ISOLATION OF SOIL BORNE MYCOFLORA FROM RHIZOSPHERE AND RHIZOPLANE OF *EUCALYPTUS* SP. AND THEIR MANAGEMENT BY USING HOMEOPATHIC MEDICINES

Kamran Ashraf, Asma Hanif and Shahnaz Dawar

Department of Botany, University of Karachi, Karachi-75250, Pakistan
Corresponding author: shahnaz_dawar@yahoo.com

ABSTRACT

Fifteen samples of rhizospheric soil of *Eucalyptus* sp. were collected from various localities of Karachi. By using serial dilution method, six fungal species (A*spergillus flavus, A. niger, A. parasiticus, A. restritus, A. terreus,* and *Rhizopus* sp.) were isolated, out of which *A. niger* found highest and dominant fungus isolated from the tested samples as compared to other fungi. Root plating of *Eucalyptus* sp. found best method for isolation of infectious fungi. Colonization of *M. phaseolina* were recorded as the most abundant in three samples; zoology department, maskun gate and M.A Jinnah road. *R. solani* found to be the most predominant fungus in two samples; botanic garden and NIPA. *F. solani* and *F. oxysporum* were observed relatively less as compared *M. phaseolina* and *R. solani* where its highest percentage found only in zoology department sample. Furthermore, thirteen homeopathic medicines (Q) were used to find out the best antifungal drugs (using paper disc and well methods) against soil borne fungal species isolated from the rhizosphere and rhizoplane soil of *Eucalyptus* sp. Out of thirteen, only two drugs *Rosmarinus officinalis* and *Arsenic album* showed significant ($p<0.05$) growth inhibition of *F. oxysporum*. Well method was found to be better in comparison of paper disc method, while other homeopathic drugs were unable to produce any inhibition in growth of pathogenic fungi.

Key-words: Soil borne fungi, homeopathic drugs, rhizosphere and rhizoplane soil

INTRODUCTION

Soil is an intricate mixed niche for a broad diversity of microbes, in which these organisms interact with each other and to their surroundings contributing plant nutrition, soil construction, productivity to plants, break down of organic matter and overcome of soil borne microbes (Kirk *et al.,* 2004; Prescott *et al.,* 2005). Soil borne fungi has been responsible for continuous decreasing vintage agriculture in Pakistan (Usman *et al.,* 2013). Plant roots provide an area of microorganisms to perform biological processes taking place and having large amount of organic compound known as rhizosphere (Barea *et al.,* 2005). Exclusion of soil borne fungi found strenuous when they generate survival structure (chlamydospores, sclerotia etc.) to adapt under harsh environmental condition (Ghaffar, 1988; Khakpour and Khara, 2012).

Eucalyptus sp. are planted in Mediterranean and tropical countries because they grow faster particularly for profitable business (Moradashahi *et al.,* 2003) due to its allelochemicals properties it possesses allelopathic effect (Turnbull, 1999; Bajwa and Nazi, 2005). Certain *Eucalyptus* spp. contain phenolic compounds and volatile oil present in their leaves and bark which act as allelopathic and destructive to other plant species growing around them (Florentine and Fox, 2003; Sasikumar *et al.,* 2002). Indispensable oil of *Eucalyptus* sp. has shown remarkable potential against a wide range of plant pathogens (Inouye *et al.,* 2001). Application of agrochemicals has been prohibited because of demerit effects in soil which kills beneficial micro-organisms (Serfoji *et al.,* 2010; Gatto*et al.,* 2011). Therefore, developments of new management strategies are required to reduce the dependency on agrochemicals (Akhtar and Malik, 2000; Choi *et al.,* 2007). Recently the use of homeopathic medicine in agriculture being introduced (Sukul and Sukul, 2004) as relatively cheaper, considered as harmless and have no ecological side effects, could offer potential advantages and because of homeopathic preparation (extremely diluted level) do not produce any toxicity in the soil ecosystem (Scofield, 1984; Elmaz *et al.,* 2004; Swati *et al.,* 2013)

Present research was conducted to isolate fungi from the rhizosphere and rhizoplane soil of *Eucalyptus* sp. and to control pathogenic fungi by using homeopathic drugs.

