

Comparative Studies Using Homeopathic Globules for Leguminous and Non-Leguminous Crop Management against Root Rot Fungi

Asma Hanif¹ & Shahnaz Dawar¹

¹ Department of Botany, University of Karachi, Karachi, Pakistan

Correspondence: Asma Hanif, Department of Botany, University of Karachi, Karachi, Pakistan. E-mail: asmahanif4@gmail.com

Received: May 17, 2016

Accepted: July 10, 2016

Online Published: August 15, 2016

doi:10.5539/jas.v8n9p205

URL: <http://dx.doi.org/10.5539/jas.v8n9p205>

Abstract

The aim of this study was to assess fungicidal potential of homeopathic globules namely *Thuja occidentalis* and *Arnica montana* (30C) on plant growth and root infecting fungi particularly *Rhizoctonia solani*, *Fusarium* spp. and *Macrophomina phaseolina*. Both *in vitro* and *in vivo* experiments had found positive results in the suppression of root rot fungi. Investigation on present study showed that *A. montana* and *T. occidentalis* globules (100, 75 and 50% v/w concentrations) reduced disease intensity caused by root rot pathogens and improved growth of test plants, but it produces negative effects on leguminous test crops in which nodules were failing to produce.

Keywords: homeopathic globules, root rot fungi, *Rhizobium* spp.

1. Introduction

Plant pathogens for example; fungi, bacteria and nematodes annually produce major economic losses in many valuable crops. Although chemical compounds have been successfully used to control plant pathogens which may lead to toxicological environment and sociological concerns, have led to the extreme reduction of efficient commercial compounds along with the use of fungicides due to the appearance of new resistant strains of pathogens (Hajieghrari et al., 2008). In some cases, root rot diseases are difficult to identify, manage and measure (Filip, 1999). *M. phaseolina* is a root inhibiting fungus which produces tuber shaped (1-8 mm) black sclerotia as a primary means of survival (Smith, 1969). Activities of exudations from the sclerotia explain the pathogenic significance of a soil borne fungus (Filnow & Lockwood, 1983). It affects the basal internodes and fibro-vascular system of the roots, impedes the transport of nutrients and water uptake causes wilting, loss of vigor, premature dying and limit yields are distinctive features of *M. phaseolina* infection and also responsible for seedling blight, damping off, early maturing, root and basal stem rot are the characteristic symptoms (Yang & Owen, 1982; Hoes, 1985) in which high losses have been reported due to the availability of low relative humidity and high atmospheric temperature (Tikhonov et al., 1976). Plant pathogenic fungus *Rhizoctonia solani* is distributed in soils worldwide (Harveson, 2003) causing symptoms of a wide range of hosts (Sneh et al., 1991) producing damping-off, foliar blight, root and crown rot (Windels & Nabben, 1989) as well as delay emergence and reduced yields (Errampalli & Johnston, 2001). *Fusarium* spp. considered the main soil borne pathogen in terms of economic damage in agricultural productions throughout the world (Saremi, 2000). Several *Fusarium* spp. produce similar symptoms on different crops, including wilting, cortical decay of the roots, root rot, premature death, yellowing and rosette on infected plants (Summerell et al., 2001; Saremi, 2005). *Fusarium* spp. lead to crops infection and yield reduction together with mycotoxin production has been reported all over the world (Korosteleva et al., 2006; Saremi & Amiri, 2010).

Recently there has been a worldwide swing of using eco-friendly methods to protect crops from plant pathogen and pests (Rao et al., 1998). Biological method for inhibiting diseases, particularly soil borne plant pathogens and nematodes have been considered more natural and environment friendly which becomes acceptable substitute to the existing chemical treatment methods (Barker & Paulitz, 1996; Eziashi et al., 2007). Homeopathic drugs are involved in biological processes of plants due to production of secondary metabolites which acts as an eco-friendly leaving no residue in an environment (Bonato & Silva, 2003) and possess antifungal properties (Shrivastava & Atri, 1998). Homeopathic drugs such as *Arnica montana* (Asteraceae) and *Thuja occidentalis* (Cupressaceae) are used intensively in the homeopathic system of medicine (Hulten & Fries,

1986; Alam, 2009). *Arnica montana* exhibits anti-inflammatory, anti-septic, anti-fungal and anti-bacterial activity (Conforti et al., 1997) whereas, *Thuja occidentalis* possess anti-viral, anti-diarrheal, anti-oxidant properties (Nam & Kang, 2005; Deb et al., 2006). Agrohomeopathy is used to control plant pathogenic fungi (Khurana & Gupta, 1981) therefore; use of homeopathic drugs in controlling root rot pathogen is now gaining importance (Hanif et al., 2015).

Therefore, the present research was carried out to explore fungicidal potential of homeopathic globules in the management of root rot fungi and improvement of crop productivity.

2. Materials and Methods

2.1 Globules Preparation with Homeopathic Drugs

Homeopathic drugs like *Arnica montana* and *Thuja occidentalis* (30C) and homeopathic globules (MEKTUM) were purchased from the medicinal market of Karachi. Slight modification of preparation of globules was made on the method given by Fontes et al. (2004). *A. montana* and *T. occidentalis* with the concentration of 100%, 75% and 50% v/v (prepared from 30C) were infuse in globules, respectively. Each globule (50 mg) contains 0.15 mL of homeopathic drug. Sterilized distilled water and absolute alcohol (MERCK) infuses with globule served as control. Homeopathic globules and non-treated globules were dried aseptically under the laminar air flow hood.

2.2 In Vitro Experiment

Homeopathic globules with the concentrations of 100%, 75% and 50% v/w of *A. montana* and *T. occidentalis* were placed on one side of the well of poured Potato Dextrose Agar (PDA) Petri plates respectively. Whereas, sterilized distilled water and absolute alcoholic globules served as control (as soon as globules interact with PDA medium it melts due to water content, therefore well was being made for it). On the other side of the Petri plate, test fungi such as; *F. oxysporum*, *M. phaseolina* and *R. solani* were inoculated respectively in each Petri plate. Each root rot fungus replicated thrice and plates were incubated for one week at room temperature (27-34 °C). Growth inhibition percent over control was calculated according to the formula given by Edington et al. (1971).

