Pak. J. Bot., 46(3): 1093-1099, 2014.

INHIBITION OF *IN VITRO* GROWTH OF SOIL-BORNE PATHOGENS BY COMPOST-INHABITING INDIGENOUS BACTERIA AND FUNGI

NADIA RAMZAN*, NAYARA NOREEN AND SALEEM SHAHZAD

Pest & Disease Research Lab., Department of Agriculture & Agribusiness Management, University of Karachi, Karachi-75270, Pakistan
Corresponding author's email: nrbotany2006@yahoo.com

Abstract

During the present studies, compost-inhabiting microorganisms including 44 fungi and 15 bacteria isolated from different compost samples were evaluated for their *in vitro* efficacy against soil-borne pathogens *viz., Fusarium solani, Macrophomina phaseolina, Pythium aphanidermatum, Rhizoctonia solani,* and *Sclerotium rolfsii.* Compost inhabiting microbes like *Trichoderma harzianum, T. virens, Bacillus cereus, B. pumilus, B. subtilis, Micrococcus varians* and *Pseudomonas fluorescens* were found to inhibit all the test pathogens. *Acrophialophora fusispora* and *Penicillium citrinum* reduced the mycelial growth of all the test pathogens except *Sclerotium rolfsii*. *Bacillus licheniformis* and *Bacillus megaterium* showed biocontrol activity against all the pathogens except *Rhizoctonia solani*. *Trichoderma harzianum* parasitized mycelia of all the tested pathogens and produced coiling around the mycelium.

Introduction

Compost is a solid mature product obtained by the controlled biological decomposition of organic matter by different microorganisms like bacteria and fungi. These microorganisms decompose the organic matter in to a stable product that contains digested organic matter and nutrients, which can be easily utilize by plants (Rebollido *et al.,* 2008). Soils generally have complex compounds and low level of nutrients. Compost amendments generally enhance the nutrient activity of soil and stimulate plant growth as well as the development of microflora (Bailey & Lazarovits, 2003; Muhammad & Amusa, 2003).

Soil-borne pathogens like *Fusarium solani*, *Macrophomina phaseolina*, *Pythium aphanidermatum*, *Rhizoctonia solani* and *Sclerotium rolfsii* are known to produce serious losses to crop plants (Ghaffar, 1998; Agrios, 2005). Chemical control is often not sufficiently helpful, and hazardous for the environment. Use of biological control provides an alternative safe method of control instead of chemicals.

Use of composts for disease suppression is economical and eco-friendly. Soil amendment by compost suppressed the diseases caused by soil-borne pathogens in different crops (Hoitink *et al.*, 2001; Reuveni *et al.*, 2002; Noble & Conventry, 2005). Lewis *et al.,* (1992) found that cotton stand improved by compost treatment that also appreciably reduced the inoculum of *R. solani* in soil. Rhizospheric and root associated bacteria isolated from suppressive compost-based plant growth media provided protection to tomato plants against *Fusarium oxysporum* (Kavroulakis *et al.,* 2010). *Phytophthora* and *Pythium* root rots in many plants were suppressed by beneficial microbes found in compost (Rijckeboer *et al.,* 2002; Postma *et al.,* 2003).

Generally, the main protection mechanism against plant pathogens depends on microbial activity in the compost. Certain microbial inhabitants of composts *viz*., *Aspergillus, Bacillus, Penicillium, Pseudomonas, Streptomyces,* and *Trichoderma* species are potential biocontrol agents (Hoitink *et al.,* 1997; De Ceuster & Hoitink, 1999; Tilston *et al.,* 2002; Fuchs & Larbi 2004). Control of pathogens by compost application may involve different mechanisms such as competition for nutrients, antibiotic production by beneficial microorganisms or activation of disease-resistance genes in plants (Hoitink & Boehm, 1999). The aim of the present study was to investigate the *in vitro* inhibition and suppression of soil-borne pathogens *viz., F. solani*, *M. phaseolina*, *P. aphanidermatum*, *R. solani*, and *S. rolfsii* by indigenous fungi and bacteria isolated from compost samples collected from Karachi.

Materials and Methods

Isolation of pathogens: *F. solani*, *R. solani*, and *M. phaseolina* were isolated from roots and seeds of mung bean (*Vigna radiata* (L.) Wilczek). Cultures of *P. aphanidermatum* and *S. rolfsii* were obtained from Karachi University Culture Collection (KUCC).

Isolation of compost inhabiting microbes: Bacteria and fungi were isolated from different compost samples using serial dilution (Waksman & Fred, 1922) and soil plate (Warcup, 1950) methods. Five days old colonies used to identify fungi after reference to Ellis (1971), Barnette & Hunter (1972), and Domsch *et al.*, (1980). Bacterial identification carried out after 2-3 days incubation on the bases of morphological as well as biochemical tests following the standard procedure describe in Bergey's manual (Holt *et al.,* 1994).

***In vitro* interactions of soil-borne pathogens with compost-inhabiting microbes:** Interactions of soil-borne pathogens with isolated microorganisms were evaluated by dual culture plate assays. A 5mm inoculum disc of a pathogen was cut from an actively growing colony on potato sucrose agar (PSA) medium and placed near the edge of a Petri plate containing PSA medium. A similar inoculums disc of a test fungus was placed at the opposite end of the Petri plate, whereas, bacteria were streaked at the opposite end. There were three replicates of each treatment. The plates were incubated at 28±2°C and colonies diameter of pathogens and test microorganisms

were measured at 24 hours intervals and the type of interaction was recorded using the following key:

A: Test microorganism inhibited growth of the pathogen and produced coiling around mycelium of the pathogen.
B: Colonies of test microorganism and pathogen met each other; No further growth of either the pathogen or the test microorganism observed.
C: Test microorganism inhibited growth of the pathogen and produced a zone of inhibition.
D: Pathogen inhibited and overgrew the colony of test microorganism.
E: Colonies of pathogen and test microorganism intermingled.