MATERIALS AND METHODS

Collection of soil samples and purchasing of homeopathic drugs

Rhizospheric soil and rhizoplane samples of *Eucalyptus* sp. were taken from the various areas of Karachi including: Kala pul, Azambasti, DHA phase 1, Malir, NIPA, Saddar, M.A Jinnah road, Korangi, Nazimabad and different locations of Karachi University campus. While, thirteen homeopathic drugs of Dr. Masood Homeopharma of mother tincture (Q) includings; *Arsenicum Album, Berberis vulgarisa, Balladonna, Calcera carbonica, Calendula officinalis, Graphite, Kreosote, Lycopodium, Phosphorus, Rosmarinus officinalis, Rhustox-radicans, Sulphur* and *Sabina* were bought from the homeopathic market of Karachi (Pakistan).

Moisture content and pH of soil samples

Moisture content of soil was calculated by the Garrett formula (1963); pH of soil was measured by using mettler Toledo MP220 pH meter (Brady, 1990).

Direct and indirect methods

In the direct method, take 1g of each area soil sample was dispersed on poured PDA plate uniformly and each treatment was replicated thrice. Plates were kept at room temperature (33-34°C) for 15 days and then fungi were identified (Warcup, 1950). After identification, pure culture was prepared for further studies.

In the serial dilution technique, 1g of rhizospheric soil was taken from each sample and suspended in sterilized distilled water (9 mL), which gave a dilution of 1:10. Serial dilution of 1: 100, 1:1000, 1:10,000 were prepared by using separate test tubes having distilled water. Then 1 mL of aliquot from 1:10,000 dilutions was added to a Petri plate supplemented with antibiotic. Each sample was replicated thrice. Plates were incubated at room temperature (31-33°C) for 7-8 days. Fungal species produced number of colonies which was multiplied by the dilution factor to attain the total number of propagules/g of soil (Waksman and Fred, 1922).

Isolation of fungi from rhizoplane of *Eucalyptus* sp.

Eucalyptus roots collected from different areas were cut into small pieces (1 cm). Root pieces were surface sterilized through 1% $Ca(OCl)_2$ and dried aseptically under sanitary conditions. Placed the five root pieces on PDA plates supplemented with antibiotics at equal distance and plates were incubated at room temperature for 7 days. Colonization % was recorded (Waksman, 1922).

Literature citation for identification

The fungal species grown on petri plates were identified by using mycology books (Raper and Fennell, 1965; Ellis, 1971; Domsch, *et al*., 1980; Nelson, *et al*., 1983).

Effect of homeo drugs on fungal growth

Filter paper disc and agar well procedure were used to investigate the inhibition of different fungal species isolated from different techniques by using homeopathic drugs. On one side of the plate, well was made through cork borer and on other side fungal disc were placed. The well was poured with mother tincture of homeopathic medicines. Similarly, for paper disc method, sterilized Whatman's filter paper disc was immersed in homeopathic mother tincture medicines for 15 minutes. Soaked discs were placed on one side of petri plate and on other side fungal disc were placed (Balouiri *et al*., 2016). Treated plates were incubated at room temperature (26-32°C) for seven days. After the specific period of incubation, growth of fungi was measured (Dellavelle *et al*., 2011).

Statistical analysis

Data was calculated as mean ± SD and estimated by using analysis of variance (ANOVA). Probability of 0.05 or less was considered significant Duncan's Multiple Range Test (Sokal andRohlf, 1995).

RESULTS

Isolation of fungi from the rhizospheric and rhizoplane soil of *Eucalyptus* sp.

