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Bacterial control of pathogenic fungi isolated from some wild plants in Taif Governorate, Saudi Arabia

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Abstract

Twenty two plants were collected from Taif Governorate, Saudi Arabia and identified. Pathogenic fungi were isolated from some of these plants and identified as *Alternaria alternata*, *Cephalosporium madurae*, *Cladosporium herbarum*, *Fusarium oxysporum*, *Humicola grisea*, *Penicillium chrysogenum*, and *Ulocladium botrytis*. Three antagonistic bacterial isolates (*Bacillus cereus*, *Bacillus firmus* and *Streptomyces alni*) were tested. Extraction with successive selective organic solvents of some plants revealed that the highest residual percentage (31.6%) attained in *Artemisia monosperma*, while the lowest percentage (17.6%) was found in *Euphorbia glomerifera*. Ethlyl alcohol was the best solvent for all species. Also preliminary phytochemical investigation of the shoot system of different plants were carried out. From the results, it is clear that *Artemisia monosperma* contained the highest secondary metabolites, mean while *Avena barbata* contained the lowest secondary material. We found that the three bacterial antagonistic isolates tested inhibit growth of the pathogenic fungi, with different ratios, but *Streptomyces leni* was the most effective one. The results indicated that the antibiotics produced by the antagonists were more effective than the bacteria itself and differ with different bacteria. Also the antibiotics produced by *Streptomyces* were more effective than that of *Bacillus*. Infiltration of plant stems with antagonist extacts reduce the severity of the disease but not prevent it in all tested pathogens.

Key words: Pathogen, antagonist, antibiotic, antimycotic bacteria.
Running title: Bacterial control of pathogenic fungi

Introduction

The phyllosphere of aboveground parts of plants is a dynamic ecosystem inhabited by specific fungi and bacteria. The interactions between microorganisms and plant hosts that lead to biocontrol can include antibiosis, competition, induction of host resistance, and predation (Sobiczewski, 2002; Stromberg *et al.*, 2000). A positive role is played by phyllosphere antagonistic microorganisms, which protect the plants from pathogenic microorganisms and in this way improve their healthiness (Patkowska, 2003). Antagonists of phytopathogenic fungi have been used to control plant diseases. Such properties are first of all exposed by the bacteria from the genera *Bacillus* and *Streptomyces* (Handelsman *et al.*, 1990; Kokalis-Burelle *et al.*, 1992; Franicevic 1993; Michereff *et al.*, 1994; Silo-Suh *et al.*, 1994; Milner *et al.*, 1996; Sonoda *et al.*, 1996; Swadling and Jeffries 1996; Larkin and Farvel, 1998; Essam *et al.*, 2006; Pengnoo *et al.*, 2006; Fagerlund *et al.*, 2008). Extraction is the first important step for the recovery and purification of active ingredients of plant materials. The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of materials and the rate of mass transfer (Wu *et al.*, 2001). Moreover, many natural products are thermally unstable and may degrade during thermal extraction. Renewed interest in plant derived drugs has led to an increased need for more efficient extraction methods (Paniwnyk *et al.*, 2001).

A general description of the vegetation of the western Saudi Arabia has been given by Vesey-Fitzgerald (1957) and recognized a number of vegetational and ecological types including littoral marshes, coastal desert plain, coastal foothills, mountain ranges and wadies. Batanouny (1979); Fayed and Zayad (1989); Mahmoud and El-Tom (1985) and Montealegre *et al.* (2000) described the vegetation of the Makkah-Taif roads and recognized a number of vegetational and ecological types mostly organized in zones. Mossallam and BaZaid (2000) showed that *P. tomentosa* is widespread in Taif and latex of its stem and leaves is irritant to the skin and eyes and can cause inflammation and pain, and if ingested can cause stomach cramps and diarrhea. In medicine it is used as expectorant and purgative.