Results

During the present studies, 20 fungi and seven bacteria showed biocontrol potential in dual culture plate assay against the soil-borne pathogens (Tables 1-5, Fig. 1).

***Fusarium solani*:** Six fungi *viz*., *Acrophialophora fusispora, Penicillium chrysogenum, P. citrinum, Stachybotrys atra, Trichoderma harzianum* and *T. virens*, and seven bacteria *viz*., *Bacillus cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, Micrococcus varians* and *Pseudomonas fluorescens* inhibited the growth of *F. solani* (Table 1).

***Macrophomina phaseolina*:** Growth of *M. phaseolina* was inhibited by 11 fungi *viz., A. fusispora, Aspergillus flavus A. fumigatus, A. niger, Drechslera hawaiiensis, Emericella nidulans, Penicillium chrysogenum, P. citrinum, S. atra, T. harzianum* and *T. virens*, and seven bacteria *viz*., *B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, M. varians* and *P. fluorescens* (Table 2).

***Pythium aphanidermatum*:** 20 fungi *viz., A. fusispora, A. flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, A. terreus* var. *flococcus, Botrytis cinerea, Chaetomium thermophilum* var. *coprophile, Conidiobolus thermophilus, Corynascus sepedonium, E. nidulans, E. nivea, E. rugulosa, Paecilomyces variotii, P. chrysogenum, P. citrinum, S. atra, T. harzianum* and *T. virens*, and seven bacteria *viz*., *B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, M. varians* and *P. fluorescens* inhibited the growth of *P. aphanidermatum* (Table 3).

***Rhizoctonia solani*:** Five fungi *viz*., *A. fusispora, A. niger, P. citrinum, T. harzianum* and *T. virens*, and five bacteria *viz., Bacillus cereus, B. pumilus, B. subtilis, M. varians* and *P. fluorescens* were antagonistic to *R. solani* (Table 4).

***Sclerotium rolfsii*:** Growth of *S. rolfsii* was inhibited by only 2 fungi *viz., T. harzianum* and *T. virens*, and seven bacteria *viz*., *B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, M. varians* and *P. fluorescens* (Table 5).

Table 1. *In vitro* interactions of *Fusarium solani* with fungi and bacteria isolated from compost.

Test microorganisms / Fungi	Days of incubation	Colony diameter (mm)		Type of interaction
		Pathogen	Test micro-organism	
Acremonium thermophilum	5	35	56	D
Acrophialophora fusispora	6	60	22	C
Alleschleria crocea	4	35	61	D
Alternaria alternata	6	59	33	D
Annelophora africana	5	42	50	D
Aspergillus flavus	6	38	50	D
Aspergillus fumigatus	5	35	54	D
Aspergillus nidulans	5	38	44	D
Aspergillus niger	6	46	34	D
Aspergillus terreus	7	59	30	D
Aspergillus terreus var. *flococcus*	7	50	38	D
Aureobasidium pullulans	4	31	60	D
Botryotrichum sp.	6	48	43	D
Botrytis cinerea	8	65	29	D
Chaetomium globossum	5	55	35	E
Chaetomium thermophilum var. *coprophile*	7	48	42	E
Chaetomium thermophilum var. *dissitum*	8	45	45	E
Conidiobolus thermophilus	7	56	37	D
Coniothyrium sp.	5	32	58	D
Corynascus sepedonium	7	56	35	D
Drechslera hawaiiensis	6	47	44	D
Emericella nidulans	7	65	26	D
Emericella nivea	8	45	44	D
Emericella rugulosa	6	57	35	D
Gilmaniella humicola	5	29	61	D
Humicola fuscoatra	5	32	60	D
Humicola grisea	5	31	60	D
Mucor fragalis	3	23	59	E
Mucor sp.	3	19	73	E
Myrothecium sp.	9	61	30	D
Paecilomyces fumosoroseus	6	46	45	D
Paecilomyces variotii	5	36	54	E
Papulaspora thermophila	5	36	56	D
Penicillium chrysogenum	7	46	42	C
Penicillium citrinum	7	53	23	C
Rhizomucor pusillus	5	30	60	E
Scytalidium sp.	6	45	45	E
Scytalidium thermophilum	8	54	37	D
Stachybotrys atra	10	70	18	C
Syncephalastrum racemosum	6	35	55	E
Talaromyces thermophilus	6	46	46	D
Thermomyces lanuginosus	5	47	44	D
Trichoderma harzianum	4	29	64	A
Trichoderma virens	4	28	60	B
Bacteria				
Aeromonas sp.	7	72	21	D
Bacillus cereus	7	60	22	C
Bacillus licheniformis	7	63	20	C
Bacillus megaterium	7	62	25	C
Bacillus pumilus	6	63	24	C
Bacillus stearothermophilus	7	65	25	E
Bacillus subtilis	7	66	19	C
Enterococcus sp.	7	69	23	D
Escherichia coli	7	66	25	D
Geobacillus toebii	7	68	23	D
Klebsiella sp.	7	69	22	D
Micrococcus varians	7	65	20	C
Pseudomonas fluorescens	7	68	22	B
Staphylococcus aureus	7	67	23	E
Thermus thermophilus	6	68	22	D

Table 2. *In vitro* interactions of *Macrophomina phaseolina* with fungi and bacteria isolated from compost.