Moisture content of tested rhizospheric soil of *Eucalyptus* sp. was ranged from 1- 4% and pH was observed slightly acidic in all samples ranging from 5-7 (Table 1). While using direct plate method, five fungal spp. were isolated from the rhizosphere soil of *Eucalyptus* sp. which includes *Aspergillus flavus, A. niger A. restritus, A. terreus* and *Rhizopus* sp. from fifteen different tested samples. *Aspergillus* spp. are the mostly recorded in all the soil samples. *Rhizopus* sp. was observed in the sample collected from woman studies (KU), cricket ground (KU), Saddar, M.A Jinnah road and Korangi (Table 2). Moreover, six fungal species were isolated by serial dilution method. *Aspergillus flavus, A. niger, A. parasiticus, A. restritus, A. terreus* and *Rhizopus* sp., were found to be highest in the sample of DHA phase 1 as compared to other localities. *Rhizopus* sp. was recorded only in the sample

of M.A Jinnah road (Table 3). Roots of *Eucalyptus* sp. collected from different localities were placed on PDA plate to check the percentage of root rot fungi (*Macrophomina phseolina, Rhizoctonia solani, Fusarium solani* and *Fusarium oxysporum*). *M. phaseolina* was recorded most dominant fungus isolated in the roots of *Eucalyptus* sp. while minimum percentage was noticed by *F. oxysporum* followed by *R. solani* (Table 4).

Screening of antifungal activity of homeopathic drugs against soil borne fungi

Homeopathic mother tincture was used to find out their activity on the growth of soil borne fungi including; *Aspergillus flavus, A. niger, A. terreus, Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium oxysporum* by using the paper disc and well techniques. Results of mother tincture of *Rosmarinus officinalis* and *Arsenic album* showed significant ($P<0.001$) suppression in growth of *F. oxysporum* in both methods. However, other medicines were failed to inhibit any fungal growth. Both methods showed significant ($p<0.01$) results in producing zone of growth inhibition but well method was found better as compared to paper disc method (Table 5).

Table 1. pH and moisture content of rhizospheric soil of *Eucalyptus* spp. from different localities of Karachi.

S.No	Locations	Soil moisture	pH
1	Zoology department (KU)	3.0±1.00	6.0±0.00
2	Woman study department (KU);	4.0±2.00	6.0±0.00
3	Kala pul, Location	2.0±0.00	7.0±0.00
4	korangi road	4.0±2.00	7.0±0.00
5	Azam basti	2.0±1.00	7.0±0.00
6	DHA phase	3.0±1.00	6.0±0.00
7	Girls hostel (KU);	3.0±1.00	6.0±0.00
8	Nazimabad	1.0±0.00	5.0±0.00
9	Malir	3.0±0.00	7.0±0.00
10	Botanic garden	2.0±1.00	7.0±0.00
11	Cricket ground (KU);	5.0±3.00	7.0±0.00
12	NIPA	1.0±0.00	6.0±0.00
13	Sadder	2.0±0.00	7.0±0.00
14	M.A Jinnah road	1.0±0.00	7.0±0.00
15	Korangi Crossing	2.0±1.00	7.0±0.00
$LSD_{0.05}$: pH = 0.00; Soil moisture = 2.0648			

Where: ± = Standard deviation

Table 1. Isolation of Rhizopheric mycoflora of *Eucalyptus* sp. by using direct method

Fungal sp.	Sample Locations														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Aspergillu niger	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
A. flavus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A. terreus	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
A. restricus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
***Rhizopus* sp.**	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

Where;Positive (**+**) = Present; Negative (-) = Absent

KEYS: Location 1= Zoology department (KU); Location 2= Woman study department (KU); Location 3= Kala pul, Location 4= korangi road; Location 5= Azambasti; Location 6= DHA phase 1; Location 7= Girls hostel (KU); Location 8= Nazimabad; Location 9= Malir; Location 10= Botanic garden (KU); Location 11= Cricket ground (KU); Location 12= NIPA; Location 13= Sadder; Location 14= M.A Jinnah road; Location 15=Korangi Crossing

Table 2. Isolation of rhizosphere mycoflora of *Eucalyptus* sp. by serial dilution method.

