated at 27 ºC and developed colonies were picked after 5 days, isolated on PDA plates and purified using the single spore and hyphal tip techniques (Dhingra and Sinclair, 1984). The isolated fungi were identified microscopically to species level according to Nelson *et al*. (1983) and Barnett and Hunter (1987). Stock cultures were maintained on PDA slants and kept at 10 ºC in refrigerator for further experiments. The identification was verified in Plant Pathology Department, Faculty of Agriculture, Cairo University.

Antibiosis of the bacterial strain towards pathogenic fungi

For the tested bacterial strain, mother culture or its filtrate was used to test the antagonistic effects against the pathogenic fungi.

Mother cultures

Petri dishes (9 cm in diameter), each containing 20 ml PDA medium were surfacely seeded with discs (5 mm in diameter) of each tested pathogen, taken from 5 day-old cultures. Discs were placed near the edge of each Petri dish and on the other side at the same time, plates were streaked with the bacterial strain. Plates inoculated only with the pathogens were used as control. Three replicates were prepared for each treatment. Plates were incubated at 27-28 °C for 5-7 days and zones of inhibition were recorded when the mycelium mats of pathogens cover the medium surface of the control plates (Siddiqui, 2001).

Culture filtrate

The bacterial strain was inoculated in Kings B broth medium and incubated at 28 °C on a shaker for 96 hrs. The liquid culture was centrifuged at 10000 rpm for 30 min and the supernatant was passed through a 0.045 µm millipore filter. The culture filtrate was kept at 4 °C for further use.

Cell-free bacterial culture was added to PDA to obtain 5, 10, 15 and 20 % concentrations of the filtrate. Thereafter, media were poured in sterile Petri dishes and allowed to solidify, PDA without culture filtrate was used as control. Media were then surfacely seeded with 5 mm discs of 5-7-day old pathogenic fungal cultures. Petri dishes were incubated for 5-7 days at 25±2 °C, inhibition zone diameters were measured. Percentages of growth inhibition were calculated using the following formula:

$$\text{Growth inhibition \%} = R1\text{-}R2/\ R1 \times 100$$

where: R_1, the radius of normal growth in control; R_2, the radius of the inhibited growth in the treatment (Abd El-Ghany, 2001).

Effect of culture filtrates of *Pseudomonas flurocences* at EC_{50} on chemical components and enzymes produced by pathogenic fungi

The EC_{50} concentration of bacterial culture filtrate was added to 100 ml of sterilized PD medium inoculated with 4 discs (5 mm) of either tested fungi and incubated at (25±2 °C). Four flasks were used for each treatment. When the mycelium growth covered the surface of media in untreated flask (control), the mycelium matrix was excluded by filtration and dried at ambient temperature overnight. The dry mycelia mates were homogenated and determined for chemical constituents.

Chemical determinations of fungi

Total soluble sugars

Total soluble sugars were determined according to Shaffer-Somogi micro method (AOAC, 1995).

Free amino acids as lysine

Samples were prepared by extracting 0.5 g of each fungal culture by 25 ml methanol 80 % (Jayaraman, 1985). A standered solution of lysine was prepared by dissolving 0.02 g lysine in 100 ml of 80 % ethanol. The color developed was measured using a spectrophotometer at wave length 570 nm.

Total protein

A ratio of 1:2.5 (w/v) of each fungus to extraction buffer (0.125M Tris-borate, pH 8.9) was used. The soluble protein concentrations were spectroscopically determined by referring to a calibration curve relating to the concentration of authentically albumin bovine at 546 nm according to Lowery *et al*. (1951).

Enzymatic activity measurements

Total amylase activity

One gram of each fungus was homogenized in a mortar with 4 ml of 0.01M Tris-HCl buffer pH 8.0 containing 0.02 M NaCl and $CaCl_2$. The supernatant was used for total amylase activity according to the method described by Dewez *et al*. (2005). The total amylase activity is expressed as mg starch consumed/15 min /1g fungus.

Peroxidase activity

One gram of each fungus was extracted two times with 0.1 M potassium phosphate buffer pH 4.7 containing 0.25 M sucrose. The colorimetric assay of total peroxidase activity was measured as recommended by Sreenivasulu *et al.* (1999). The peroxidase activity was expressed in a unit of the increase of absorption at 470 nm for 5 min per g fungus, designated as OD 470 nm /5 min/g fungus.