Test microorganisms / Fungi	Days of incubation	Colony diameter (mm)		Type of interaction
		Pathogen	Test micro-organism	
Acremonium thermophilum	4	52	40	D
Acrophialophora fusispora	4	68	17	C
Alleschleria crocea	4	58	36	D
Alternaria alternata	4	67	24	D
Annelophora africana	4	47	45	D
Aspergillus flavus	4	59	30	C
Aspergillus fumigatus	3	54	33	C
Aspergillus nidulans	5	51	39	E
Aspergillus niger	4	53	54	B
Aspergillus terreus	4	68	23	D
Aspergillus terreus var. *flococcus*	5	68	22	E
Aureobasidium pullulans	4	26	65	D
Botryotrichum sp.	4	61	29	D
Botrytis cinerea	4	76	15	D
Chaetomium globossum	4	64	26	E
Chaetomium thermophilum var. *coprophile*	4	67	23	E
Chaetomium thermophilum var. *dissitum*	4	70	20	E
Conidiobolus thermophilus	4	57	40	E
Coniothyrium sp.	4	32	58	D
Corynascus sepedonium	4	66	24	E
Drechslera hawaiiensis	5	63	26	B
Emericella nidulans	5	66	18	C
Emericella nivea	4	67	23	E
Emericella rugulosa	4	66	24	E
Gilmaniella humicola	4	36	54	D
Humicola fuscoatra	4	41	49	E
Humicola grisea	4	35	55	E
Mucor fragalis	3	46	46	E
Mucor sp.	3	34	57	E
Myrothecium sp.	5	73	17	E
Paecilomyces fumosoroseus	4	47	44	D
Paecilomyces variotii	4	56	34	E
Papulaspora thermophila	4	34	56	D
Penicillium chrysogenum	4	67	24	B
Penicillium citrinum	4	64	20	C
Rhizomucor pusillus	3	44	46	E
Scytalidium sp.	4	63	27	E
Scytalidium thermophilum	4	65	25	D
Stachybotrys atra	5	69	17	C
Syncephalastrum racemosum	4	58	38	E
Talaromyces thermophilus	4	51	39	D
Thermomyces lanuginosus	4	49	42	D
Trichoderma harzianum	3	35	53	A
Trichoderma virens	4	52	45	B
Bacteria				
Aeromonas sp.	4	67	24	D
Bacillus cereus	4	66	18	C
Bacillus licheniformis	4	64	20	C
Bacillus megaterium	4	65	22	C
Bacillus pumilus	4	63	25	C
Bacillus stearothermophilus	5	65	25	E
Bacillus subtilis	4	65	20	C
Enterococcus sp.	4	70	23	D
Escherichia coli	4	68	25	D
Geobacillus toebii	4	68	24	D
Klebsiella sp.	4	69	22	D
Micrococcus varians	4	60	26	C
Pseudomonas fluorescens	4	66	22	C
Staphylococcus aureus	5	67	23	E
Thermus thermophilus	5	69	21	D

Table 3. *In vitro* interactions of *Pythium aphanidermatum* with fungi and bacteria isolated from compost.

Test microorganisms / Fungi	Days of incubation	Colony diameter (mm)		Type of interaction
		Pathogen	Test micro-organism	
Acremonium thermophilum	3	56	27	D
Acrophialophora fusispora	4	70	17	C
Alleschleria crocea	4	57	46	D
Alternaria alternata	3	69	21	E
Annelophora africana	4	57	45	D
Aspergillus flavus	3	63	25	C
Aspergillus fumigatus	3	60	31	B
Aspergillus nidulans	3	62	30	B
Aspergillus niger	3	57	29	C
Aspergillus terreus	3	67	20	C
Aspergillus terreus var. *flococcus*	4	64	21	C
Aureobasidium pullulans	3	32	57	D
Botryotrichum sp.	3	62	28	D
Botrytis cinerea	3	62	17	C
Chaetomium globossum	5	64	26	E
Chaetomium thermophilum var. *coprophile*	4	59	25	C
Chaetomium thermophilum var. *dissitum*	4	72	19	D
Conidiobolus thermophilus	3	48	37	C
Coniothyrium sp.	2	36	55	D
Corynascus sepedonium	4	66	20	C
Drechslera hawaiiensis	4	65	26	D
Emericella nidulans	4	73	16	C
Emericella nivea	4	62	23	C
Emericella rugulosa	4	64	24	C
Gilmaniella humicola	3	36	55	D
Humicola fuscoatra	3	29	61	D
Humicola grisea	3	38	52	D
Mucor fragalis	3	54	44	E
Mucor sp.	3	54	42	E
Myrothecium sp.	4	77	14	D
Paecilomyces fumosoroseus	4	44	46	D
Paecilomyces variotii	3	55	35	B
Papulaspora thermophila	4	35	56	D
Penicillium chrysogenum	4	68	20	C
Penicillium citrinum	3	66	19	C
Rhizomucor pusillus	3	50	40	E
Scytalidium sp.	4	63	30	D
Scytalidium thermophilum	3	63	28	D
Stachybotrys atra	4	74	13	C
Syncephalastrum racemosum	4	60	30	E
Talaromyces thermophilus	4	49	45	D
Thermomyces lanuginosus	4	48	42	D
Trichoderma harzianum	3	46	56	A
Trichoderma virens	3	47	55	B
Bacteria				
Aeromonas sp.	4	68	23	D
Bacillus cereus	4	60	18	C
Bacillus licheniformis	4	65	15	C
Bacillus megaterium	3	60	22	C
Bacillus pumilus	4	55	25	C
Bacillus stearothermophilus	4	67	23	E
Bacillus subtilis	3	60	25	C
Enterococcus sp.	4	78	18	D
Escherichia coli	3	79	17	D
Geobacillus toebii	4	67	24	D
Klebsiella sp.	3	71	20	D
Micrococcus varians	3	54	24	C
Pseudomonas fluorescens	3	60	21	C
Staphylococcus aureus	4	70	22	D
Thermus thermophilus	3	69	22	D

Table 4. *In vitro* interactions of *Rhizoctonia solani* with fungi and bacteria isolated from compost.