2.3 In Vivo Experiment

Pot experiment was conducted at the screen house of Botany department (KU). The soil consists of natural infestation having *R. solani* 25% (Wilhelm, 1955), 6-8 sclerotia g⁻¹ by *M. phaseolina* (Sheikh & Ghaffar, 1975), *Fusarium* spp. 3700 cfu g⁻¹ (Nash & Synder, 1962). Soil used for the experiment was sandy loam having 72% sand, 13% clay and 15% silt of pH 7.8 and organic matter 1.10%, amended with 0.5 g homeopathic globules (≥ 10 globules ≈ 500 mg) of *A. montana* and *T. occidentalis* at 75% and 50% v/w concentrations (prepared from 30C) separately in each plot. Non-treated globules were regarded as control. Each treatment was replicated thrice. Five seeds of mung bean (*Vigna radiata* (L.) R. Wilczek. cv. NM-2006), mash bean (*Vigna mungo* (L.) Hepper cv. NM-97), okra (*Abelmoschus esculentus* (L.) Moench cv. Arka anamika) and sunflower (*Helianthus annuus* L. cv. Hysun-38) were sown in plastic pots (80 mm diameter) having 300 g of soil and watered regularly for one month to maintain sufficient moisture essential for the plant growth.

2.4 Isolation of Root Rot Fungi from Roots

Uproot the plants after one month of germination and record the growth parameters. Roots were washed in running tap water and dried on blotter paper. Then carefully cut into five pieces. These pieces of roots after surface sterilization with 1% Ca(ClO)₂, for 5-10 minutes, transferred to poured potato dextrose agar (PDA) medium supplemented with antibiotics (penicillin at 200 mg and streptomycin at 200 mg/L) to inhibit the growth of bacteria. Incubate the Petri plates for one week at room temperature (28-35 °C) and the colonization of root infecting fungi from each root segment was recorded.

2.5 Isolation of Rhizobia from Nodules

Nodules were removed from the root of mature mung bean plant. Nodules were washed with a camel hair brush in running tap water to remove adhering soil particles and surface sterilized with acidified 0.1% mercuric chloride for 2-3 minutes, followed by washing with 70% alcohol for 2-4 minutes and finally washed with sterilized distilled water. Nodules were then placed in another sterilized Petri plate containing 1 mL sterile distilled water and crushed with sterile forcep. The exudate was mixed with sterile water and a loopful of exudate was transferred to a Petri plate containing YMA (Yeast extract mannitol agar) media. The dishes were incubated at room temperature (28-32 °C) and observed after 48 hours (Aneja, 2003). Colonies were identified by the Gram staining method and observed under the microscope (Vincent, 1970).

2.6 Paper Disc Diffusion Method for Determination of Antibacterial Activity

Rhizobium spp. were inoculated into 10 mL of sterile broth and shake thoroughly. Incubated at 34 °C for 24 hours, which was designated as the working stocks used mainly for antibacterial studies. Crushed the globules and treated globules containing homeopathic drugs by using a pestle and mortar (0.5 g in 1-2 mL alcohol) respectively to make a paste. Sterilized Whatmann filter paper (No. 1) discs (6 mm in diameter) and were soaked in 2-3 mL different concentrations of globules separately, whereas sterilized discs were soaked in different concentrations of *A. montana* and *T. occidentalis*, sterilized water and absolute alcohol (control) for 10-15 minutes. 1 mL of bacterial suspension was taken and diluted in 10 mL sterilized distilled water. Bacterial suspension was inoculated on YMA (Yeast extract mannitol agar) medium by lawn culture method (Bailey & Scott, 1974). Treated paper discs were inoculated at the center of each Petri plates having a bacterial lawn. All the Petri plates were incubated at 32-36 °C for 24 hours and zone of inhibition around each disc was observed and measured (Banjara et al., 2012).

2.7 Statistical Analysis

Data were analyzed to two way analysis (ANOVA) as per experimental design separately followed by the least significant difference (LSD) test at $P = 0.05$ and Duncan's multiple range tests to compare treatment means (Sokal & Rohlf, 1995).

3. Results

In vitro experiment, homeopathic globules of *A. montana* and *T. occidentalis* (100%, 75% and 50% v/w concentrations) were examined to check the growth of root rot fungi namely; *R. solani*, *F. oxysporum* and *M. phaseolina*. *A. montana* globules at 100% v/w concentration inhibited growth of test fungi and produced highest zone of inhibition against *F. oxysporum*, *R. solani* and *M. phaseolina*, respectively followed by *T. occidentalis* globules when used at 100% v/w concentration. *A. montana* globules (75% v/w conc.) showed greater zone of *F. oxysporum* inhibition, whereas maximum control of *R. solani* and *M. phaseolina* were observed. However, *T. occidentalis* globules showed significant ($P < 0.001$) suppression of *F. oxysporum* followed by *M. phaseolina* and *R. solani*. Minimum inhibition of test fungi was recorded by both homeopathic globules when used at 50% v/w concentrations, respectively. Results showed that *A. montana* globules in 100% v/w concentrations found to be the best in the inhibition of root rot fungi, whereas *T. occidentalis* in all concentrations showed better results as compared to control (Table 1).

Table 1. *In vitro*, growth inhibitions of root rot fungi by different concentrations of homeopathic globules

Homeopathic drugs (30C)	Concentrations/Growth inhibition (MIC)														
	<i>Fusarium oxysporum</i> (%)					<i>Rhizoctonia solani</i> (%)					<i>Macrophomina phaseolina</i> (%)				
	Control		A	B	C	Control		A	B	C	Control		A	B	C
	1	2				1	2				1	2			
<i>Arnica montana</i>	0.0	0.0	14.10	40.00	87.41	0.0	0.0	13.33	21.86	68.52	0.0	0.0	10.40	20.37	57.0
	±0.0	±0.0	±4.16	±9.17	±3.05	±0.0	±0.0	±2.00	±4.73	±3.51	±0.0	±0.0	±1.53	±4.04	±6.43
<i>Thuja occidentalis</i>	0.0	0.0	42.22	49.63	61.86	0.0	0.0	23.70	52.97	57.0	0.0	0.0	9.63	54.08	60.0
	±0.0	±0.0	±6.00	±3.06	±2.52	±0.0	±0.0	±3.05	±3.79	±3.05	±0.0	±0.0	±3.05	±5.03	±3.00
LSD _{0.05} (Concentration)=	5.513					3.663					4.406				
LSD _{0.05} (Drug)=	3.898					2.590					3.115				

Note. MIC = Minimum inhibitory concentration; ± Standard deviation and Concentration of drug: A = 50% v/v, B = 75% v/v, C = 100% v/v, Control 1 = Sterilized distilled water, Control 2 = absolute alcohol.

