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ORIGINAL ARTICLES

Antimicrobial Efficacy of Secondary Metabolites of *Beauveria Bassiana* Against Selected Bacteria and Phytopathogenic Fungi

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ABSTRACT

Fungi are known to produce a vast array of secondary metabolites that are importance for biotechnological applications. Therefore, the focus of this study was to determine the antibacterial and antifungal activity of crude ethyl acetate extract of *Beauvaria bassiana* (B.b.) using agar filter paper bioassay method. It is found that crude extract exhibited antibacterial activity by any concentration used on different strains of G+ and G- bacteria. However, these antibacterial activities against *Bacillus cereus, B. subtilis, Micrococcus leteus and Streptococcus aureus* (v-) and *Escherechia coli* and *Aeromonas* sp. (v+)were shown to be less active when compared to the control streptomycin and penicillin at 100 µg/ml. Among gram positive bacteria *St. aureus* was the most susceptible, while *B. subtilis* was the least susceptible. On the other hand, crude extract of *B. bassiana* exhibited moderate antifungal activity at concentrations between 1200-1600µg/ml against *Alternaria tenuis, Fusarium avenaceum and F. graminearum* without significant difference and against *Aspergillus paraziticus*, *F. moniliforme* and F. *oxysporum* with significant difference.

Key words: Fungi, bacteria, antimicrobial activity, secondary metabolites, Entomopathogenic fungi.

Introduction

Fungi play a major ecosystems along with bacteria, small invertebrates and plant., through complex trophic interactions. Most soil fungi are regarded as, decomposing organic matter and contributing to nutrient cycling. Also, recognized as prolific secondary metabolite producers, fungi have provided several bioactive compounds and chemical models currently used as pharmaceutical. Soils are traditionally the main source of fungal genetic resources for bioprospection programs (Adrio *et al*., 2003).

Fungi produce a wide range of secondary metabolites with high therapeutic value as antibiotics, cytotoxic substances, insecticides, compounds that promote or inhibit growth, attractor and reoellent and are gaining importance for their biotechnological applications. (Demain, 1999, Kishore *et al.*, 2007 and Mabrouk *et al*., 2008). Secondary metabolites produced from fungi vary in production, function and specify to a particular fungus (Keller *et al*., 2002). Entomopathogenic fungi (EPFs) are classified as fungi that infect, invade and eventually kill their insects (Singkaravanit *et al.,* 2010). EPFs have been investigated for use against a broad range of insect pests (Butt *et al*., 2001; Lacey *et al*., 2000 and Ansari *et al*., 2004). Diverse toxic metabolites have been described in several fungal biological control agents including *Beauvaria, Metarhizium and Pacilomyces* (Vey *et al.,* 2001). Some of these metabolites have been found to display antibiotic, fungicidal or insecticidal properties against insect pests and diseases (Kershaw *et al*., 1999, Vey *et al*., 2001 and Ross, 2005). Production of oosporein, beauvencin, bassianolide, and cyclosporine A were commonly observed in some cultures of entomopathogenic fungi (Boucias and Pendland, 1998 and Strasser *et al.,* 2000). Secondary metabolites of *B. bassiana* are reported to have antifungal and antibacterial properties against some pathogens (Parine *et al.,* 2010).

Hence, the purpose of this study to evaluate the bioeffect of *B. bassiana* culture filtrate against selected bacteria and phytopathogenic fungal strains.

Material and Methods

Cultures:

Beauvaria bassiana isolate kindly obtained from Prof. Dr. Alian Vey, (mycology unite control, National De La Research Scientifique, Univ. Montpellier) was selected for the experiment.

Eight fungal organisms pathogenic to plant, i.e., *Alternaria tenuis, Aspergillus niger, Aspergillus paraziticus, Fusarium avenaceum, Fusarium moniliform, Fusarium oxysporum, Fusarium graminearum* and

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J. Appl. Sci. Res., 8(3): 1441-1444, 2012

Penicillium sp. and six bacterial cultures, i.e., *Bacillus cereus, B. subtilis, Micrococcus leteus and Streptococcus aureus* (Gram-) and *Escherechia coli* and *Aeromonas* sp. (Gram+) were used. All fungal and bacterial cultures were obtained from Plant pathology Dept., NRC of Egypt. Cultures were maintained on potato dextrose agar slant for fungi and nutrient agar slant for bacteria and were sub-cultured in Petri-dishes prior to testing.