Test microorganisms / Fungi	Days of incubation	Colony diameter (mm)		Type of interaction
		Pathogen	Test micro-organism	
Acremonium thermophilum	3	53	42	D
Acrophialophora fusispora	4	69	17	C
Alleschleria crocea	3	40	57	D
Alternaria alternata	4	71	21	D
Annelophora africana	3	48	46	D
Aspergillus flavus	4	58	32	E
Aspergillus fumigatus	3	61	32	D
Aspergillus nidulans	3	68	25	D
Aspergillus niger	4	52	36	C
Aspergillus terreus	4	67	25	D
Aspergillus terreus var. *flococcus*	4	70	23	D
Aureobasidium pullulans	3	29	62	D
Botryotrichum sp.	3	63	28	D
Botrytis cinerea	4	76	16	D
Chaetomium globossum	4	70	20	D
Chaetomium thermophilum var. *coprophile*	4	65	26	D
Chaetomium thermophilum var. *dissitum*	4	74	17	D
Conidiobolus thermophilus	3	63	30	D
Coniothyrium sp.	3	36	55	D
Corynascus sepedonium	4	73	21	D
Drechslera hawaiiensis	3	67	27	D
Emericella nidulans	4	78	13	D
Emericella nivea	4	71	23	D
Emericella rugulosa	4	68	22	E
Gilmaniella humicola	3	35	55	D
Humicola fuscoatra	3	44	48	D
Humicola grisea	3	39	52	D
Mucor fragalis	3	48	45	D
Mucor sp.	3	49	49	D
Myrothecium sp.	4	75	16	D
Paecilomyces fumosoroseus	3	47	44	D
Paecilomyces variotii	3	61	30	D
Papulaspora thermophila	4	41	53	D
Penicillium chrysogenum	3	72	19	D
Penicillium citrinum	4	67	20	C
Rhizomucor pusillus	3	61	41	D
Scytalidium sp.	3	63	28	D
Scytalidium thermophilum	3	64	27	D
Stachybotrys atra	4	78	12	D
Syncephalastrum racemosum	3	62	34	D
Talaromyces thermophilus	4	58	33	D
Thermomyces lanuginosus	4	46	47	D
Trichoderma harzianum	4	51	50	A
Trichoderma virens	4	50	55	B
Bacteria				
Aeromonas sp.	4	70	22	D
Bacillus cereus	3	64	25	C
Bacillus licheniformis	3	65	25	E
Bacillus megaterium	3	70	20	D
Bacillus pumilus	3	65	21	C
Bacillus stearothermophilus	4	65	25	D
Bacillus subtilis	3	69	20	B
Enterococcus sp.	4	79	15	D
Escherichia coli	4	76	15	D
Geobacillus toebii	4	74	18	D
Klebsiella sp.	4	71	20	D
Micrococcus varians	3	64	20	C
Pseudomonas fluorescens	3	68	21	C
Staphylococcus aureus	3	73	20	D
Thermus thermophilus	4	68	23	D

Table 5. *In vitro* interactions of *Sclerotium rolfsii* with fungi and bacteria isolated from compost.

Test microorganisms / Fungi	Days of incubation	Colony diameter (mm)		Type of interaction
		Pathogen	Test micro-organism	
Acremonium thermophilum	4	55	40	D
Acrophialophora fusispora	4	71	19	D
Alleschleria crocea	3	61	31	D
Alternaria alternata	4	68	24	D
Annelophora africana	4	47	46	D
Aspergillus flavus	4	64	30	D
Aspergillus fumigatus	4	56	36	D
Aspergillus nidulans	4	61	32	D
Aspergillus niger	4	64	32	D
Aspergillus terreus	4	66	25	D
Aspergillus terreus var. *flococcus*	4	73	24	D
Aureobasidium pullulans	4	32	58	D
Botryotrichum sp.	4	68	26	D
Botrytis cinerea	5	80	15	D
Chaetomium globossum	5	70	21	D
Chaetomium thermophilum var. *coprophile*	4	65	31	D
Chaetomium thermophilum var. *dissitum*	4	71	24	D
Conidiobolus thermophilus	3	54	39	D
Coniothyrium sp.	3	41	51	D
Corynascus sepedonium	4	78	15	D
Drechslera hawaiiensis	4	67	27	D
Emericella nidulans	4	80	12	D
Emericella nivea	4	70	23	D
Emericella rugulosa	4	67	25	D
Gilmaniella humicola	3	35	57	D
Humicola fuscoatra	3	44	47	D
Humicola grisea	3	36	56	D
Mucor fragalis	4	51	44	D
Mucor sp.	4	49	53	D
Myrothecium sp.	5	74	17	D
Paecilomyces fumosoroseus	4	47	44	D
Paecilomyces variotii	4	61	32	D
Papulaspora thermophila	4	55	36	D
Penicillium chrysogenum	4	74	23	D
Penicillium citrinum	4	68	25	D
Rhizomucor pusillus	3	47	45	D
Scytalidium sp.	4	59	32	D
Scytalidium thermophilum	4	61	29	D
Stachybotrys atra	5	85	15	D
Syncephalastrum racemosum	3	61	33	D
Talaromyces thermophilus	4	56	39	D
Thermomyces lanuginosus	3	50	42	D
Trichoderma harzianum	3	37	54	A
Trichoderma virens	4	53	56	B
Bacteria				
Aeromonas sp.	3	78	14	D
Bacillus cereus	4	55	20	C
Bacillus licheniformis	4	53	23	C
Bacillus megaterium	4	56	24	C
Bacillus pumilus	4	54	28	C
Bacillus stearothermophilus	4	78	12	D
Bacillus subtilis	4	55	25	C
Enterococcus sp.	4	66	25	D
Escherichia coli	3	67	22	D
Geobacillus toebii	4	75	26	D
Klebsiella sp.	4	70	22	D
Micrococcus varians	4	50	20	C
Pseudomonas fluorescens	4	74	12	C
Staphylococcus aureus	4	72	22	D
Thermus thermophilus	4	68	23	E