Many medicinal herbs in nature like *Pulicaria crispa*, *Launaea sonchoides*, *Forsskalea*

tenacissima, Capparis decidua, Prunus persica and *Avena barbata* may be infected by many diseases (Zhenying *et al.*, 2004). So the objective of this work is to protect these plants from fungal diseases by antagonistic bacteria.

Materials and Methods

Surveying and monitoring of some wild plants in Al Taif area

Wild plants were collected from different regions of Taif Governorate, and identified according to Boulos & El-Hadidi (1994) and Boulos (2002).

1- Collection and identification of plants

Collected plants were sorted, cleaned of debris and gently washed to remove as much epiphytic growth as possible. The shoot system of different plants were air-dried and ground to fine powder.

2- Extraction with successive selective organic solvents

A known weight of the fine powder of shoot systems of each plant was successively extracted using petroleum ether, ether, chloroform, acetone and ethyl alcohol. Each of the obtained extracts was dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure. The residues were dried in vacuum desiccators, the amount of the residues was calculated as percentage of air dried plant materials and was added together to get total of the residues (Abd El-Fattah and Galal, 1993).

3- Qualitative tests

The qualitative tests for tannins, unsaturated sterols, terpens, flavonoids, alkaloids, glycosides, saponins, resins, chlorides and sulphates were carried out following the methods described by Wall *et al.* (1964) and Brieskorn *et al.* (1961).

Isolation and Identification of pathogenic fungi from Taif plants

Pieces of plants that showed symptoms of the disease were submerged in 5% sodium hypochloride for five minutes. After this treatment, they were extensively washed with sterile distilled water and placed on Petri dishes containing potato-dextrose-agar (PDA, Difco) amended with streptomycin sulphate (30 mg/liter) and rose bengal (3.3 ml of 1% (w/v)) to eliminate bacterial contamination and incubated at 25^0C for 72 hrs. according to Ismail and Aly (1997); Montealergre *et al.* (2003) and Abou-Zeid *et al.* (2004). The isolated fungal strains were purified and identified, according to Ellis (1976); Booth (1977); Alexopouls & Mims (1979); Domsch, *et al.* (1980), Pitt (1988); Burgess *et al.* (1988) and Klich & Pitt (1988).

In vitro evaluation of the antagonistic potential of the bacterial bioagents tested

Antagonistic reactions between the causal pathogens and the bacterial bioagents were studied *in vitro*. 100 µl of each tested bacterial suspensions were placed in PDA medium inoculated with pathogenic fungus (Montealergre *et al.*, 2003) This experiment was conducted in 3 replicates for each bioagent and plates were incubated for 3 days. Clear zones of growth inhibition were evaluated and inhibition percentage were calculated.

Production of diffusible antibiotics

PDA plates, covered with a cellophane membrane, were inoculated in the center with 100 µl of a bioantagonistic bacterial suspension. After incubation for 72 hrs at 37 ^{o}C, the membrane with the grown organism was removed, and the plate was inoculated in the middle with a 10-mm disk of a pure culture of the pathogen. Plates were further incubated at 25 ^{o}C for 48 hrs and the growth of the pathogen was measured. Controls were run as described above by replacing the antagonists with sterile distilled water (Montealegre *et al.*, 2003).

Control of pathogen by antagonist extracts

The basal portions of plant stems were treated with extracts of various bacterial antagonists before planting in sand beds infected with pathogen. Control was run as mentioned above by replacing the antagonisms by sterile distilled water, and the percent of inhibition was calculated (Jone and Pettit 1987).

Results and Discussion

Ten plant species were collected from Shafa and twelve from Southern route of Taif Governorate and were identified as listed in Tables (1).