Protease activity

Protease activity was determined by the method described by Dewez *et al.* (2005). The blue color developed was determined after 5 min at 625 nm.

Effects of *Pseudomonas flurocesnces* metabolites

Extraction of antifungal compounds

The bacterial strain was allowed to grow in 200 ml of pigment production broth medium (peptone, 20 g; glycerol, 20 ml; NaCl, 5 g; KNO_3, 1 g; distilled water 1 l; pH 7.2) for 4 days on rotary shaker at 30 °C. The fermentation broth was centrifuged at 3500 rpm for five minutes and the supernatant was collected. It was acidified to pH 2.0 with 1 N HCl then extracted with an equal volume of ethyl acetate. The ethyl acetate extract was reduced to dryness in vacuo. The residues were dissolved in methanol and kept at 4 °C until use for TLC (Mallaiah *et al*., 2017).

Antifungal activity of ethyl acetate crude extract

The residues of ethyl acetate extract was dissolved in DEMSO to determine the antagonistic activity of crude extract at the concentrations 25, 50,75 and 100 mg ml^{-1}, added to PDA media, poured in sterile Petri dishes and allowed to solidify, PDA without crude extract was used as control. Media were then surfacely seeded with 5 mm discs of 5-7-day old pathogenic fungal cultures. Petri dishes were incubated for 5-7 days at 25±2 °C, inhibition zone diameters were measured. Percentages of growth inhibition were calculated using the following formula:

Growth inhibition % = R_1-R_2/ R_1 X 100

where : R_1,the radius of normal growth in control; R_2,the radius of the inhibited growth in the treatment (Abd El-Ghany, 2001).

Scanning electron microscopy of the hyphal morphology

The protocols described by Trolezi *et al*. (2017) was followed for SEM analysis. Hyphal fragments were collected and washed three times in 0.1 M phosphate buffer solution (PBS), then, fixed in 2.5 % glutaraldehyde (Merck) in 0.1M PBS at 4 °C for 1h and washed in the same buffer. Samples were dehydrated with a graded ethanol series. The Emitech K500X sputter coater (Quorum

Technologies) was used for gold sputtering of SEM samples. The hyphal morphology was evaluated by the S-3000N scanning electron microscope (Hitachi).

Purification of the ethyl acetate crude extract

The crude extract was applied to thin layer chromatography (TLC) plate for further purification using the mixture of acetonitrile / methanol /water (1:1:1) as a solvent system. Crude extract was separated by puriflash column 30 silica HP -25.0 g (22 bar). The dried concentrated sample was dissolved in 5 ml of hexane and loaded onto the silica gel column and solvent system comprising hexane and ethyl acetate at a flow rate of 15 ml min^{-1}. The eluted fractions were collected. The fractions were pooled and concentrated by evaporation at 40 °C using rotary evaporator.

Thin-layer chromatography (TLC)

Thin layer chromatography (TLC) was performed on silica gel TLC- cards G25-UV 254 plates for purification using a mixture of acetonitrile / methanol /water (1:1:1) as a solvent system (Rosales *et al*.,1995). TLC of the prepared crude extract was performed with precoated silica and was observed under UV light (254 nm).

Mass spectroscopy

The molecular weight of the fraction 1 was determined using Quick mass determination (Probe / TLC-MS) at Nawah Scientific Research, Cairo, Egypt.

Fourier-Transform Infrared Spectroscopy (FTIR Spectroscopy)

The purified fraction 1 was subjected to FTIR spectroscopic analysis. FTIR was carried out by mixing the sample with finely grounded KBr. The parameters used in FTIR analysis were spectral range between 4000 and 400 cm^{-1}. Upon pressing under 2000 kPa, pellet disc obtained was analyzed using JASCO FTIR -3600 infrared spectrometer by employing KBr pellet technique, equipped with KBr beam splitter with DTCS (Deuterated triglycine sulfate detector (7800-350 cm^{-1}) at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984), followed by Duncan's multiple range tests to compare means (Duncan, 1955).

Results and Discussion

Isolation, purification and identification of fungi

The isolated fungi were identified as *Fusarium solani, Fusarium oxysporium, Fusarium semitectium, Rhizoctonia solani* and *Sclerotium rolfsii.* All fungi were isolated from root, stem and rhizosphere of tomato seedlings, while *Botrytis cinerea* was isolated from the fruit of tomato plant.