In vivo experiment, *T. occidentalis* and *A. montana* globules at 75% and 50% v/w concentrations mixed with soil respectively, for the control of root rot fungi. In case of sunflower plants, growth parameters including shoot length and weight, root length and weight were increased when *A. montana* used at 75% v/w concentration along with greater inhibition of root infecting fungi were observed. When 50% v/w of both homeopathic globules applied in soil, it not only showed better plant height and weight as well as reduced the colonization of *Fusarium* spp., *R. solani* and *M. phaseolina*. In okra plants, *T. occidentalis* used at 75% v/w increased shoot length and weight, whereas *A. montana* used at 75% v/w increased root length and weight. Greater reduction in colonization of root rot fungi was observed by both homeopathic globules when used at 75% v/w followed by 50% v/w concentration, which not only increased the plant height and weight but also showed significant ($P < 0.001$)

suppression of *R. solani*, *M. phaseolina* and *Fusarium* spp. colonization (Table 2 and Figure 1). Significant ($P < 0.001$) enhancement of shoot length and weight were recorded in mung bean plants, when *T. occidentalis* used at 75% v/w followed by 50% v/w concentration. Although *A. montana* in both concentrations showed highest root length and weight. Greater inhibition of *Fusarium* spp., *R. solani* and *M. phaseolina* colonization was shown by both homeopathic globules (75% v/w conc.). However, maximum suppression of root rot fungi was observed by *A. montana* and *T. occidentalis* at 50% v/w concentration. Whereas in mash bean plants, when both homeopathic globules were used at 75% and 50% v/w concentrations respectively it increased the shoot length, shoot weight, root length and root weight. Highest shoot and root weight were noticed by *T. occidentalis* at 75% v/w concentration which also significantly ($P < 0.001$) reduced the colonization of root infecting fungi followed by 50% v/w concentration. While *A. montana* when used in both concentrations, showed greater control of *R. solani* and *M. phaseolina* colonization as compared to control (Table 2 and Figure 2). It was striking to observe that in mung bean and mash bean plants nodules formation was found to be absent. It was observed that by using globules alone or treated with both concentrations of homeopathic drugs it showed effective zone of inhibition against *Rhizobium* spp. However, sterilized water, absolute alcohol, *A. montana* and *T. occidentalis* (75% and 50% v/v concentrations) found to be ineffective and failed to show zone of inhibition. Experimental results proved that due to the use of globules *Rhizobium* spp. inhibited, therefore nodules were absent in leguminous plants (Table 3 and Figure 3).

Table 2. Effect of *Arnica montana* and *Thuja occidentalis* globules (30C) on growth parameters of crop plants

Treatments	Shoot length (cm) ±SD	Shoot weight (g) ±SD	Root length (cm) ±SD	Root weight (g) ±SD	Number of nodules ±SD
<i>Mash bean (Vigna mungo (L.) Hepper)</i>					
Control (Ster. DW. Globules)	16.1±1.99	0.71±0.15	18.5±1.77	0.25±0.031	0.0±0.0
<i>A. montana</i> at 75% v/w	26.4±1.11	0.95±0.031	24.83±2.237	0.49±0.03	0.0±0.0
<i>A. montana</i> at 50% v/w	25.53±0.75	0.93±0.01	25.07±3.46	0.42±0.02	0.0±0.0
<i>T. occidentalis</i> at 75% v/w	26.27±2.49	0.97±0.076	24.53±1.22	0.52±0.04	0.0±0.0
<i>T. occidentalis</i> at 50% v/w	25.17±1.64	0.92±0.045	24.20±2.19	0.44±0.021	0.0±0.0
LSD _{0.05} (Concentration) =	2.221	0.121	2.793	0.037	0.00
LSD _{0.05} (Drug) =	1.814	0.099	2.280	0.030	0.00
<i>Mung bean (Vigna radiata (L.) R. Wilczek.)</i>					
Control (Ster. DW. Globules)	16.23±0.93	0.57±0.05	15.9±4.12	0.35±0.03	0.0±0.0
<i>A. montana</i> at 75% v/w	24.03±1.72	0.99±0.042	27.1±0.61	0.46±0.02	0.0±0.0
<i>A. montana</i> at 50% v/w	23.13±0.31	0.93±0.04	25.13±1.51	0.45±0.035	0.0±0.0
<i>T. occidentalis</i> at 75% v/w	26.63±0.93	1.08±0.06	24.57±2.61	0.48±0.047	0.0±0.0
<i>T. occidentalis</i> at 50% v/w	25.83±1.23	1.03±0.044	23.56±0.91	0.51±0.031	0.0±0.0
LSD _{0.05} (Concentration) =	1.373	0.061	3.417	0.042	0.00
LSD _{0.05} (Drug) =	1.121	0.049	2.789	0.034	0.00
<i>Okra (Abelmoschus esculentus (L.) Moench)</i>					
Control (Ster. DW. Globules)	11.73±1.4	0.82±0.13	10.6±0.92	0.18±0.02	-
<i>A. montana</i> at 75% v/w	15.2±0.92	1.16±0.032	14.07±0.76	0.36±0.015	-
<i>A. montana</i> at 50% v/w	14.93±0.61	1.10±0.04	14.4±1.31	0.32±0.045	-
<i>T. occidentalis</i> at 75% v/w	16.23±0.38	1.21±0.042	13.4±0.92	0.35±0.032	-
<i>T. occidentalis</i> at 50% v/w	15.67±0.5	1.11±0.072	14.83±0.85	0.31±0.025	-
LSD _{0.05} (Concentration) =	1.211	0.107	1.208	0.035	-
LSD _{0.05} (Drug) =	0.988	0.088	0.987	0.028	-
<i>Sunflower (Helianthus annuus L.)</i>					
Control (Ster. DW. Globules)	19.0±0.72	0.94±0.053	9.8±0.4	0.42±0.045	-
<i>A. montana</i> at 75% v/w	25.03±0.49	1.41±0.064	14.23±0.93	0.75±0.067	-
<i>A. montana</i> at 50% v/w	24.5±0.85	1.28±0.067	13.87±0.95	0.69±0.035	-
<i>T. occidentalis</i> at 75% v/w	25.8±2.23	1.37±0.076	17.37±1.59	0.67±0.05	-
<i>T. occidentalis</i> at 50% v/w	23.63±1.06	1.16±0.085	14.73±0.5	0.62±0.035	-
LSD _{0.05} (Concentration) =	1.461	0.085	1.131	0.059	-
LSD _{0.05} (Drug) =	1.193	0.069	0.924	0.049	-

Note. Ster. DW = Sterilized distilled water; ± Standard deviation.