Extraction with successive selective organic solvents

One hundred grams of the powdered air dried shoots of eight species was used. The data obtained (Table 2) show that collected plants attained different residual percentages. The highest residual percentage (31.6%) was obtained in *Artemisia monosperma*, while the lowest percentage (17.6%) was in *Euphorbia*

glomerifera. This may be attributed to the habitat condition i.e. *Artemisia monosperma* dominated in bed and plains while *Euphorbia glomerifera* dominated on slopes habitat. In this connection Abd El-Fattah and Galal (1993) recorded that plants collected from Wadi bed habitat attained higher residual percentage than those of Rocky habitat.

Table 1: plants collected and identified from Taif Governorate

regions of isolation in Taif	
shafa	**Southern route**
1- *Artemisia monosperma*	1- *Aerva lanata*
2- *Capparis decidua*	2- *Arnebia hispidissima*
3- *Eucalyptus lobules*	3- *Artemisia judaica*
4- *Euphorbia glomerifera*	4- *Asphodelus aestives*
5- *Juniperus procera*	5- *Avena barbata*
6- *Launaea mucronata*	6- *Foeniculum vulgare*
7- *Medicago sativa*	7- *Forsskalea tenacissima*
8- *Opuntia ficus*	8- *Launea sonchoides*
9- *Prunus persica*	9- *Phagnalon sinaicum*
10-*Punica granatum*	10- *Pulicaria crispa*
	11- *Rumex dentatus*
	12-*Trichodesma calathiforme*

Table 2: The amount of residues in successive extraction of species collected from Taif area.

Species	**Solvent**										
	Pet.Ether		Ether		Chlorof.		Acetone		Eth.Alc.		Total
	%	C	%	C	%	C	%	C	%	C	%
E. glomerifera	3.2	gr	.8	gi	1.6	gr	4.2	bi	7.8	br	17.6
C. decidua	5.1	gi	1.4	gi	1.8	gr	5.9	bi	10.1	br	24.3
P. persica	3.5	bi	1.1	bi	1.4	bi	5.2	bi	6.7	br	17.9
A. monosperma	6.8	br	1.9	bi	3.1	br	7.3	bi	12.5	br	31.6
P. crispa	4.6	ye	1.3	yi	2.1	gi	5.4	bi	8.5	br	21.9
L. sonchoides	5.9	gi	1.6	gi	2.3	gr	6.7	bi	11.2	br	27.7
F. tenacissima	4.2	gi	1	gi	1.6	gr	5.1	bi	8.2	br	20.1
Avena barbata	5.0	gi	1.2	gi	2.2	gr	5.8	bi	9.8	br	24

% = % of air-dry weight, C = colour, gr = green, gi = greenish, br = brown, bi = brownish, ye = yellow, yi = yellowish

The results obtained revealed also that ethlyl alcohol was the best solvent for all species, i.e in case of *A. monosperma* the amount of residues in ethyl alcohol extract was 12.5% out of 31.6% of total residue. The ethyl alcohol was the best solvent for all plant materials due to the higher polarity (Vinatoru *et al.*, 1999)

Qualitative tests

The results of preliminary phytochemical investigations of the shoot system of different plants are given in Table, 3. The obtained results showed the presence of glucosides and or carbohydrates, tannins, terpens, chlorides and sulphates in all studied species. While flavonoides were recorded only in *Artemisia monosperma, Euphorbia glomerifera, Forsskalea tenacissima, Launea sonchoides* and *Pulicaria crispa*. Saponins was recorded in *Avena barbata, Capparis decidua, Prunus persica* and *Pulicaria crispa*. Sterols were recorded in *Artemisia monosperma, Forsskalea tenacissima, Launae sonchoides, Prunus peorsica* and *Pulicaria crispa*. Alkaloides were recorded only in *Artemisia monosperma* and *Euphorbia glomerifera*. Resins were found only in *Artemisia monosperma, Capparis decidua, Euphorbia glomerifera, Launea sonchoides* and *Prunus persica*.

From the results, it is clear that *Artemisia monosperma* attained the highest percentage of secondary metabolites, meanwhile *Avena barbata* contained the lowest secondary metabolites.