Antagonistic effect of *P. fluorescens* against fungal pathogens

Fusarium solani, Rhizoctonia solani and *Fusarium semitectium* were the main fungi causing root rot disease in several plants (Karima *et al*., 2012). While *Botrytis cinerea*, the agent of gray mold, is a facultative phytopathogenic fungus that attacks flowers, fruits, leaves and stem of more than 200 plant species (Li Hue *et al*., 2018). Disease caused by this fungus produces considerable losses to crops in the field and during storage. *Sclerotium rolfsii*, is a destructive stem and crown rot of tomato. White mycelium spread over stems of infected plants and formed sclerotia on the old lesions nearby soil surface. *Fusarium oxysporium* f. sp *lycoperisici* causes soil borne vascular wilt disease in the tomato plant (Van der Does *et al*., 2018).

The previous fungi traditionally controlled by commercial fungicides has caused serious problem, such as the appearance of highly resistant strains and the contamination of soil and water, while the bacterial bioagents are an alternative to chemical fungicides. For this reasons, this study investigats the effects of the bacterial bioagent *Pseudomonas flurocences,* against the phytopathogenic fungi. *P. fluorescens* significally inhibited the mycelium growth of all tested fungi. The highest

inhibition zone was recorded against *Botrytis cinerea* (23 mm) followed by *Fusarium oxysporium* (21 mm). On the other hand, *Pseudomonas flurocences* showed the lowest inhibition zone with *R. solani* (9 mm). Fluorescent *pseudomonads* have an antagonistic effect that is not necessarily associated with the antibiotics that were searched for here. Several reports on plant-associated pseudomonads indicated also a role of siderophores, lytic enzymes, hydrogen cyanide and ammonia as well as organic volatiles in the inhibition of fungal phytopathogens (Alimi *et al*., 2012; Loper *et al*., 2012; Zhang *et al*., 2014; De Vrieze *et al*., 2015; Yasmin *et al*., 2016; Wagner *et al*., 2018).

Effect of *Pseudomonas fluorescens* culture filtrate on the linear growth of phytopathogenic fungi

Data presented in Table (1) show the effect of *Pseudomonas fluorescens* culture filtrate on the tested phytopathogenic fungi. The culture filtrate had inhibitory effects on the pathogenic fungi except the fungus *Sclerotium rolfsii*. The highest percentage of reduction in mycelial growth (72) was obtained at 20 % concentration on *F. semitecctium,* followed by *F. solani* and *F.oxysporium* (70.4 and 70 % respectively) at the same concentration, whereas the lowest effect was recorded on *R. solani* (19.4 %).

The lowest EC_{50} (4.53) was recorded with *F. oxysporium* followed by *F. solani* and *F. semitectium* (7.052 and 8.79 respectively) whereas the highest EC_{50} was recorded with *B. cinerea* (10.67). Data proved that *S. rolfsii* and *R. solani* seem to be resistant or tolerant to *P. fluorescens* culture filtrate than *B. cinerea* and *Fusarim* species. Rohit *et al*. (2011) proposed that application of culture filtrate of plant growth promoting rhizobacteria (PGPRs) *i.e. Bacillus subtilis* MA-2 and *Pseudomonas fluorescens* MA-4 inhibited the growth of phtytopathogens infecting selected medicinal and aromatic plants, indicating that suppression was due to antifungal compounds in the filtrate.

Table 1: Inhibitory effects (%) of *Pseudomonas fluorescens* culture filtrate at different concentrations against pathogenic fungi

Phytopathogenic fungi	Concentration (%)				
	20	**15**	**10**	**5**	**EC_{50}**
F. solani	70.4^{ab}	62^{b}	57.4^{b}	43.5^{b}	7.052
F. oxysporium	70^{ab}	65.7^{a}	60^{a}	51.8^{a}	4.53
F. semitecctium	72^{a}	64.4^{a}	55.5^{b}	33^{c}	8.79
R. solani	19.4^{c}	0^{d}	0^{d}	0^{d}	-
S. rolfisii	0^{d}	0^{d}	0^{d}	0^{d}	-
B. cinerea	66.6^{b}	50^{c}	43^{c}	42.6^{b}	10.67
Control	0^{d}	0^{d}	0^{d}	0^{d}	-

Means follow by the same letters are statistically insignificant.