Fungal Growth And Extraction Of Crude Extract:

B. bassiana cultures grown on Sabouraud's Dextrose Agar for 14 days were used. Conidia were harvested by lightly scraping the fungal surface with a sterile scalpe. One ml of aqueous conidial suspension (10^7 conidia/ml) was inoculated in 100 ml liquid medium in 250 ml Erlenmeyer flasks (Goettel and Inglis, 1984). The flasks were incubated on a rotary shaker (180 rpm) for 7 days at 27 ± 2 ºC.

The fermented broth (3 liters) was treated with ethyl acetate (2 liters) for 2 hours followed by cheesecloth filtration to remove the biomass. The organic extract was separated and dried over anhydrous sodium sulfate and concentrated *in vacuo* to yield a crude yellow solid (0.3g).

Antibacterial And Antifungal Studies:

Streptomycin and Pencillin G (100µg/ml) were used for Gram positive and for Gram negative bacteria respectively as controls. For fungi, fungicide (8 hydroxy quinolin, 100µg/ml) was used as control. The crude extract of *B. bassiana* was made to different concentrations (400-1600 µg/ml) and tested against the bacterial and fungal strains, following the procedure of Kishore *et al*. (2007). The diameter of the zone of inhibition was measured in mm. For each test four replicates were performed.

Statistical Analysis:

Data obtained was statistical analysed using Duncan's multiple range test according to snedecor and Cochran (1980).

Results and Discussion

Antibacterial Activity:

Fungi are well known to show antibacterial, antifungal, larvicidal and antioxidant activities ext. (Demain, 1999; Keller *et al.,* 2002 and Kishore *et al.*, 2007). In the present study different concentrations of crude ethyl acetate extract of *B. bassiana* were tested against 6 bacterial strains and 8 fungal strains. The antibacterial activity of the tested entomopathogenic fungi was apparent in the six bacterial organisms (Table, 1). It is found from the table that there was significant antibacterial activity exhibited by any concentration of *B. bassiana* crude extract on different strains of Gram (+ve and -ve) bacteria (Fig.1). Variation in susceptibility to the culture filtrate extract of the fungus between Gram ve^+ and ve^- bacteria shown in this study could partly be attributed to differences in chemical composition of their cell wall.

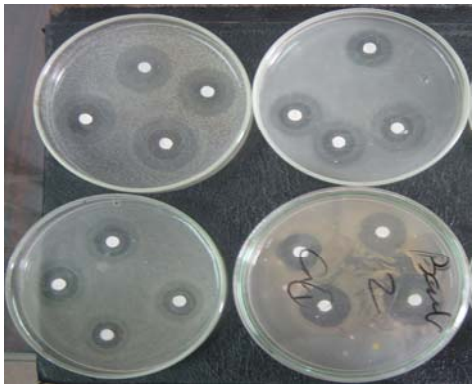

Fig. 1: Zone of inhibition caused by *B. bassiana* culture filterate against some selected gram (+ve and -ve) bacterial strains.

J. Appl. Sci. Res., 8(3): 1441-1444, 2012

Table 1: Testing of antibacterial activity of *B. bassiana* culture filterate against some selected gram (+ve and -ve) bacterial strains (Zone of inhibition values in mm).

Tested organisms	Concentration (µg/ml)				
	400	800	1200	1600	*Antibiotic 100µg/ml
Gram positive					
Micrococcus leteus	9.5 O	14.50 N	17.75 LM	26.25 DEF	46.50 A
Streptococcus aureus	20.5 IJKL	20.25IJKL	21.00IJK	28.50 D	44.75 A
Bacillus cereus	19.75 JKL	23.0 GHI	24.25 FGH	26.50 DEF	33.25 C
Bacillus subtilis	8.50 O	9.50 O	11.50 N	18.25 KLM	44.75 A
Gram negative					
Escherichia coli	13.50 N	14.75 N	17.0 LM	20.75 IJKL	33.25 C
Aeromonas sp.	12.25 N	16.0 MN	21.00 IJK	21.25 IJK	35.50 C

*Positive control for G. ve+ bacteria = Streptomycin ; For G ve- bacteria = Pencillin-G
-Nagative control: Ethyl acetate
-Four replicates were used for each treatment
-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.