Table 3: Preliminary phytochemical screening of species collected from Taif area, S. A.

	Species							
Contituents	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Glucosides and or carbohydrates	+	+	+	+	+	+	+	+
Flavonoides	+	-	-	+	+	+	+	-
Tannins	+	+	+	+	+	+	+	+
Saponins	-	+	+	+	+	-	-	+
Sterols	-	-	+	+	+	+	+	-
Terpens	+	+	+	+	+	+	+	+
Alkaloids	+	-	-	+	-	-	-	-
Resins	+	+	+	+	-	+	-	-
Chlorides	+	+	+	+	+	+	+	+
Sulphates	+	+	+	+	+	+	+	+

1= *E. glomerifera*, 2= *C. deciduas*, 3= *P. peorsica*, 4= *A. monosperma*, 5= *P. crispa*, 6= *L. sonchoides*, 7= *F. tenacissima*, 8= *A. barbata*

From the results, it is also clear that *Artemisia monosperma* contained the highest amount of secondary metabolites, meanwhile *Avena barbata* had lowest amount of these materials in this connection. El-Hady (1990) recorded that in *Centaurea scoparia* collected from wadi bed habitat contained more secondary metabolites than those of rocky ones, which support our results.

Fifteen crude extracts prepared from seven Ethiopian medicinal plants used to treat various infectious diseases were assessed for their ability to inhibit the growth of *Mycobacterium tuberculosis*. A preliminary screening of the crude extracts against *M. tuberculosis* types humanus (ATCC 27294) was done by dilution assay using Löwenstein-Jensen medium. None of the tested extracts except the acetone fraction obtained from the stem bark of *Combretum molle* (R. Br. ex G. Don.) Engl & Diels (Combretaceae) showed significant inhibitory action against this strain (Asres *et al.*, 2001). Dried leaves, flowers and seeds of *Argemone subfusiformis* are also used in Argentina and Peru as febrifuge (Ramirez *et al.*, 1988). *Argemone subfusiformis*, like many other *Argemone* species is characterised by a rich content in isoquinoleinic alkaloids. In above ground parts, protopine, berberine and allocryptopine have been identified. According to Sriwilaijareon *et al.*, (2002) berberine prevents the development of *Plasmodium falciparum* by inhibition of its telomerase activity, so this observation could explain the activity detected with *A. subfusiformis*.

The quantitative insight in processes underlying yield and concentrations of interesting secondary metabolites in crops is still limited. Yet, this insight is essential to further improve commercial production of target metabolites. *Artemisia monosperma* L. (annual or sweet wormwood from Asteraceae) attained the highest secondary metabolites. *A. monosperma* is an annual herb producing the antimalarial artemisinin, a sesquiterpene lactone with an endoperoxide bridge. Artemisinin is predominantly produced in glandular trichomes present on the leaves and inflorescences. Leaves are the most important organs harvested for its commercial production (Lommen *et al.*, 2006). *Avena barbata* attained the lowest value of secondary material, may be due to the behaviour of the plant. Sherrard and Maherali, (2006) showed that *Avena barbata* from the drought strongly influences plant productivity. Decreased photosynthetic capacity (A_{max}) was maladaptive in the dry environment, perhaps because of the respiratory cost associated with maintaining excess enzyme and substrate capacity.

Antagonistic bacteria

Three bacterial strains were selected: *Streptomyces alni* (TU*Sa*120) and 2 isolates belonging to *Bacillus* spp., *B. cereus* (ZU*Bc*71) and *B. firmus* (ZU*Bf*72).

Antagonistic effect of different bacterial strains

The antagonistic effect of different bacterial strains was measured by inhibition zone diameter (Table 4 and Fig. 1) and antibiotic production Table (5). From Table 4 and Fig. 1, we found that *Streptomyces alni* significantly inhibited the growth of all pathogenic fungi tested. The maximum inhibition zones were reported with

Ulocladium botrytis (4.07 cm) and *Fusarium oxysporum* (3.9 cm), followed by *Alternaria alternata* strains and *Humicola grisea* (3.0 cm), while the lowest inhibition zones of 1.8 & 1.77 cm were detected in *Penicillium chrysogenum* and *Cladosporium herbarum* respectively.