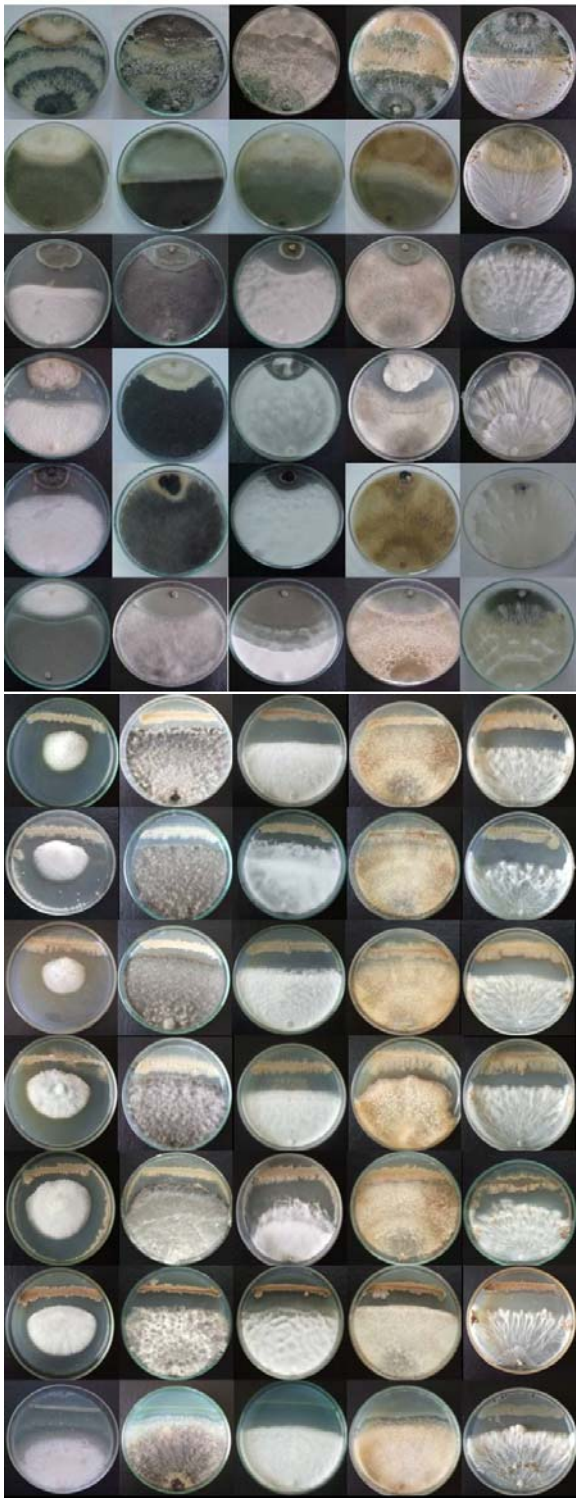

Fig. 1. Interactions of soil-borne pathogens with microorganisms isolated from compost (Pathogens at the bottom and antagonists at the top side of the plates).

Columns (from left): *Fusarium solani, Macrophomina phaseolina, Pythium aphanidermatum, Rhizoctonia solani,* and *Sclerotium rolfsii.*

Rows (from top): *Trichoderma harzianum, T. virens, Penicillium citrinum, Acrophialophora fusispora, Stachybotrys atra, Aspergillus fumigatus, Bacillus cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis, Micrococcus varians* and *Pseudomonas fluorescens.*

Discussion

Of the 44 fungi and 15 bacteria isolated from different compost samples, 20 species of fungi belonging to 12 genera *viz*., *Acrophialophora, Aspergillus, Botrytis, Chaetomium, Conidiobolus, Corynascus, Drechslera, Emericella, Paecilomyces, Penicillium, Stachybotrys* and *Trichoderma,* and seven species of bacteria belonging to three genera *viz*., *Bacillus, Micrococcus* and *Pseudomonas* showed antagonism against different soil-borne pathogens used during the present studies. No previous report on antagonistic activity of *M. varians* is available but in our studies, it successfully inhibited the growth of all the pathogens. *Trichoderma harzianum* parasitized all the pathogens and produced coiling around their mycelia, whereas, *Trichoderma virens* did not produce any coiling around the mycelium of the pathogens. Bacteria *viz*., *B. cereus, B. pumilus, B. subtilis, M. varians* and *P. fluorescens* inhibited all the test pathogens and produced zones of inhibition. *B. licheniformis* and *B. megaterium* showed biocontrol activity against all the pathogens except *R. solani. A. fusispora* and *P. citrinum* also produced zones of inhibition against all the pathogens except *S. rolfsii*. The maximum zones of inhibition were produced by *P. citrinum* against *F. solani* and *M. varians* against *M. phaseolina, P. aphanidermatum, R. solani* and *S. rolfsii*.