Name of Fungi	Population of fungi x 10^3 cfu / g soil														
	Sample Locations														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aspergillus niger	0.33± 0.57	17.66± 4.72	4.66± 1.52	13.66± 2.88	8.33± 2.08	5.0± 1.0	3.0± 1.73	1.0± 1.0	6.66± 1.15	8.66± 1.52	0.66± 1.15	1.66± 1.52	0.66± 0.57	2.33± 2.08	4.33± 4.04
Aspergillus terreus	1.66± 1.52	2.66± 4.61	8.33± 4.04	29.0± 7.81	20.33± 2.51	0.33± 0.57	1.66± 1.52	1.66± 1.52	18.33± 11.93	4.66± 4.16	16.33± 2.08	1.66± 2.08	17.66± 3.05	19.33± 2.08	1.66± 2.08
Aspergillus flavus	1.0± 1.73	2.0± 1.0	0.33± 0.57	0.66± 1.15	1.0± 1.0	1.33± 0.57	0.66± 1.15	1.33± 1.52	1.33± 1.52	3.33± 1.52	2.66± 3.05	1.33± 2.30	1.66± 1.52	2.0± 1.73	2.33± 2.51
Aspergillus ostrictus	0.0± 0.0	0.66± 1.54	0.0± 0.0	1.0± 1.73	0.33± 0.57	0.0± 0.0	0.33± 0.57	0.33± 0.57	0.0± 0.0	0.33± 0.57	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
Aspergillus parasiticus	0.0± 0.0	0.33± 0.57	0.0± 0.0	0.0± 0.0	0.66± 1.15	0.0± 0.0	0.33± 0.57	0.33± 0.57	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.33± 0.57	0.0± 0.0	0.0± 0.0	0.0± 0.0
Rhizopus sp	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	1.0± 1.73	0.0± 0.0
$LSD_{0.05}$: Samples=1.42; Fungi= 0.98															

Where: ± = Standard deviation

KEYS: Location 1= Zoology department (KU); Location 2= Woman study department (KU); Location 3= Kala pul, Location 4= korangi road; Location 5= Azambasti; Location 6= DHA phase 1; Location 7= Girls hostel (KU); Location 8= Nazimabad; Location 9= Malir; Location 10= Botanic garden (KU); Location 11= Cricket ground (KU); Location 12= NIPA; Location 13= Sadder; Location 14= M.A Jinnah road; Location 5=Korangi Crossing

Table 3. Isolation of root rot fungi by using rhizoplane method from *Eucalyptus* sp.

Name of Fungi	Colonization percentage														
	Sample Locations														
	1	2	3	4	5	6	7	8	9	1	11	12	13	14	15
M phaseolina	50.0 ± 25.0	33.33 ± 14.43	16.66 ± 14.43	25.0 ± 25.0	8.33 ± 14.43	8.33 ± 14.43	50.0 ± 0.0	0.0 ± 0.0	16.66 ± 14.43	25.0 ± 25.0	16.66±1 4.43	41.66± 14.43	25.0 ±25.0	50.0 ±0.0	8.33±14 .43
R solani	8.33 ± 14.43	16.66 ± 14.43	8.33 ± 14.43	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	50.0 ± 43.30	8.33 ± 14.43	8.33 ± 14.43	75.0 ± 0.0	25.0 ±25.0	41.66± 28.86	16.66±1 4.43	8.33±14 .43	16.66±1 4.43
F solani	41.66 ± 38.18	8.33 ± 14.43	0.0 ± 0.0	16.66 ± 14.43	0.0 ± 0.0	0.0 ± 0.0	16.66 ± 28.86	0.0 ± 0.0	8.33 ± 14.43	25.0 ± 25.0	8.33±14 .43	8.33±1 4.43	8.33±14 .43	0.0 ± 0.0	16.66±1 4.43
F oxysporum	0.0 ± 0.0	8.33 ± 14.43	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	8.33 ± 14.43	8.33 ± 14.43	0.0 ± 0.0	8.33 ± 14.43	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	8.33 ± 14.43	0.0 ± 0.0
$LSD_{0.05}$: Samples=2.60; Fungi= 1.78															

Where: ± = Standard deviation

KEYS: Location 1= Zoology department (KU); Location 2= Woman study department (KU); Location 3= Kala pul, Location 4= korangi road; Location 5= Azambasti; Location 6= DHA phase 1; Location 7= Girls hostel (KU); Location 8= Nazimabad; Location 9= Malir; Location 10= Botanic garden (KU); Location 11= Cricket ground (KU); Location 12= NIPA; Location 13= Sadder; Location 14= M.A Jinnah road; Location 5=Korangi Crossing

Table 4. Effect of homeopathic medicines (Q) against fungal pathogens by using paper disc and well methods.