With respect to the effect of antibiotics produced by *S. alni*, as shown in Table (5), the growth of all pathogens were completely inhibited by the antibiotic.

The antagonistic effect of *Bacillus firmus* on fungal pathogens was presented in Table 4 and Fig. 1. The highest inhibition zones were found in *Fusarium oxysporum* (2.17 cm), *Penicillium chrysogenum* (2.1 cm) and *Cephalosporium madura* (1.9 cm), followed by *Humicola grisea* (1.77 cm), while the lowest inhibition zone (1.4 cm) was reported with *Cladosporium herbarum* and *Alternaria alternata* isolated from *Launaea sonchoides*.

With respect to the effect of antibiotics produced by *B. firmus* against pathogenic fungi, we found that the highest inhibition percentage (78.55) was reported by *A. alternata* isolated from *Forsskalea tenacissima*, followed by *U. botrytis* where percentage of 66.76 was detected; *Cephalosporium madura* with 63.42 I% and *A. alternata* with 63.16 isolated from *Prunus persica*. While the lowest percentage of 36.41 and 28.57 were detected with *H. grisea* isolated from *A. monosperma* and *A. alternata* isolated from *Avena barbata* respectively (Table 5).

The antagonistic effect of *Bacillus cereus* on fungal pathogen growth was also presented in Table 4 and Fig. 1. The highest inhibition zone (1.53 cm) was presented in *P. chrysogenum*, followed by *U. botrytis* (1.43 cm) and *A. alternata* (1.4 cm) and the lowest inhibition zone of 0.2 cm was reported in *Cladosporium herbarum*.

With respect to the effect of antibiotics produced by *B. cereus*. As shown in Table 5, it was found that the highest inhibition percent of 49.87 was reported with *A. alternata* isolated from *Forsskalea tenacissima*, followed by *P. chrysogenum* (I% 41.2) and *F. oxysporum* of 37.5 I% and the lowest I% of 3.55 was reported by *Cladosporium herbarum*.

Table 4: *In vitro* antagonistic potential of *Streptomyces alni*, *Bacillus cereus* and *B. firmus* on the causal pathogens

Pathogen	**Source of isolation**	**Inhibition zone diameter (cm)**		
		S. alni	***B. cereus***	***B. firmus***
Ulocladium botrytis	*Forsskalea tenacissima*	4.07	1.43	1.67
Alternaria alternata	*Prunus persica*	3.43	1.1	1.87
Alternaria alternata	*Euphorbia glomerifera*	3.3	1.17	1.43
Alternaria alternata	*Avena barbata*	3.27	1.17	1.5
Alternaria alternata	*Forsskalea tenacissima*	3.5	1.4	1.87
Alternaria alternata	*Launaea sonchoides*	2.67	1.1	1.4
Cladosporium herbarum	*Pulicaria crispa*	1.77	0.2	1.4
Cephalosporium madurae	*Launaea sonchoides*	2.43	0.9	1.9
Penicillium chrysogenum	*Capparis decidua*	1.83	1.53	2.1
Fusarium oxysporum	*Prunus persica*	3.9	1.3	2.17
Humicola grisea	*Artemisia monosperma*	3.0	1.23	1.77

Table 5: *In vitro* antagonistic potential of antibiotics produced by *Streptomyces alni*, *Bacillus cereus* and *B. firmus* on the causal pathogens.