A. thermophilum, A. crocea, A. alternata, A. africana, A. pullulans, Botryotrichum sp., *C. thermophilum* var. *dissitum, Coniothyrium* sp., *G. humicola, H. fuscoatra, H. grisea, Mucor* sp., *Myrothecium* sp., *P. fumosoroseus, P. thermophila, R. pusillus, Scytalidium* sp., *S. racemosum, T. thermophilus* and *T. lanuginosus* showed 'D' type of interaction with all the pathogens, where the pathogens inhibited and overgrew the colonies of the test microorganisms. Similarly, bacteria *viz., Aeromonas* sp., *B. stearothermophilus, Enterococcus* sp., *E. coli, Klebsiella* sp., *G. toebii* and *T. thermophilus* did not show antagonistic activity against any pathogen.

Muhammad & Amusa (2003) found that compost-inhabiting microbes inhibited the growth of pathogens and thus suppressed the diseases. During the present studies, growth of *Fusarium solani* was inhibited by *T. harzianum, T. virens, P. chrysogenum, P. citrinum* and *S. atra* that corroborates well with the results reported by Akrami *et al.,* (2012) and Dwivedi & Dwivedi (2012). Suppression in growth of *F. solani* by *B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis* and *P. fluorescens* during the present studies supports the findings of Amalraj *et al.,* (2012) and Haggag & El-Gamal (2012).

Growth of *Macrophomina phaseolina* was found to be inhibited by *T. harzianum, T. virens, A. niger, A. fumigatus, Penicillium* sp., *B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis* and *P. fluorescens.* Similar results have been reported by Shivakumar (2007), El-Mougy & Abdel-Kader (2008), Hajieghrari *et al.,* (2008), Bandopadhyay *et al.,* (2011), Mahalingam, *et al.,* (2011), Amalraj *et al.,* (2012), and Gajera *et al.,* (2012).

Rhizoctonia solani was inhibited by *A. fusispora, A. niger, P. citrinum, T. harzianum, T. virens, B. cereus, B. pumilus, B. subtilis, M. varians* and *P. fluorescens.* There are several reports where growth of *R. solani* was inhibited by *T. harzianum* (Demirci *et al.,* 2011), *T. virens* and *A. niger* (Vibha, 2010), *B. subtilis* and *P. fluorescens* (Haggag & El-Gamal, 2012). Ganesan & Sekar (2011) reported that *B. pumilus* and *B. licheniformis* inhibited the mycelial growth of *R. solani in vitro*, but in present studies, *B. licheniformis* did not find to inhibit the mycelial growth of *R. solani*.

Pythium aphanidermatum was the most sensitive among all the pathogens tested since 20 fungi and seven bacteria inhibited its growth. Similar results have been reported by Kamil *et al.,* (2007), Mishra (2010), Gomathi & Ambikapathy (2011), El-Mohamedy *et al.,* (2011), Basurto-Cadena *et al.,* (2012), and El-Bramawy *et al.,* (2012). On the other hand, *Sclerotium rolfsii* showed highest tolerance to the antagonistic effects of microorganisms since only *T. harzianum* and *T. virens* were able to inhibit its growth, whereas, all other fungi and bacteria were over grown by *S. rolfsii*. Inhibition in growth of *S. rolfsii* has also been reported by Yaqub & Shahzad (2005) and Bhuiyan *et al.,* (2012). Bosah *et al.,* (2010) reported that *A. niger* reduced the mycelial growth of *S. rolfsii* but *Penicillium* did not. In our studies, *A. niger* and *Penicillium* did not inhibit the mycelial growth of *S. rolfsii*. *B. cereus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis* and *P. fluorescens* reduced the mycelial growth of *S. rolfsii*. Similar results were observed by Nalisha *et al.,* (2006), Shivakumar (2007), and Amalraj *et al.,* (2012).

The growth inhibition of pathogen may be due to hyperparasitism, antibiosis (We *et al.,* 1986) or production of chitinase and B-1,3-glucanase enzymes which degrade the cell wall of the pathogens (Ahmed & Baker, 1987). *Trichoderma* generate many enzymes that are used against cell walls of fungi to utilize the fragment of pathogens (Grosch *et al.,* 2006). *B. subtilis* produces five antibiotics *viz.,* bacillin, bacitracin, bacilomycin, subtilin and subtenolin. Muhammad & Amusa (2003) reported that two mechanisms were involved during the interactions between the antagonist and the pathogen: First one, the production of biologically active metabolites, and the other one, the rapid growth of biocontrol agent on moist surface of agar, which inhibited the growth of pathogens. Various *Trichoderma* and *Bacillus* species have the potential to control plant pathogens (Utkhede *et al.,* 1999; Begum & Begum, 2010). Inhibition of *Rhizoctonia solani* growth by *Bacillus subtilis* is attributed to the production of lipopeptides such as iturin and surfactin (Yu *et al.,* 2002). The results of the present study confirm that compost contains a large number of indigenous beneficial microbes capable of suppressing soil-borne pathogens. This character of the compost can be utilized to overcome the difficulties in mass production of biocontrol agents for field application and may lead to suppression of diseases leading to increased crop productivity.

Acknowledgements

Higher Education Commission, Pakistan, provided the financial support for this work. Identification of bacterial cultures was confirmed by Dr. Muhammad Sohail, Assistant Professor, Department of Microbiology, University of Karachi.

References

Agrios, J.C. 2005. *Plant Pathology*. Fifth ed. Academic Press.

Ahmed, J.S. and R. Baker. 1987. Competitive saprophytic ability and cellulolytic activity of rhizosphere competent of *Trichoderma harzianum*. *Phytopathology*, 77: 358-362.

Akrami, M., H.K. Khiavi, H. Shikhlinski and H. Khoshvaghtei. 2012. Biocontrolling two pathogens of chickpea *Fusarium solani* and *Fusarium oxysporum* by different combinations of *Trichoderma harzianum*, *Trichoderma asperellum* and *Trichoderma virens* under field conditions. *International Journal of Agricultural Science Research*, 1(3): 41-45.