	Homeopathic mother tinctures(Q)	Fungal growth (mm)											
		Paper disc method						Well method					
		F. oxysporum	*M. phaseolina*	*R. solani*	*A.flavus*	*A.terreus*	*A.niger*	*F. oxysporum*	*M. phaseolina*	*R. solani*	*A.flavus*	*A.terreus*	*A.niger*
1	**Control**	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
2	*Arsenicum album*	**42.33±0.57**	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	**40.66±1.52**	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
3	*Berberis vulgaris*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
4	*Belladonna*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0± .00	45.0±0.0	45.0±0.0
5	*Calcerearub ousa*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
6	*Calendula officinalis*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
7	*Graphite*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
8	*Kreosote*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
9	*Lycopodium*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
10	*Phosphorus*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
11	*Rosmarinusof ficinalis*	**41.0±1.0**	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	**41.0±1.0**	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
12	*Rhustoxi codendron*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
13	*Sulphur*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0
14	*Sabina*	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0	45.0±0.0

LSD $_{0.05}$: **Homeopathic drugs**= 0.0773; **Methods**= 0.0292; **Fungi**= = 0.0506

Where: ± = Standard deviation

DISCUSSION

By using serial dilution and direct plating methods of rhizospheric soil of *Eucalyptus* sp. showed variety of fungi present. The present results showed that highest numbers of fungi were obtained by the serial dilution technique. Result showed that *Aspergillus* species were the most common fungi observed throughout the soil samples of *Eucalyptus* sp. *A. flavus* and *A. niger* were showed highest incidence of occurrence by using serial dilution method. Similar results were obtained by Mehdi and Saifullah (2000) and Manzoor *et al*. (2004) who mentioned that *Aspergillus* was found to be the most diverse genus. Soil borne fungi are injurious to crops production causing heavy losses in country economy (Chitwood, 2003). Among them, *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp. are the most important pathogenic fungi (Ghaffar, 1988; Ehtsham-ul-Haque and Ghaffar, 1993). Many researchers showing importance on the use of herbal drugs which attained from medicinal plants (Vulto and Smet, 1988; Mentz and Schenkel, 1989). Medicinal plants have been used as remedy during historic periods to treat many diseases in human (Samuelesson, 2004). Therefore researcher discloses that herbal remedies obtained from medicinal plants delineate to be harmless devoid of any contrary side effects (World Health Organization, 1977; 2001). Homeopathic medicines have potential to control diseases of plant (Kumar, 1980). In our present research of *in vitro* experiment, thirteen homeopathic mother tinctures were checked against *M. phaseolina, R. solani, F. oxysporum, A. niger, A. flavus*and *A. terreus.* Out of 13 homeopathic medicines, *Rosmarinus officinalis* and *Arsenic album* have shown significant zone of inhibition against *F. oxysporum*.

Rosmarinus officinalis (Lamiaceae) commonly known as rosemary (Rotblatt, 2000). Rosemary extract (E392) reported harmless having useful natural antioxidant (Food Standards Agency, 2006). Essential oil of *R. officinali* possesses antifungal activity that was studied, especially show inhibitory effect on the growth and aflatoxin production of *A. parasiticus* at 450ppm. On analysis of *R. officinalis,* oil was found major components of antimicrobial properties including; piperotone (23.65%), Alpha- pinene (14.94%), Limonene (14.89%), 1, 8 Cineole (7.43%), and Thymol (37.2%), P- Cymene (32.3%), Gamma- Terpinene (27.3%), respectively (Calvo *et al*., 2011; Zhang *et al*., 2014). Extract of this plant many pharmacological activities including anti-inflammatory (Yu *et al*., 2013) antioxidant (Pérez-Fons *et al*., 2010), antitumor (Cheung and Tai, 2007; Yesil *et al.,* 2010) and antibacterial (Bozin *et al.,* 2007). *In vitro,* studies carnosic acid, carnosol, rosmarinic acid, oleanolic acid, urcolic acid and essential oil of *R. officinalis* tested against *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus subtilis* (gram positive bacteria) and *Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa* (gram negative bacteria) in addition with fungi (*Candida albicans* and *Aspergillus niger*) proving positive efficiency of *R. officinalis* as an antifungal and antiviral activity (Shin *et al.,* 2013). The present study also proved *R.officinalis* as an antifungal against *Fusarium oxysporum* which also support the research of Gupta and Srivastava (2002). *In vitro,* excellent result showed by *Thuja occidentalis* against the *A. flavus* by treating with 30M and 200M potency. 50M potency used against *A.niger* which proved to exhibit antifungal activity. Similarly, by using *R. officinalis* against *F.oxysporum* showed impressive suppression of fungal mycelium when disc and well methods were used. Similarly, Ahmad *et al*., (2010) confirmed antibacterial activity against *Pseudomonas aeruginosa, Yersinia aldovae, Citrobacter, Staphylococus, Escherichia coli* as well as showed antifungal activity against *Aspergillus parasitcus, Candida albicans, Saccharomyces cereviciae, Trichophyon rubrum, Fusarium solani* and *Macrophomina phaseolina* when homeopathic medicine namely *Thuja occidentalis* was used.