Pathogen	Source of isolation	Inhibition percentage		
		S. alni	***B. cereus***	***B. firmus***
Ulocladium botrytis	*Forsskalea tenacissima*	100	21.62	66.76
Alternaria alternata	*Prunus persica*	100	16.67	63.16
Alternaria alternata	*Euphorbia glomerifera*	100	20.21	30.61
Alternaria alternata	*Avena barbata*	100	21.32	28.57
Alternaria alternata	*Forsskalea tenacissima*	100	49.87	78.55
Alternaria alternata	*Launaea sonchoides*	100	9.3	33.5
Cladosporium herbarum	*Pulicaria crispa*	100	3.55	59.4
Cephalosporium madurae	*Launaea sonchoides*	100	9.43	63.42
Penicillium chrysogenum	*Capparis decidua*	100	41.21	61.2
Fusarium oxysporum	*Prunus persica*	100	37.51	53.68
Humicola grisea	*Artemisia monosperma*	100	17.52	36.41

So we can concluded that the inhibition effect of bacteria on the tested pathogenic fungi differed with different bacterial strains. The highest inhibition percentage was reported by *Streptomyces alni* followed by *Bacillus firmus* and the lowest effect was recorded by *B. cereus* . Also the effects of antibiotics produced by the antagonists were more effective than the bacterial strain itself and differed with different bacteria.

Control of pathogen by antagonist extracts

It was found that infiltration of plant stems with antagonist extracts reduced the severity of the disease but did not prevent it in all tested pathogens.

Streptomyces and *Bacillus* are a group of antibiotic-producing bacteria that are used for biological control of plant diseases. Isolates of *Bacillus* and *Pseudomonas* spp., in daual culture on agar plates, produced a zone of inhibition, an area of browning of the pathogens, or grew rapidly over the pathogen and inhibited their growth (Franicevic, 1993). Bacterial biocontrol agents belonging to the genera *Agrobacterium, Bacillus, Pseudomonas,* and *Streptomyces*, have been found by observing zones of inhibition in Petri plates (Larkin and Fravel, 1998). Theses results agree with our results.

Bacillus spp. isolates have shown the capacity to control early leaf spot of peanut (Kokalis-Burelle *et al.*, 1992), yam leaf spot (Michereff *et al.*, 1994), grey mould of strawberries (Swadling and Jeffries 1996), and post-bloom fruit drop of citrus (Sonoda *et al.*, 1996), which support our results.

Pengnoo *et al.* (2006) found that 16 isolates of *Bacillus* spp. had the ability to inhibit mycelial growth of *Rhizoctonia solani*, causal agent of leaf blight of bambara groundnut. Among these isolates, *Bacillus firmus* had the greatest activity in anti-microbial tests against *Rhizoctonia solani*. Also our results indicated that our *B. firmus* strain was more active than *B. cereus* strain. On the other hand Essam *et al.* (2006) found that *Bacillius subtilis* 1020 and *B. cereus* 1080 showed highest antifungal activity against the pathogen, *Penicillium italicum.*

Handelsman *et al.* (1990) found that of the 700 bacterial isolates tested , for biological control of alfalfa (*Medicago sativa* L.) damping-off caused by *Phytophthora megasperma* f. sp. *Medicaginis*, only *Bacillus cereus* strain UW85, reduced seedling mortality to 0% in the initial screen and in two secondary screens. Silo-Suh *et al.* (1994) purified two antibiotics produced by *B. cereus* and showed that one of them, designated zwittermicin A, was an aminopolyol of 396 Da that was cationic at pH 7.0; the second, designated as antibiotic B, appeared to be an aminoglycoside containing a disaccharide. Both antibiotics prevented disease of alfalfa seedlings caused by *P. Medicaginis*. Milner *et al.* (1996) determined the chemical structure, regulation, and the target range of one of the antibiotics. The antibiotic was identified as 3-amino-3-deoxy-D-glucose, also known as kanosamine. Kanosamine was highly inhibitory to growth of plant-pathogenic oomycetes and moderately inhibitory to certain fungi and inhibited few bacterial species tested. All these

results supported our data. Fagerlund *et al.* (2008) proposed that *Bacillus cereus* produced three putative enterotoxins, haemolysin BL (Hbl), cytotoxin K and non-haemolytic enterotoxin (Nhe). Both Hbl and Nhe are three-component cytotoxins and maximal cytotoxicity of Nhe against epithelia is dependent on all three components.