Aly, A.A., A.M.M. El-Shazly, R.M. Youseef and M.R. Omar. 2001. Chemical and biological control of charcoal rot of cotton caused by *Macrophomina phaseolina. J. Agric. Sci.* Mansoura Univ., 26: 7661-7674.

Amalraj, E.L.D., S. Maiyappan and J.A. Peter. 2012. *In vivo* and *In vitro* studies of *Bacillus megaterium var. phosphaticum* on nutrient mobilization, antagonism and plant growth promoting traits. *Journal of Ecobiotechnology*, 4(1): 35-42.

Bailey, K.L. and G. Lazarovits. 2003. Suppressing soil-borne diseases with residue management and organic amendments. *Soil Tillage Res.*, 72: 169-180.

Bandopadhyay, A., A.K. Bandopadhyay and N. Samajpati. 2011. *In vitro* antifungal activity of some biocontrol fungi against jute pathogen *Macrophomina phaseolina*. *Indian Phytopathology*, 61(2): 204-211.

Barnett, H.L. and B.B. Hunter. 1972. *Illustrated genera of imperfect fungi*. Burgess Pub. Co., Minnesota. 241 pp.

Basurto-Cadena, M.G.L., M. Vazquez-Arista, J. Garcia-Jimenez, R. Salcedo-Hernandez, D.K. Bideshi and J.E. Barboza-Corona. 2012. Isolation of a new Mexican strain of *Bacillus subtilis* with antifungal and antibacterial activities. *The Scientific World Journal*, 10: 1-7.

Begum, H.A. and S.A. Begum. 2010. Management of collar rot of tomato by fungicides and *Trichoderma* spp. *J. Bangladesh Soc. Agric. Sci. Technol.*, 7(1&2): 55-58.

Bhuiyan, M.A.H.B., M.T. Rahman and K.A. Bhuiyan. 2012. *In vitro* screening of fungicides and antagonists against *Sclerotium rolfsii*. *African Journal of Biotechnology*, 11(82): 14822-14827.

Bosah, O., C.A. Igeleke and V.I. Omorusi. 2010. *In vitro* microbial control of pathogenic *Sclerotium rolfsii*. *Int. J. of Agric. Biol.*, 12: 474-476.

De Ceuster, T.J.J. and H.A.J. Hoitink. 1999. Using compost to control plant diseases. *Biocycle Magazine*, 6: 61-64.

Demirci, E., E. Dane and C. Eken. 2011. *In vitro* antagonistic activity of fungi isolated from sclerotia on potato tubers against *Rhizoctonia solani*. *Turk. J. Biol.*, 35: 457-462.

Domsch, K.H., W. Gams and T.H. Anderson. 1980. *Compendium of soil fungi*. Vol. 1. Academic Press.

Dwivedi, S.K. and N. Dwivedi. 2012. *In vitro* bio efficacy of some selected fungal antagonists against guava wilt pathogen. *IOSR Journal of Engineering*, 2(5): 1217-1223.

El-Bramawy, M.A.S. and E.E. El-Sarag. 2012. Enhancement of seed yield and its components in some promising sesame lines using antagonism of *Trichoderma* spp., against soil-borne fungal diseases. *Int. J. Forest, Soil & Erosion*, 2(3): 148-154.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 608 pp.

El-Mohamedy, R.S.R., A. El-Samad, A.M. Hoda, T.S.H. Habib and F. El-Bab. 2011. Effect of using biocontrol agents on growth, yield, head quality and root rot control in Broccoli plants. *International Journal of Academic Res.*, 3: 71-80.

El-Mougy, N.S. and M.M. Abdel-Kader. 2008. Long term activity of bio-priming seed treatment for biological control of faba bean root rot pathogens. *Australasian Plant Pathology*, 37: 464- 471.

Fuchs, J. and M. Larbi. 2004. Disease control with quality compost in pot and field trials. Paper presented at International Conference on soil and composts eco-biology. *Soil ACE, Biomase Peninsular.* León-Spain. pp. 157-166.

Gajera, H.P., R.P. Bambharolia, S.V. Patel, T.J. Khatrani and B.A. Goalkiya. 2012. Antagonism of *Trichoderma* spp., against *Macrophomina phaseolina*: Evaluation of coiling and cell wall degrading enzymatic activities. *Journal of Plant Pathol. Microb.,* 3(7): 1-7.

Ganesan, S. and R. Sekar. 2011. Screening of biocontrol agents against *Rhizoctonia solani* causing web blight disease of groundnut (*Arachis hypogaea* L.). *Pesticides in the modern world*. pp. 127-140.

Ghaffar, A.1998. *Soil-borne Disease Research Center, Final Research Report.* Department of Botany, University of Karachi.75270, Pakistan 111 pp.

Gomathi, S. and V. Ambikapathy. 2011. Antagonistic activity of fungi against *Pythium debaryanum* (Hesse) isolated from chilli field soil. *Advances in Applied Science Research,* 2(4): 291-297.

Grosch, R., K. Scherwinski, J. Lottmann and G. Berg. 2006. Fungal antagonists of the plant pathogen *Rhizoctonia solani*: selection, control efficacy and influence on the indigenous microbial community. *Mycol. Res*., 110: 1464-1474.

Haggag, K.H.E. and N. El-Gamal 2012. *In vitro* study on *Fusarium solani* and *Rhizoctonia solani* isolates causing the damping off and root rot diseases in tomatoes. *Nature and Science,* 10(11): 16-25.

Hajieghrari, B., M. Torabi-Giglou, M.Z. Mohammadi and M. Davari. 2008. Biological potential of some Iranian *Trichoderma* isolates in the control of soil-borne plant pathogenic fungi. *Afr. J. Biotechnol.*, 7(8): 967-972.