Effect of culture filtrate of *Pseudomonas fluorescens* on the biochemical components produced by phytopathogenic fungi

Results in Table (2) proved that, the PD liquid medium treated with *P. fluorescens* culture filtrate induced significant decreases in total soluble sugars for four tested fungi comparing with untreated one. Such effects were highly pronounced in case of treated *F. oxysporium* and *F. semitectium* with culture filtrate which reached 46.6 and 41.7 % respectively of that recorded in untreated one. Moderate decreases in total soluble sugars for *F. solani* and *B. cinerea* reached 26.8 and 24.28 % respectively.

The reduction of free amino acids for phytopathogenic fungi was observed due to treatment with the culture filtrate. Such effect was highly significant in case of *F. oxysporium* and *F. semitectium*. The reduction percentages of free amino acids for *F. semitectium, F. oxysporium, F. solani* and *B. cinerea* were 36.35, 35.3, 16.56 and 18.2 respectively. The same trend was found with the reduction in total protein for the four tested fungi. The reduction of total protein was highly significant in case of *F. oxysporium* which reached 44.64 % of that recorded in untreated.

Amylase activity in *F. oxysporium* and *F. solani* sharply reduced due to treatment with the culture filtrate comparing with untreated which reached 48 and 40 % respectively. The lowest effect was observed in case of *B. cinerea* which recorded 16 % reduction in amylase activity of that obtained in untreated corresponding. Both protease and peroxidase activities for all tested fungi showed moderate reductions compared with controls fungi.

Table 2: Effects of *Ps. fluorescens* culture filtrate on total soluble sugars, free amino acids, total protiens and enzymes activities produced by phytopathogenic fungi

Phytopathogenic fungi	Biochemical component					
	Total soluble sugars (g/1g fungus)	Free amino acids (mg/1g Fungus)	Total protein (mg/1g Fungus)	Amylase activity (mg starch/1g Fungus)	Protease (O.D/ 5min)	Peroxidase activity (O.D/ 5 min)
F. solani	0.030 [b]	40.7 [b]	2.967 [b]	1.2 [b]	0.40 [b]	0.2467 [b]
Control	0.041 [a]	50.6 [a]	4.5 [a]	2.5 [a]	0.53 [a]	0.3367 [a]
% Reduction	26.8	19.56	33.3	40	23.07	26.73
F. oxysporium	0.016 [b]	33.1 [b]	3.1[b]	1.3 [b]	0.30 [b]	0.40 [b]
Control	0.030 [a]	51.2 [a]	5.6 [a]	2.5 [a]	0.41 [a]	0.506 [a]
% Reduction	46.6	35.3	44.64	48	21.95	20.9
F. semitecctium	0.1166 [b]	25.67 [b]	5.6 b	3.2 [b]	0.30 [b]	0.4500 [b]
Control	0.20 [a]	40.33 [a]	7.16 [a]	4.30 [a]	0.4 [a]	0.550 [a]
% Reduction	41.7	36.35	22.2	25.58	25	18.2
B. cinerea	0.053 [b]	13.5 [b]	10.700 [b]	0.68 [b]	0.3000 [b]	0.5000 [b]
Control	0.070 [a]	16.5 [a]	13.6 a	0.81[a]	0.40667 [a]	0.631[a]
% Reduction	24.28	18.2	21.32	16	26.22	20.7

Means follow by the same letters are statistically insignificant.

Antifungal activity of ethyl acetate crude extract

The crude antibiotics of *P. fluorescens* were tested for their antifungal activities against the phytopathogenic fungi at the concentrations 25, 50, 75 and 100 mg ml^{-1} in (Table 3 and Fig. 1). The crude antibiotics were found to record maximum (100 %) reduction of mycelial growth against *F. oxysporium* and *S. rolfsii* at the highest concentration. All tested concentrations reduced the mycelia growth of tested fungi. At the concentrations less than 25 mg ml^{-1}, no inhibitory influence was observed against all tested fungi except *S. rolfsii* being very sensitive to all concentrations. Also, *S. rolfsii* recorded the lowest EC_{50} (21.54). Kavitha (2004) reported that crude antibiotics of *B. subtilis* and *P. fluorescens* inhibited the growth of *P. aphanidermatum* in turmeric. The crude antibiotics produced by *P. fluorescens* (CHA0) suppressed damping off disease in cucumber (Maurhofer *et al*., 1992). Indumathi (2012) also reported that crude antibiotic from *P. aeruginosa* (P1) showed maximum inhibition of mycelial growth of *F. oxysporum* f. sp. *dianthi*. In addition, *B. amyloliquefaciens* and *P. fluorescens* were regarded as non-pathogenic bacterial species. Thus, the use of *Pseudomonas* as a biocontrol agent may be an environmentally safe way to suppress this plant disease.