Several *Streptomyces* spp. or unidentified isolates belonging to actinomycetes have been shown to possess an antifungal activity against some *Alternaria* species *in vitro* (Sharma and Sinha, 1989). On the other hand, numerous *Streptomyces* isolates have been reported to produce antibiotics that are more or less successful in controlling plant diseases caused by *Alternaria*, particularly by seed treatment (Tahvonen and Avikainen, 1987). The two non-glucosidic antifungal macrolides (Galbonolidies A and B) produced by *Streptomyces galbus* were active against broad spectrum of fungi including several plant pathogens (Fauth *et al.*, 1986).

Also Smither-Kopperl *et al.* (2001) reported *Streptomyces* isolates that have significant antifungal activity against fungal pathogens, including *Fusarium, Pythium, Colletotrichum and Rhizoctonia*. The effectiveness of two *Streptomyces* spp. strains to control pathogenic fungi was studied in stored maize grain (Bressan 2003). Treatments with *Streptomyces* strains alone effectively suppressed the development of *Aspergillus* spp., *Curvularia lunata*, and *Drechslera maydis* and significantly ($p < 0,05$) reduced the incidence of *Fusarium subglutinans* and *Cephalosporium acremonium*. All these results agreed with the present results. Thirty one bacterial isolates (eubacteria and actinomycetes) showed antifungal activity against the fungal pathogen, *Penicillium italicum* (Essam *et al.*, 2006). The most active antifungal actinomycetes was *Streptomyces alni*. This result completely support our results which indicated that *S. alni* was the most active strain tested.

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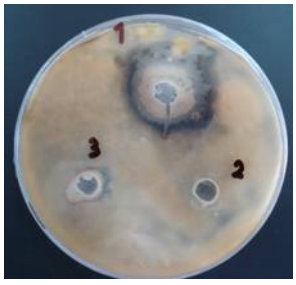
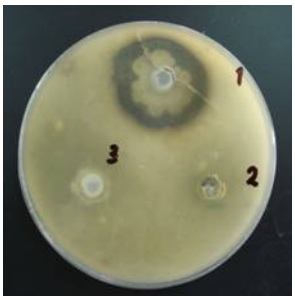
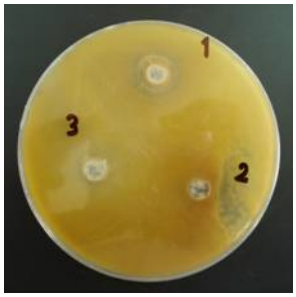
Growth inhibition of *U. bo*trytis by different bacterial strains



Growth inhibition of *A. alternata* (*A. barbata*) by different bacterial strains

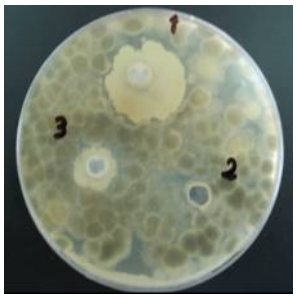


Growth inhibition of *A. alternata* (*E. glomerifera*) by different bacterial strains



Growth inhibition of *Cl. herbarum* by different bacterial strains

Growth inhibition of *C. madurae* by different bactrial strains



Growth inhibition of *H. grisa* by different bacterial strains

Figure 1: Examples *in vitro* inhibition assay. Different bacterial isolates [*Streptomyces alni* (1); *Bacillus cereus* (2) and *B. firmus* (3)] are tested for their ability to inhibit the growth of some tested pathogenic fungi. Zones of growth inhibition can be detected around bacterial strains.

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