There was also significant increase in antibacterial activities found from 400 to 1600 µg/ml against *M. letues* and B. cereus (176.3% and 34.3% increase respectively) ; from 800 to 1600 µg/ml against *St. aureus* and *E. coli* (40.7% and 40.7% increase respectively); from 1200 to 1600 µg/ml against *B. subtilis* (58.7% increase) and 800 to 1200 µg/ml against *Aeromonas* sp. (31.3% increase). According to the finding of Parine *et al*. (2010) the different antibacterial properties of crude extracts of *B. bassiana* depend on the active compounds in the specific bacteria. However, these antibacterial activities against these bacteria were shown to be less active when compared to the control streptomycin and penicillin at 100 µg/ml. As, antibiotic produced about 46.5, 44.8, 33.3, 44.8, 33.3, and 35.5mm diameter of zone of inhibition against *M. leteus, St. aureus, B. cereus, B. subtilis, E.coli and Aeromonas* sp. respectively, while culture filtrate of 1600 µg/ml produced less zone of inhibition by 43.6%, 57.0%, 20.3%, 59.2%, 37.6% and 40.1% respectively.

Among gram positive bacteria *St. aureus* was the most susceptible, while *B. subtilis* was the least susceptible one. This differential action of antibacterial property of crude extract of *B. bassiana* may be depending upon the active compounds on the specific bacteria.

Antifungal Activity:

Table (2) and Fig. (2) show the antifungal activity of *B. bassiana* culture filtrate against some selected phytopathogenic fungal strains. The data showed that there was moderate antifungal activity exhibited by some concentrations of *B. bassiana* crude extract. These moderate antifungal activities were found at concentrations between 1200-1600 µg/ml against *A. tenuis, F. avenaceum and F. graminearum* without significant difference and against *Asp. paraziticus*, *F. moniliforme* and F. *oxysporum* with significant difference.

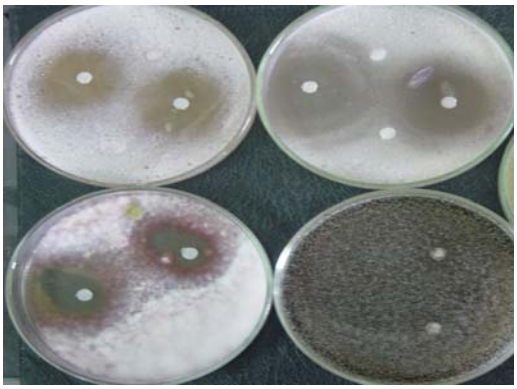

Fig. 2: Zone of inhibition caused by *B. bassiana* culture filterate against some selected phytopathogenic fungi.

However, the *in-vitro* antifungal activity of the culture filtrate extract against a number of phytopathogenic fungi was not promising as that of its activity against test bacteria and this indicate that the extract has a wide spectrum activity. This result is in line with Bitew (2010).

However, the antifungal activities against these fungi were shown to be less activity when compared to the control (hydroxyl quinoline fungicide). As, fungicide (100 µg/ml) produced about 43.8, 37.5, 36.5, 22.5, 43.0 and 43.0mm diameter of zone of inhibition against the phytopathogenic fungi A.*tenuis, Asp. Paraziticus, F. avenaceum, F. graminearum, F. moniliforme* and *F. oxysporum* respectively. while, at 1600 µg/ml produced

less zone of inhibition by 71.4%, 82.0%, 68.5%, 33.3%, 74.4% and 81.4% respectively than the control. Hence, the present study gives an idea to test more number of organisms and to find out the actual antibacterial and antifungal compounds from *B. bassiana*. A vast number of fungi have been utilized for biotransformation process and many more to be explored for isolation of some potential compounds (Vay *et al.,* 2001 and Ansari *et al.,* 2004).

Table 2: Testing of antifungal activity of *B. bassiana* culture filterate against some selected phytopathogenic fungal strains (Zone of inhibition values in mm).

Tested organisms	Concentration (µg/ml)				
	400	800	1200	1600	*Fungicide 100µg/ml
Alternaria tenuis	0 D	0 D	7.25 C	12.5 C	43.75 A
Aspergillus niger	0 D	0 D	0 D	0 D	36.50 B
Aspergillus paraziticus	0 D	0 D	0 D	6.75 C	37.50 B
Fusarium avenaceum	0 D	0 D	7.00 C	11.50C	36.50 B
Fusarium graminearum	0 D	6.50 C	10.00 C	12.00 C	22.50 B
Fusarium moniliform	0 D	0 D	0 D	11.00 C	43.00 A
Fusarium oxysporum	0 D	0 D	0 D	8.50 C	43.00 A
Penicillium sp.	0 D	0 D	0 D	0 D	44.75 A

*Nagative control: Ethyl acetate
-Positive control for fungi= 8 hydroxy quinoline sulphate
-Four replicates were used for each treatment
-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.

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