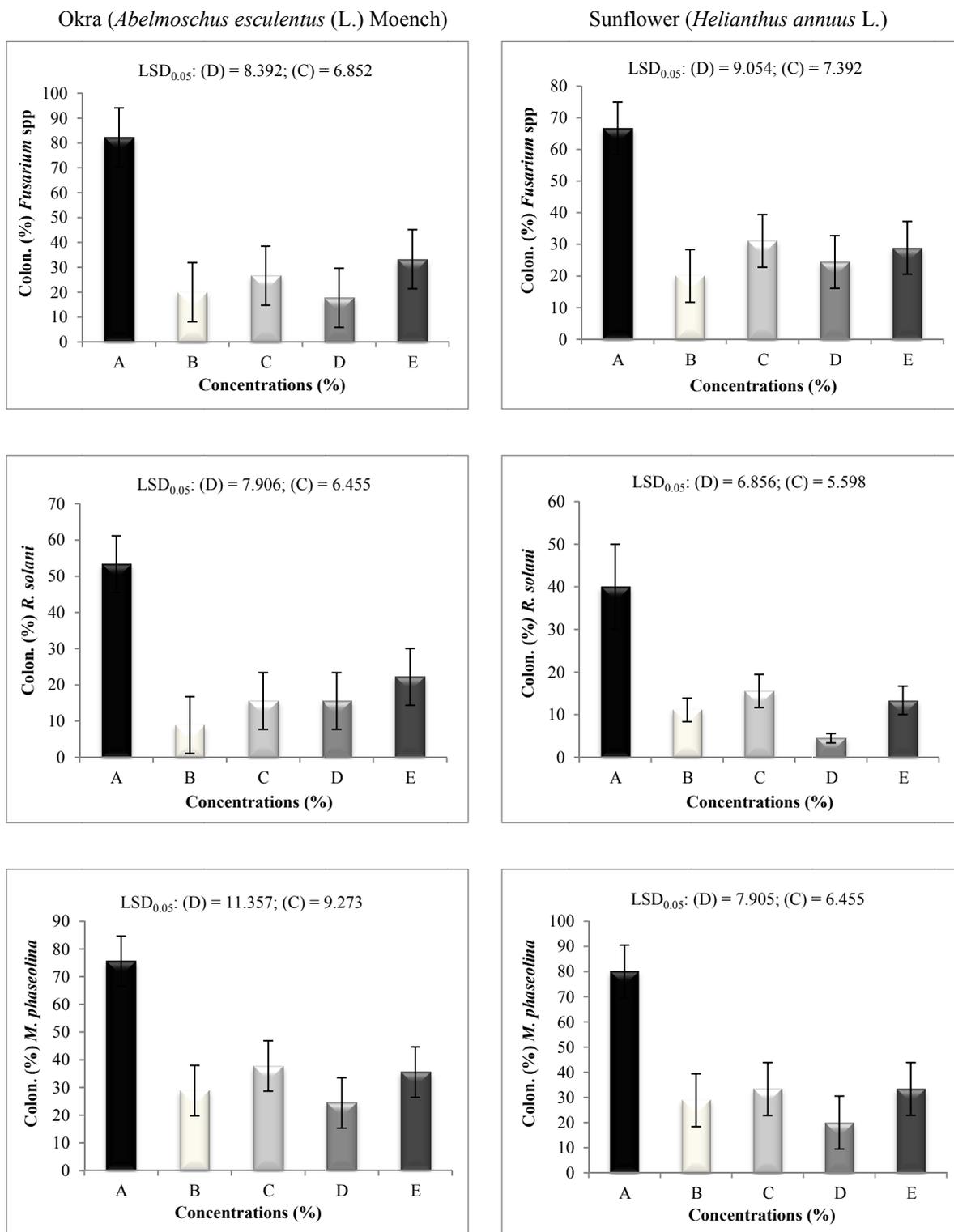


Figure 1. Effect of *Arnica montana* and *Thuja occidentalis* globules (30C) in the control of root rot fungi on non-leguminous crops

Note. (C) = Concentrations; (D) = Drugs; Colon. (%) = Colonization percentage; a = Control; b = A at 75%; c = A at 50%; d = T at 75%; e = T at 50% v/w concentrations (Prepared from 30C).