Table 3: Efficacy of crude antibiotics produced by *P. fluorescens* against phytopathogenic fungi

Concentration mg ml-1	Reduction percentage of mycelia growth					
	F. solani	***F. oxysporium***	***F. Semitectium***	***R. solani***	***S. rolfisii***	***B. cinerea***
100	87[b]	100.0[a]	77.7[d]	92.6[c]	100.0[a]	87.4[b]
75	57.4[d]	81.48[b]	72.9[c]	76.0[c]	100.0[a]	75.9[c]
50	43.5[c]	64.82[b]	59.2[b]	49.0[c]	100.0[a]	62.9[b]
25	14.8[d]	36.11[c]	35.37[c]	16.0[d]	71.5[a]	42.6[b]
20	-	-	-	-	49.0[b]	-
15	-	-	-	-	33.33[c]	-
10	-	-	-	-	25.9[d]	-
EC_{50}	55.44	34.68	38.35	47.61	21.54	32.3

Means follow by the same letters are statistically insignificant

Scanning electron microscope

Microscopic examination (SEM) of the control (untreated) showed that hyphae, were present in their normal condition. However, morphological abnormalities such as rupturing of hyphae and shrinkage, twisting, hydrolysis were observed on hyphae treated with crude antibiotics of *P. fluorescens* (Fig.2.).

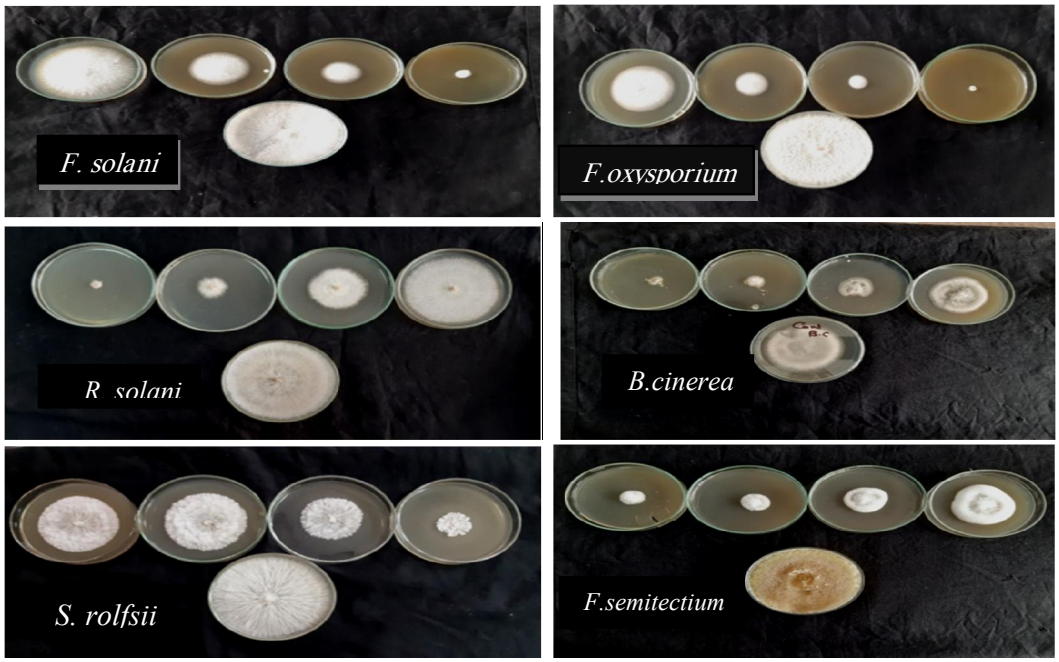

Fig. 1: Effects of crude antibiotics of *P. fluorescens* against phytopathogenic fungi.