Arsenicum album in homeopathy is a drug prepared by diluting aqueous *Arsenic trioxide*. This is used by homeopathic physician to manage and treat various symptoms including digestive disorders (Lockie and Geddes, 1995). No results were found in the literature where *A. album* was used to control diseases of plants. In the present study *A. album* showed significant result against *Fusarium oxysporum* and inhibited the mycelial growth *in vitro* method.

Fungicides control plant pathogenic fungi and showed success in short period of time, but these chemicals are costly and produced detrimental effects on the soil ecosystem. Nowadays, substitute measures are present to amplify worldwide demand for chemical free food. Present research *in vitro* testing showed that *Rosmarinus officinalis* and *Asenicum album* exhibited antifungal activity against *F.oxysporum* isolated from the rhizospheric soil of *Eucalyptus* spp. from the various areas of Karachi. A large number of homeopathic medicines are still not tested against root rot pathogens and there is need to test more and more homeopathic medicine against root rot pathogens which can be implemented on agricultural field, would provide benefits to soil ecology by controlling root pathogenic fungi as a replacement of fungicides.

REFERENCES

Ahmad, M.S.A., M. Ashraf and Q. Ali (2010). Soil salinity as a selection pressure is a key determinant for the evolution of salt tolerance in blue panic grass (*Panicum antidotale* Retz.). *Flora.,* 205: 37-45.

Akhtar, M. and A. Malik (2000). Roles of organic amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bio.Res Technol*., 74: 35- 47.

Bajwa, R. and I. Nazi (2005). Allelopathic effects of *Eucalyptus citriodora* on growth, nodulation and AM colonization of *Vigna adiata* (L) Wilczek, *Allelopathy Journal*, 15: 237-246.

Balouriri, M., M. Sadiki and S.K. Ibnsouda (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6: 71-79.

Barea, J., M.J. Pozo and C. Azcon-Aguilar (2005). Microbial co-operation in the rhizosphere. *Journal of Experimental Botany*, 56(417): 1761-1778.

Bozin, B., N. Mimica-Dukic, I. Samojlik and E. Jovin (2007).Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. *J. Agric. Food Chem.* 55(19): 7879–7885.

Brady, N.C. (1990). *The Nature and properties of soils*.10th ed. Macmillan pub.Company. New York.

Calvo, M.I., S. Akerreta and R.Y. Cavero (2011). Pharmaceutical ethnobotany in the riverside of Navarra (Iberian Peninsula) *J. Ethnopharmacol*., 135(1):22–33.

Chitwood, D.J. (2003). Research on plant parasitic nematode biology conducted by the United States Department of Agriculture-Agricultural Research Service. *Pest Manag. Sci*., 9: 748-753.

Cheung, S. and J. Tai (2007). Anti-proliferative and antioxidant properties of rosemary *Rosmarinus officinalis. Oncol. Rep*., 17(6):1525–1531.

Choi, I. H., S.C. Shin and I.K. Park (2007). Nematicidal activity of onion (*Allium cepa*) oil and it's components against the pine wood nematode (*Bursaphelenchus xylophilus*). *Nematology*, 9: 231- 235.