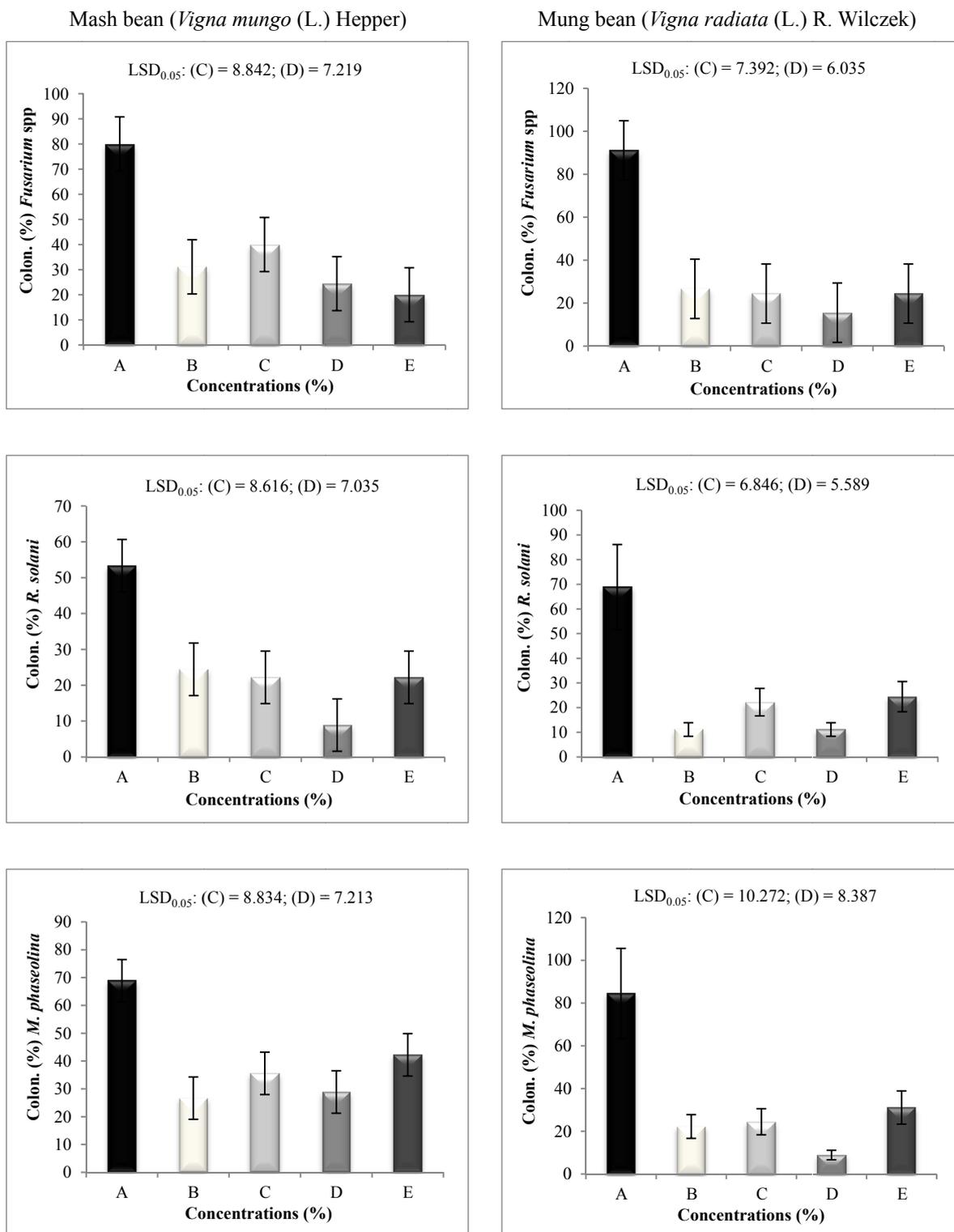


Figure 2. Effect of *Arnica montana* and *Thuja occidentalis* globules (30C) in the control of root rot fungi on leguminous crops

Note. (C) = Concentrations; (D) = Drugs; Colon. (%) = Colonization percentage; a = Control; b = A at 75%; c = A at 50%; d = T at 75%; e = T at 50% v/w concentrations (Prepared from 30C).

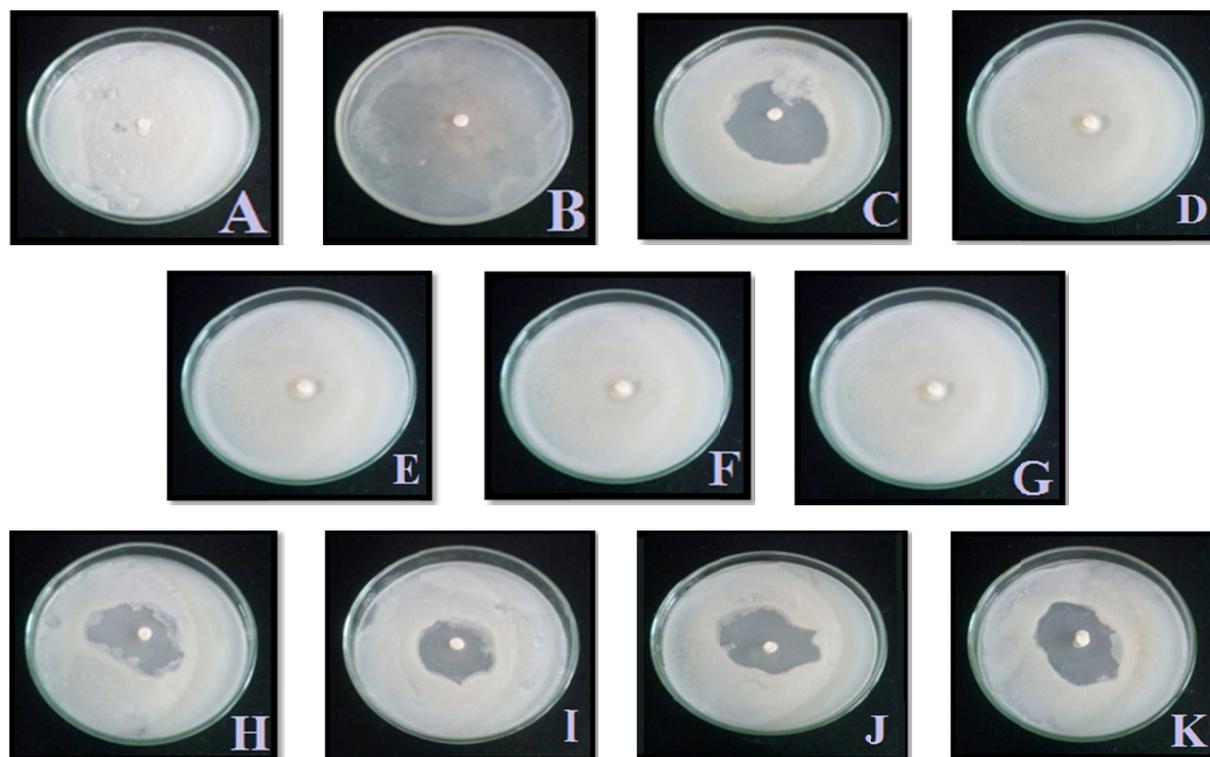


Figure 3. *In vitro*, paper disc diffusion method for determination of antibacterial activity of globules against *Rhizobium* spp.

Note. A = Sterilized water (control); B = Absolute alcohol (control); C = Globules (control); D = A at 75% v/v, E = A at 50% v/v; F = T at 75% v/v; G = T at 50% v/v; H = A at 75% v/w (globules); I = A at 50% v/w (globules), J = T at 75% v/w (globules); K = T at 50% v/w (globules).

Table 3. Level of zone of inhibition of homeopathic globules against *Rhizobium* spp.

Treatments	Conc. (%)	Zone of inhibition (mm) \pm SD	Treatments	Conc. (%)	Zone of inhibition (mm) \pm SD
Control			Globules		
Sterilized water	0	0.00 \pm 0.00	<i>A. montana</i>	75	75.19 \pm 4.51
Absolute alcohol	0	0.00 \pm 0.00	<i>A. montana</i>	50	73.70 \pm 3.78
Globules	0	85.95 \pm 4.16	<i>T. occidentalis</i>	75	81.86 \pm 4.04
Homeopathic drugs			<i>T. occidentalis</i>	50	71.48 \pm 1.53
<i>A. montana</i>	75	0.00 \pm 0.00			
<i>A. montana</i>	50	0.00 \pm 0.00			
<i>T. occidentalis</i>	75	0.00 \pm 0.00			
<i>T. occidentalis</i>	50	0.00 \pm 0.00			

LSD.05 (Concentration) = 2.965
LSD.05 (Drug) = 1.585

Note. Conc. = Concentration; \pm SD = Standard deviation.