Hoitink H.A.J. and M.J. Boehm.1999. Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Ann. Rev. Phytopathol.*, 37: 427-446.

Hoitink, H.A.J., A.G. Stone and D.Y. Han .1997. Suppression of plant diseases by composts. *Hort Science.*, 32(2): 184-187.

Hoitink, H.A.J., M.S. Krause and D.Y. Han. 2001. Spectrum and mechanisms of plant disease control with composts. In: *Compost Utilization in Horticultural Cropping Systems.* (Eds.): P.J. Stoffela & B.A. Kahn. Lewis, Boca Raton, Florida, pp. 263-273.

Holt J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. William. 1994. *Bergey's manual of determinative bacteriology.* Williams and Wilkins (Eds.) 9th ed., Wavely Company, Baltimore, pp. 787.

Kamil, Z., M. Rizk, M. Saleh and S. Moustafa. 2007. Isolation and identification of rhizosphere soil chitinolytic bacteria and their potential in antifungal biocontrol. *Global J. Mol. Sci.*, 2(2): 57-66.

Kavroulakis, N., S. Ntougias, M.I. Besi, P. Katsou, A. Damaskinou, C. Ehaliotis, G.I. Zervakis and K.K. Papadopoulou. 2010. Antagonistic bacteria of composted agro-industrial residues exhibit antibiosis against soil-borne fungal plant pathogens and protection of tomato plants from *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Plant and Soil,* 333: 233-247.

Lewis, J.A., R.D. Lumsden, P.D. Millner and A.P. Keinath.1992. Suppression of damping-off of peas and cotton in the field with composted sewage sludge. *Crop Protect.*, 11: 260-266.

Mahalingam, R., L. Prince, V. Ambikapathy and A. Panneerselvam. 2011. *In vitro* studies on biocontrol measures of seedling blight disease in sugarcane. *World Journal of Science and Technology,* 1(9): 18-20.

Mishra, V.K. 2010. *In vitro* antagonism of *Trichoderma* species against *Pythium aphanidermatum*. *Journal of Phytology,* 2(9): 28-35.

Muhammad, S. and N.A. Amusa. 2003. *In vitro* inhibition of some seedling blight inducing pathogens by compost inhabiting microbes. *African J. Biotechnol.*, 2(6):161-164.

Nalisha, I., M. Muskhazli and T. Nor Frizan. 2006. Production of bioactive compounds by *Bacillus subtilis* against *Sclerotium rolfsii*. *Malaysian Journal of Microbiology,* 2(2): 19-23.

Noble, R. and E. Coventry. 2005. Suppression of soil-borne plant diseases with composts: a review. *Biocontrol Sci. Technol.,* 15: 3-20.

Postma, J., M. Montanari, H.J.F. Paul and V.D. Boogert. 2003. Microbial enrichment to enhance the disease suppressive activity of compost. *Soil Biology,* 39: 157-163.

Rebollido, R., J. Martinez, Y. Aguilera, K. Melchor, I. Koerner and R. Stegmann. 2008. Microbial populations during composting process of organic fraction of municipal solid waste. *Applied Ecology and Environmental Research,* 6(3): 61-67.

Reuveni, R., M. Raviv, A. Krasnovsky, L. Freiman, S. Medina, A. Bar and D. Orion. 2002. Compost induces protection against *Fusarium oxysporum* in sweet Basil. *Crop Prot.*, 21: 583-587.

Rijckeboer, J., K. Vaes, L. Vanwynsberghe and J. Coosemans. 2002. The suitability of mature biowaste and yardwaste compost as carrier substrates for bacterial and fungal biocontrol agents. In: *Proceeding of International Symposium on Composting and Compost Utilization,* (Eds.): F.J. Michel, R.F. Rynk & H.A.J. Hoitink. Columbus, Ohio, 6-8 May 2002, JG Press, Emmaus, USA.

Shivakumar, B. 2007. *Biocontrol potential and plant growth promotional activity of fluorescent Pseudomonas of western ghats.* MS thesis. Department of Agriculture Microbiology, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad.

Tilston, E.L., D. Pitt and A.C. Groenhof. 2002. Composted recycled organic matter suppresses soil-borne diseases of field crops. *New Phytologist,* 154: 731-740.

Utkhede, R.S., C.A. Koch and G.J. Menzies. 1999. Rhizobacterial growth and yield promotion of cucumber plants inoculated with *Pythium aphanidermatum*. *Can. J. Plant Pathol.,* 21: 265-271.

Vibha. 2010. Effect of fungal metabolites and amendments on mycelial growth of *Rhizoctonia solani*. *Journal of Plant Protection Research,* 50(1): 93-97.

Waksman, S.A. and E.B. Fred.1922. A tentative outline of the plate method for determining the number of microorganism in the soil. *Soil Sci.*, 14: 27-28.

Warcup, J.H. 1950. The soil plate method for isolation of fungi from soil. *Nature London*, 166: 117-118.

We, W.S., S.D. Liu and S. Tschen. 1986. Hyperparasitic relationship between antagonists and *Rhizoctonia solani. Plant Prot. Bull.,* 28: 91-100.

Yaqub, F. and S. Shahzad. 2005. Pathogenecity of *Sclerotium rolfsii* on different crops and effect of inoculums density on colonization of mungbean and sunflower roots. *Pak. J. Bot.,* 37(1): 175-180.

Yu, G.Y., J.B. Sinclair, G.L. Hartman and B.L. Bertagnolli. 2002. Production of iturine A by *Bacillus amylolequefaciens* suppressing *Rhizoctonia solani. Soil Biol. Biochem.,* 34: 955-63.

(Received for publication 19 September 2012)