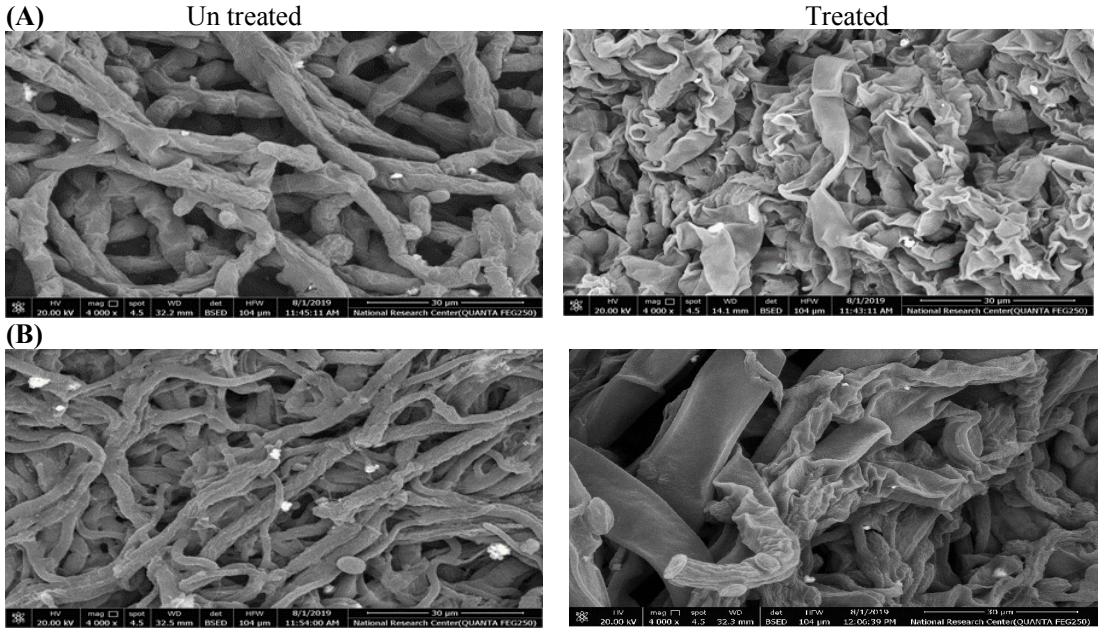

Fig. 2: Scanning electron micrographs of (A) *R. solani* and (B) *B. cinerea* treated with crude antibiotics of *P. fluorescens*.

Identification and purification of antifungal compounds

TLC of the metabolites produced by *P. fluorescens* showed five compounds. These compounds were purified and tested for antifungal activity by agar disk diffusion method (Mounyr *et al*., 2016). The five fractions were obtained from the column puriflash, Fraction no 1 showed in Fig. (3) has potential antifungal activity against phytopathogenic fungi.

Fig. 3: TLC of the major fraction that has antifungal effect.

Characterization and identification of antifungal compound

Mass spectroscopy

Results revealed that the molecular weight of the antifungal compound was 255.4. m/z (Fig. 4). This confirms that the antifungal compound extracted from *P. fluorescens* is pyrrolnitrin with the formula $C_{10}H_6Cl_2N_2O_2$. These results are in accordance with El-Banna and Winkelmann (1998) mass spectroscopy (negative ion spectrum) of PRN further confirmed (mass-to-charge ratio; m/z) at 256. High-resolution mass spectrometry of the two molecular ions gave m/z 255.9826 and 257.9777, respectively, indicating the molecular formula of $C_{10}H_6N_2O_2{}^{35}C_{12}$ and $C_{10}H_6N_2O_2$ $^{35}Cl^{37}Cl$ (Cartwright *et al*., 1995).