4. Discussion

Homeopathic globules of *T. occidentalis* and *A. montana* (75% and 50% v/w concentrations) showed an enhancement in growth parameters and reduced root rot fungi on test crops. Although using *A. montana* and *T. occidentalis* (30C) as seed treatment and soil drenching methods showed nodule formation (Hanif & Dawar, 2015a) but present research showed that using homeopathic globules on leguminous crops, it inhibits the nodule formation. Process of nitrogen fixation occurs in the roots of leguminous plants by specialized structures called nodules form by the soil bacteria of the Rhizobiaceae family (Lepek & D'Antuono, 2005) which made it

possible through the exchange of molecular signals (Chataigné, 2007). *Rhizobium* spp. produced exopolysaccharides (EPS) are macromolecular complexes required for a symbiotic relationship between *Rhizobium* and leguminous plants (Mendrygal & Gonzalez, 2000). EPS consider as signaling molecules which play key steps in the formation of root nodules (Chuang-Yien, 2000). Carbon source is one of the factors which influence the biosynthesis of EPS for the conditions of bacterial growth (Ruas-Madiedo & de Los Reyes-Gavilán, 2005). Homeopathic globules are made from an inert substance often sugars, typically lactose (Ernst, 2005). Razika et al. (2012) reported that when five strains (S1, S2, S3, S4, and A6) of *Rhizobium sultae* were tested, galactose and lactose showed a less stimulating effect on nodulation and low production of EPS except strain S4 gave maximum nodules in the presence of lactose. *Rhizobium* utilizes glucose as carbon source which also showed confirm test (Kucuk et al., 2006). Singh et al. (2008) showed that pure *Rhizobium* isolates were unable to grow on lactose. Lactose metabolism has not been studied extensively in *Rhizobium* spp., by adding lactose to a culture of 104A14 (pMK320) growing in YMB (Yeast mannitol broth) it inhibited cell growth rapidly. No inhibition was observed, when lactose was added to cultures of 104A14 (pMK330). Observations indicated that addition of lactose to 104A14 (pMK320) was found to be bactericidal. When lactose treated cultures were examined under a microscope, abnormal cells/cell lysis was not observed (Timblin & Kahn, 1984). Utilization of carbohydrates by *Rhizobium* has been a subject of extensive studies in the past (Baldwin & Fred, 1927; Georgi & Ettinger, 1941; Graham & Parker, 1964). Graham (1976), Trinick (1982) and Irisarri et al. (1996) reported that fast growing rhizobia utilize a wider range of sugars than slow growing rhizobia, as the later were more specialized in their sugar requirement. Stowers and Elkan (1984) showed that cowpea rhizobia behave uniformly on carbon substrate that is either all strains grew on a carbon substrate or none of the strain grew however, all strains showed limited growth response with maltose, lactose, arbutol and 2-ketogluconate substrates. Nodules isolates from *A. lebbbeck* and *S. saman* did not utilize fructose and salicin (Qadri, 2000). Dykhuizen and Hartl (1978) reported that by the addition of high levels of lactose or galactose to *E. coli* cultures have killed 90% of the bacteria. Gupta (2002) proved that using *Thuja* (30 and 200C) found to be effectual against *Aspergillus flavus*, whereas *Thuja* (50M) against *Aspergillus niger* in human. Asha et al. (2014) reported that all potencies of *Thuja* (Q, 30C, 200C, 1M, 10M and 50M) showed excellent inhibition against *Bipolaris* spp., followed by *Curvularia* spp., *Exserohilum* spp. and *Aspergillus flavus*. Similarly, it was reported that *Thuja* and *Natrum muriaticum* were effective on *Fusarium* spp (Hussain et al., 2000). *T. occidentalis* extracts showed antifungal activity against *Aspergillus parasiticus*, *Fusarium solani*, *Macrophomina*, *Candida albicans*, *Trichophyton rubrum* and *Saccharomyces cerevisiae* (Jahan et al., 2010). *T. occidentalis* showed significant results *in vitro* against *Aspergillus flavus* (30 and 200M) whereas 50M, against *Aspergillus niger* (Gupta & Srivastava, 2002). *Arnica montana* (3, 6 and 12 CH) was used to improve plant growth (Bonfim et al., 2008). *T. occidentalis* and *A. montana* pellets (30C) showed positive results against root rot fungi when used *in vitro* and *in vivo* experiments (Hanif et al., 2015). Seed treatment with homeopathic drugs and soil amendment with fertilizers found increased in plant growth and suppressing root rot fungi (Hanif & Dawar, 2015b). Methanolic extract of *T. occidentalis* (10% v/v concentration) showed significant results in the inhibition of *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Microsporium* spp. (Nam & Kang, 2005).

Application of amendments provides energy and nutrients in soil, which enhanced the plant growth (Muchovej & Pacovsky, 1997). Several organic amendments showed inhibition of soil borne pathogens by releasing compounds such as phenols which affects plant pathogens. (Rodriguez-Kabana, 1986; Ali et al., 2001). Thus, based on the finding of present investigation, *T. occidentalis* and *A. montana* globules (30C) amended in the soil released fungicidal compounds which reduced the colonization of root rot fungi and promotes growth productivity.

5. Conclusion

Although used of homeopathic globules found positive results in reducing root rot fungi and increased growth of plants, but it showed a negative effect on nodule formation in leguminous plants. Therefore, it is suggested that it is applicable only on non-leguminous crops and should be applied to large scale due to non hazardous and environment friendly.

Acknowledgements

I take this opportunity to sincerely acknowledge Prof. Dr. M. Javed Zaki and Prof. Dr. Raiha Qadri (Department of Botany, University of Karachi) as well as Dr. Marium Tariq (M.A.H. Qadri Biological Research Centre, University of Karachi) for their kind cooperation, valuable constructive suggestions and execution of my research work.