Dellavalle, P.D., A. Cabrera, D. Alem, P. Larrañaga, F. Ferreira and M.D. Rizza (2011).Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. *Chil. J. Agric. Res*., 71 (2): 231-239.

Domsch, K..H., W. Gams and T.H. Anderson (1980). *Compendium of Soil Fungi.* Academic Press, New York, USA, pp. 1156.

Elmaz, O., H. Cerit, M. Ozcelik and S. Ulas (2004). Impact of organic agriculture on the environment. *Fresen. Environ. Bull*, 13: 1072-1078.

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

Ehteshamul-Haque, S. and A. Ghaffar (1993). Use of rhizobia in the control of root rot diseases of sunflower, okra, soyabean and mungbean. *J. Phytopath*., 138: 157-163.

Florentine, S.K. and J.E.D. Fox (2003). Allelopathic effects of *Eucalyptus victrix* L. on *Eucalyptus* species and grasses. *Allelopathy Journal*, 11: 77-83.

Food Standards Agency (2006). Current EU approved additives and their E-Numbers. Food Standards Agency.

Garret, S.D. (1963).*Soil fungi and fertility*.Oxford peramon press.

Gatto, M.A., A. Ippolito, V. Linsalata, N.A. Cascarano, F. Nigro, S. Vanadia and D. Di-venere (2011). Activity of extracts from wild edible herbs against post harvest fungal diseases of fruit and vegetables. *Postharvest Biol. Technol*., 61: 72-82.

Ghaffar, A. (1988). *Soil borne Diseases: Final Research Report*. Department of Botany, University of Karachi-75270, Pakistan.

Gupta, G. and A.K. Srivastava (2002). *In vitro* activity of *Thuja occidentalis* Linn. against human pathogensic *Aspergillus*. *The Homeopathic Heritage*.

Inouye, S., T. Takizawa and U. Yamaguchi (2001). Antibacterial activity of essential oil and their major constituents against respiratory tract pathogens by gaseous contact. *J. Antimicrobial chemotherapy*, 47: 565-573.

Kirk, J.L., L.A. Beaudette, M. Hart, P. Moutoglis, J.N. Khironomos, H. Lee and J.T. Trevors (2004). Methods of studying soil microbial diversity. *Journal of microbiological methods*, 58: 169-188.

Khakpour, O. and J. Khara (2012). Spore density and root colonization by arbuscular mycorrhizal fungi in some species in the Northwest of Iran. *International Research Journal of Applied and Basic Sciences*, 3: 977-982.

Kumar, R.S. (1980). Effect for certain homeopathic medicine on fungal growth and conidial germination. *Indian Phytopathol*., 33: 620-622.

Lockie, A. and N. Geddes (1995). *Homeopathy: The Principles and Practice of Treatment*. Dorling Kindersley Publishing. pp. 52–53.

Manzoor, S., S. Dawar and S.S Shaukat (2004). Studies on the soil mycoflora of Karachi cost. *Int. J. Biol. Biotech*., 1(4): 597-602.

Mehdi, F.S. and M.S. Saifullah (2000). Species diversity and seasonal occurrence of on seedlings of *Avicenia marina* (Forsk.)Vierh. *Pak. J. Biol. Sci.* 3(2):265-268.

Mentz, L.A. and E.P. Schenkel (1989). Medicinal plants: coherence and confidence of therapeutic indications. *Caderno de Farmácia*, 5: 93-119.

Moradshahi, A., H. Ghadiri and F. Ebrahimikia (2003). Allelopathic effects of crude volatile oil and aqueous extracts *of Eucalyptus camaldulensis* Dehnh.leaves on crops and weeds. *Allelopathy Journal*, 12: 189-195.

Nelson, P.E., T.A. Tousson and W.F.O. Marasas (1983). *Fusarium Species: an illustrated manual for identification.* University Park, Pennsylvania: Pennsylvania State University Press.

Prescott, L.M., J.P. Harley and D.A. Klein (2005). The epidemiology of infectious disease. In: *Microbiology*. Prescott, L.M.., J.P. Harley and D.A. Klein (Eds.). McGraw-Hill, New York, USA, 6th edition. pp. 821-843.