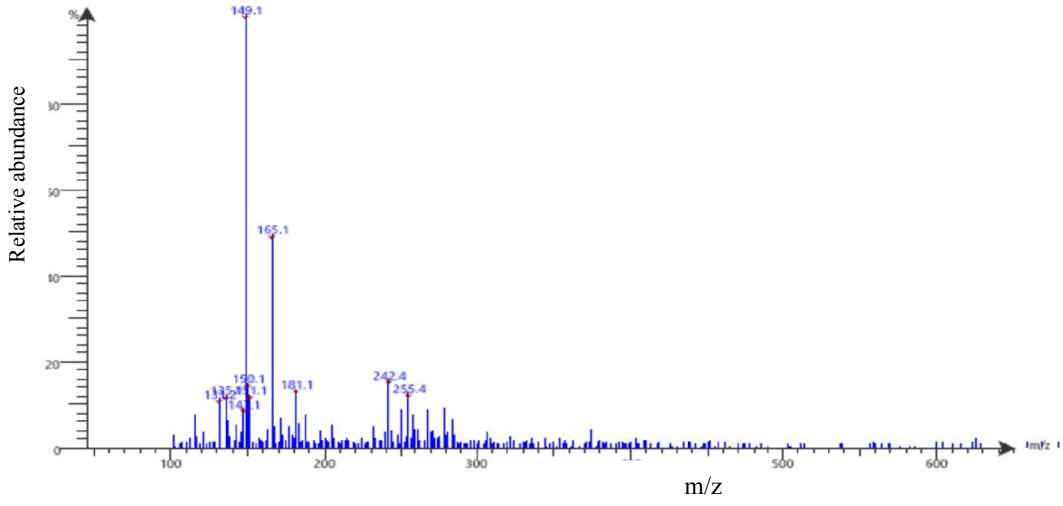

Fig. 4: TLC mass spectroscopy of pyrrolnitrin produced by *P. fluorescens.*

Fourier Transform Infrared (FTIR) spectrum of antifungal compound

In FTIR analysis, antifungal compound extracted from *P. fluorescens* revealed absorption at 3318.13 per cm pyrrole ring and CH3 (stretch) (1450.2) and C=C aromatic weak intensity (1662.44), C-Cl_2 (624.81). These results confirms that the antifungal compound is pyrrolinitrin (Fig. 5). These results are in the same trend of Shraddha *et al*. (2019).

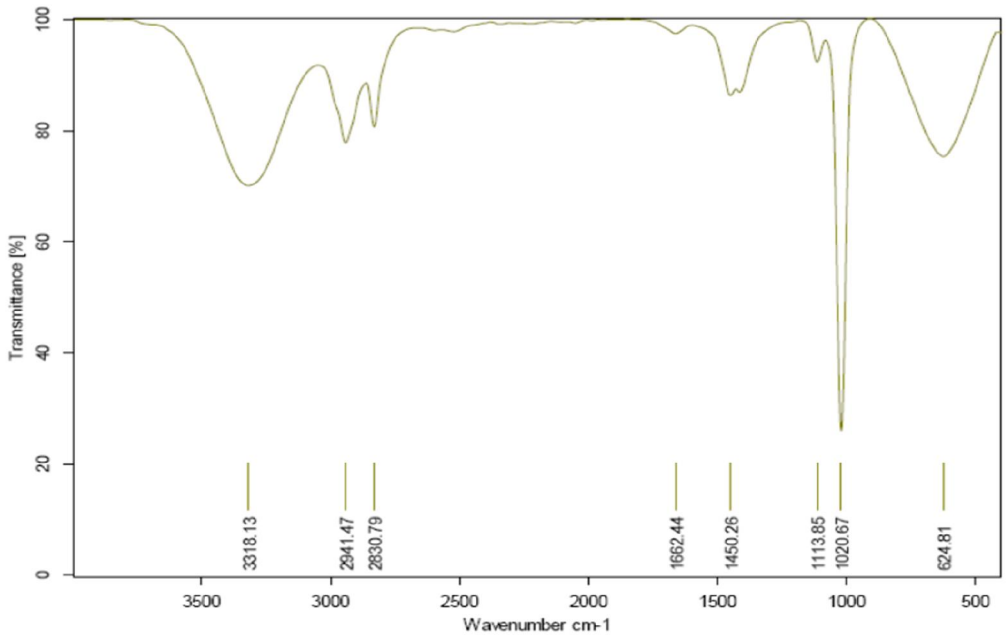

Fig. 5: FTIR for pyrrolnitrin produced by *P. fluorescens.*

From the previous results, the major compound in the antibiotic crude extract is pyrrolnitrin and this antibiotic affects the fungal cell through several mechanisms, the primary target of PRN lies in the cell membrane to impede protein, RNA, DNA synthesis and uncouple the normal electron flow in the respiratory electron transport chain (Warden and Edwards (1976). The metabolite has demonstrated biological activity at low concentration and act as an uncoupler of oxidative phosphorylation in *Neurospora crassa*. High concentration of PRN causes impairment of electron transport in flavin region and cytochrome c oxidase; accumulation of glycerol; synthesis of triacyl glycerol leading to leakage of cell membrane and inhibition of cell growth; *in vitro* activity against bacteria and fungi in the range of 1–100 µg mL^{-1} (Di Santo *et al*., 1998). PRN also functions as a signal molecule, beyond its role as a bioactive molecule to suppress fungal and affected cell motility (Liu *et al*., 2018).

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