References

- Alam, S. M. (2009). *Investigation on the different malignancies curing properties of herbal homeopathic drugs, Thuja occidentalis, Taraxacum officinale, Chelidonium majus, Cistus Canadensis, etc.* (Ph.D. Thesis, Department of Pharmacognosy, University of Karachi, Pakistan).
- Ali, N. I., Siddiqui, I. A., Zaki, M. J., & Shaukat, S. S. (2001). Nematicidal potential of *Lantana camara* against *Meloidogyne javanica* in mung bean. *Nematologica Mediterranea*, 29(1), 99-102.
- Aneja, K. R. (2003). *Experiments in Microbiology Plant Pathology and Biotechnology* (4th ed.). New Age International Publishers, New Delhi, India.
- Asha, R., Nisha, P., Suneer, K., Mythili, A., Shafeeq, H. A., Panneer, S. K., ... Shobana, C. S. (2014). *In Vitro* activity of various potencies of homeopathic drug *Thuja* against molds involved in mycotic keratitis. *International Journal of Pharmacy and Pharmaceutical Science*, 6(10), 555-559.
- Bailey, W. R., & Scott, E. G. (1974). *Diagnostic microbiology* (4th ed.). St. Louis, USA: C.V. Mosby.
- Baldwin, I. L., & Fred, E. B. (1927). The fermentation characters of the root nodule bacteria of Leguminosae. *Soil Science*, 24, 217-230. <http://dx.doi.org/10.1097/00010694-192709000-00006>
- Banjara, R. A., Jadhav, S. K., & Bhoite, S. A. (2012). Antibacterial activity of di-2-ethylaniline phosphate screened by paper disc diffusion method. *Applied Pharmaceutical Science*, 2(7), 230-233. <http://dx.doi.org/10.7324/JAPS.2012.2720>
- Barker, R., & Paulitz, T. C. (1996). Theoretical basis for microbial interactions leading to biological control of soil borne plant pathogens. In R. Hall (Ed.), *Principals and practice of managing soil borne plant pathogens* (pp. 50-79). American Phythopathology Society, St. Paul, Mn.
- Bonato, C. M., & Silva, E. P. (2003). Effect of the homeopathic solution *Sulphur* on the growth and productivity of radish. *Acta Scientiarum. Agronomy*, 25, 259-263.
- Bonfim, F. P. G., Martins, E. R., Dores, R. G. R., Barbosa, C. K. R., Casali, V. W. D., & Honorio, I. C. G. (2008). Use of homeopathic *Arnica montana* for the issuance of roots of *Rosmarinus officuinalis* and *Lippa alba* (Mill). *N.E. International Journal High Dilution Research*, 7, 72-76.
- Chataigné, G. (2007). Détermination structurale des lipopolysaccharides de surface chez *Sinorhizobium* (Ph.D. thesis, University of Toulouse, Toulouse III - Paul Sabatier University).
- Chuang-Yien, L. J. (2000). Expression Studies on the exoY Promoter Region in *Rhizobium meliloti*. *BUG Journal*, 3, 182-187.
- Conforti, A., Bertani, S., Metelmann, H., Chirumbolo, S., Lussignoli, S., & Bellavite, P. (1997). Experimental studies of the anti-inflammatory activity of a homeopathic preparation. *Biomedical Therapy*, 15(1), 28-31.
- Deb, L., Dubey, S. K., Jain, A. K., Jain, A., Pandian, G. S., & Rout, S. P. (2006). Anti diarrhoeal activity of *Thuja occidentalis* Linn Ethanol Extract on Experimental Animal. *Indian Drugs*, 44(4), 319-321.
- Dykhuizen, D., & Hartl, D. (1978). Transport by the lactose permease of *Escherichia coli* as the basis of lactose killing. *Journal of Bacteriology*, 135, 876-882.
- Edington, L. V., Khew, K. L., & Barron, G. I. (1971). Fungitoxic spectrum of benzimidazole compounds. *Phytopathology*, 61, 42-44. <http://dx.doi.org/10.1094/Phyto-61-42>
- Ernst, E. (2005). Is homeopathy a clinically valuable approach? *Trends in Pharmacological Sciences*, 26(11), 547-548. <http://dx.doi.org/10.1016/j.tips.2005.09.003>
- Errampalli, D., & Johnston, H. W. (2001). Control of tuber-borne black scurf [*Rhizoctonia solani*] and common scab [*Streptomyces scabies*] of potatoes with a combination of sodium hypochlorite and thiophanatemethyl preplanting seed tuber treatment. *Canadian Journal of Plant Pathology*, 23, 68-77. <http://dx.doi.org/10.1080/07060660109506911>
- Eziashi, E. I., Omamor, I. B., & Odigie, E. E. (2007). Antagonism of *Trichoderma viridae* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*. *African Journal of Biotechnology*, 6(4), 388-392.
- Filip, G. M. (1999). *Ecology, Identification, and Management of Forest Root Diseases in Oregon* (p. 11). Oregon State University Extension Service.

- Filnow, A. B., & Lockwood, L. J. (1983). Mycostasis in relation to the microbial nutrient sinks of five soils. *Soil Biology and Biochemistry*, 15, 557-565. [http://dx.doi.org/10.1016/0038-0717\(83\)90050-0](http://dx.doi.org/10.1016/0038-0717(83)90050-0)
- Fontes, O. L., Araujo, T. L., Mazzi, J. L., Chaud, M. V., & Gutierrez, M. A. (2004). Validation of techniques and methods for the impregnation of homeopathic globules. *Cultura Homeopathica*, 3(9), 8-16.
- Georgi, C. E., & Ettinger, J. M. (1941). Utilization of carbohydrates and sugar acids by rhizobia. *Journal of Bacteriology*, 41, 232-240.
- Graham, P. H. (1976). Identification and classification of root nodule bacteria. In P. S. Nutmen (Ed.), *Symbiotic Nitrogen Fixation in plant* (pp. 99-112). Cambridge University Press, London.
- Graham, P. H., & Parker, C. A. (1964). Diagnostic features in the characterization of the root nodule bacteria of legumes. *Plant and Soil*, 20, 383-396. <http://dx.doi.org/10.1007/BF01373828>
- Gupta, G. (2002). *In vitro* antimycotic potential of *Thuja occidentalis* against *Curvulara lunata* causing Phaeophomycosis in Human. *National Journal of Homoeopathy*, 4(3), 5-12.
- Gupta, G., & Srivastava, A. K. (2002). *In vitro* activity of *Tuja occidentalis* Linn. against human pathogenic *Aspergilla*. *The Homeopathic Heritage*, 27(1), 5-12.
- Hajjegrari, B., Torabi-Giglou, M., Mohammadi, M. R., & Davari, M. (2008). Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *African Journal of Biotechnology*, 7(8), 967-972.
- Hanif, A., & Dawar, S. (2015a). Fungicidal effects of homeopathic drugs in the control of root rot fungi and growth of leguminous and non leguminous crops. *International Journal of Biology and Biotechnology*, 12(1), 97-105.
- Hanif, A., & Dawar, S. (2015b). Use of homeopathic drugs in combination with fertilizers for the control of root rot fungi. *Pakistan Journal of Botany*, 47(6), 2455-2462.
- Hanif, A., Dawar, S., Tariq, M., & Imtiaz, F. (2015). Fungicidal potential of homeopathic pellets in the inhibition of root rot fungi and for promotion of crop plants productivity. *European Journal of Biology and Medical Science Research*, 3(6), 26-39.
- Harveson, B. (2003). *Rhizoctonia crown/root rot*. Plant Disease Centre UNL Extension Service.
- Hoes, J. A. (1985). *Macrophomina phaseolina* causal agent of charcoal rot of sunflower and other crops. Agriculture Research Station, Modren Manitoba, Canada.
- Hulten, E., & Fries, M. (1986). Atlas of north European vascular plants: North of the tropic of cancer. *Konigstin*, 1, 498.
- Hussain, S. Z., Anandam, R. J., & Rao, A. S. (2000). Effect of different fungicides and homeopathic drugs on seed borne fungi of sunflower (*Helianthus annuus* L.). *Indian Journal of Plant Protection*, 28(2), 148-151.
- Irisarri, P., Milnitsky, F., Monza, J., & Bedmar, E. J. (1996). Characterization of rhizobia nodulating *Lotus subbiflorus* from Uruguayan soils. *Plant and Soil*, 180, 39-47. <http://dx.doi.org/10.1007/BF00015409>
- Jahan, N., Ahmad, M., Mehjabeen, M., Zia-ul-haq, S., Alam, L., & Quereshi, M. (2010). Antimicrobial screening of some medicinal plants of Pakistan. *Pakistan Journal of Botany*, 42(6), 4281-4284.
- Khurana, S. M. P., & Gupta, G. C. (1981). Homœopathy: Promise and prospects for plant protection. *Advancing Homœopathy*, 1, 107-116.
- Korosteleva, S. N., Smith, T. K., & Boermans, H. J. (2006). Effects of Feedborne *Fusarium* Mycotoxins on the Performance, Metabolism, and Immunity of Dairy Cows. *Journal of Dairy Science*, 165, 297-311. <http://dx.doi.org/10.3168/jds.2007-0162>
- Kucuk, C., Kivanc, M., & Kinaci, E. (2006). Characterization of *Rhizobium* Sp. isolated from Bean. *Turkish Journal of Biology*, 30, 127-132.
- Lepek, C. V., & D'Antuono, L. A. (2005). Bacterial surface polysaccharides and their role in the rhizobia-legume association. *Lotus Newsletter*, 35, 93-105.
- Mendrygal, K. E., & Gonzalez, J. E. (2000). Environmental Regulation of Exopolysaccharide Production in *Sinorhizobium meliloti*. *Journal of Bacteriology*, 182(3), 599-606. <http://dx.doi.org/10.1128/JB.182.3.599-606.2000>