Pérez-Fons, L., M.T. Garzón and V. Micol (2010). Relationship between the antioxidant capacity and effect of rosemary (*Rosmarinusofficinalis* L.) polyphenols on membrane phospholipid order. *J. Agric. Food Chem*., 58(1):161-171.

Raper, K. B. and D.I. Fennell (1965). *Aspergillus fumigatus* group in *The genus Aspergillus.* eds Raper K. B., Fennell D. I. (The William & Wilkins Co. Baltimore, Md), pp 238–268.

Rotblatt, M. (2000). Herbal medicine: expanded commission E-monographs. *Ann. Intern. Med.* 133(6):487.

Samuelsson, G. (2004). *Drugs of natural origin: A textbook of pharmacognosy*, 5th Swedish pharmaceutical press, Stockholm.

Sasikumar, K., C. Vijayalakshmi and K.T. Parthiban (2002). Allelopathic effects of *Eucalyptu*s on blackgram (*Phaseolus mungo* L.). *Allelopathy Journal*, 9: 205-214.

Scofield, A. (1984). Homeopathy and its potential role in agriculture, a critical view. *Bio. Agric. Hort*., 2: 1-50.

Serfoji, P., S. Rajeshkumar and T. Selvaraj (2010). Management of root-knot nematode, *Meloidogyne incognita* on tomato CV Pusa Ruby by using vermicopost, AM Fungus, *Glormus aggregatum* and mycorrhiza helper bacterium, *bacillus coagulans*. *Journal of Agricultural technology*, 61(168): 51-62.

Shin, H.B., M.S. Choi and B. Ryu (2013). Antiviral activity of carnosic acid against respiratory syncytial virus. *Virol. J*. 10(1): 303.

Sokal, R.R. and F.J. Rohlf (1995) *Biometry: The principles and practice of statistics in biological research*. 3rd Edition, W.H. Freeman and Co., New York.

Sukul, N.C. and A. Sukul (2004). High dilution effects: Physical and biochemical basis. Dordrecht: *Kluwer Academic Publishers*. pp. 11-38.

Swati, S.P., S. Mohanty and K.D. Nabin (2013). Effects of potentised homeopathic medicines on the germination, growth and photosynthetic activity of *Pisumsativum* L.*Recent research in Science and Technology*, 5(4): 11-14.

Turnbull, J.W. (1999). *Eucalyptus* plantations. *New Forest*, 17:37-52.

Usman, B. A., J.O. Adefila and I.J. Musa (2013).Impact of rural road transport on agricultural production in kwara state, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*., 9(2): 20-25.

Vulto, A.G and P.A.G.M. Smet (1988). Drug used in non-orthodox medicine, In: *Meyler's side effects of drugs*, Eleventh edition, Published by Elsevier Amsterdam, 999-1005.

Waksman, S.A. and E.B. Fred (1922). A tentative outline of the plate method for determining the number of micro-organisms in the soil. *Soil Science*, 14(1): 27-28.

Warcup, J.H. (1950). The soil plate method for isolation of fungi from soil. *Nature*, 166(4211):117-118.

Waksman, S.A. (1922). A method for counting the number of fungi in the soil. *J. Bact*., 7 (3): 339-341.

World Health Organization (1977). *Resolution-promotion and development of training and research in traditional medicine*. WHO document No: 30-49.

World Health Organization (2001). *Legal status of traditional medicine and complementary/alternative medicine: A world wide review*. WHO Publishing 1.

Yesil-Celiktas, O., C. Sevimli, E. Bedir and F. Vardar-Sukan (2010). Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines. *Plant foods Hum. Nutr*. 65(2):158–163.

Yu, M.H., J.H. Choi and I.G. Chae (2013). Suppression of LPS-induced inflammatory activities by *Rosmarinus officinalis* L. *Food Chem*., 136(2):1047–1054.

Zhang, Y., T.A. Adelakun and L. Qu (2014). New terpenoid glycosides obtained from *Rosmarinus officinalis* L. aerial parts. *Fitoterapia*, 99:78–85.

(*Accepted for publication October 2019*)