- Muchovej, R. M. C., & Pacovsky, R. S. (1997). Future directions of by-products and wastes in agriculture. In J. E. Rechcigl & G. C. MacKinnon (Eds.), *Agricultural Uses of By-Products and Wastes* (pp. 1-19). ACS Symposium Series, American Chemical Society Washington, DC, USA. <http://dx.doi.org/10.1021/bk-1997-0668.ch001>
- Nam, S. H., & Kang, M. Y. (2005). Anti-oxidant activity of Medicinal Plants, *Pharmaceutical Biotechnology, Medicinal and Aromatic Plant Abstracts*, 42(6), 409-415.
- Nash, S. M., & Synder, W. C. (1962). Quantitative estimations by plate counts of propagules of the bean root rot, *Fusarium* in field soils. *Phytopathology*, 52, 567-572.
- Qadri, R. (2000). *Comparative anatomy and microbiology of root nodules of some tree legumes found in Karachi* (pp. 72-73, Ph.D. thesis, Department of Botany, University of Karachi, Pakistan).
- Rao, M. S., Reddy, P. P., & Nagesh, M. (1998). Evaluation of plant based formulations on *Trichoderma harzianum* for the management of *Meloidogyn incognita* on egg plant. *Nematologia Mediterranea*, 26, 59-62.
- Razika, G., Amira, B., Yacine, B., & Ammar, B. (2012). Influence of carbon source on the production of exopolysaccharides by *Rhizobium sultae* and on the nodulation of *Hedysarum coronarium* L. legume. *African Journal of Microbiology Research*, 6(30), 5940-5946.
- Rodriguez-Kabana, R. (1986). Organic and inorganic amendment of soil as nematode suppressent. *Journal of Nematology*, 18, 129-135.
- Ruas-Madiedo, P., & de Los Reyes-Gavilán, C. G. (2005). Invited Review: Methods for the Screening, Isolation and Characterization of Exopolysaccharides Produced by Lactic Acid Bacteria. *Journal of Dairy Science*, 88, 843-856. [http://dx.doi.org/10.3168/jds.s0022-0302\(05\)72750-8](http://dx.doi.org/10.3168/jds.s0022-0302(05)72750-8)
- Saremi, H. (2000). *Plant Diseases Caused by Fusarium Species* (p. 160) Jihad Daneshgahi, Ferdosy Mashhad University, Iran.
- Saremi, H. (2005). *Fusarium, biology, ecology and taxonomy* (p. 152). Jihad Daneshgahi, Ferdosy Mashhad University, Iran.
- Saremi, H., & Amiri, M. E. (2010). Exploration of potato cultivar resistant to the major fungal pathogen on potato wilting disease in Iran. *Journal of Food, Agriculture and Environment*, 8(2), 821-826.
- Sheikh, A. H., & Ghaffar, A. (1975). Population study of sclerotia of *Macrophomina phaseolina* in cotton field. *Pakistan Journal of Botany*, 7, 13-17.
- Shrivastava, J., & Atri, D. C. (1998). Effect of the homoeopathic drugs on the production of aflatoxin B1 by *Aspergillus flavus*. *Journal of Phytology Research*, 11(1), 45-49.
- Singh, B., Kaur, R., & Singh, K. (2008). Characterization of *Rhizobium* strain isolated from the roots of *Trigonella foenumgraecum* (fenugreek). *African journal of Biotechnology*, 7(20), 3671-3676.
- Smith, W. H. (1969). Germination of *Macro-phomina phaseolina* sclerotia as affected by *Pinus lamberitina* root exudes. *Canadian Journal of Microbiology*, 15(12), 1387-1391. <http://dx.doi.org/10.1139/m69-250>
- Sneh, B., Burpee, L., & Ogoshi, A. (1991). *Identification of Rhizoctonia species*. APS Press, St. Paul.
- Sokal, R.R. & Rohlf, F.J. (1995). *Biometry: The principles and practices of statistics in biological research* (p. 887). Freeman, New York.
- Stowers, M. D., & Elkan, G. H. (1984). Growth and nutritional characteristics of cowpea rhizobia. *Plant and Soil*, 80, 191-200. <http://dx.doi.org/10.1007/BF02161175>
- Summerell, B. A., Leslie, J. F., Backhouse, D., Bryden, W. L., & Burgess, L. W. (2001). *Fusarium: Paul E. Nelson Memorial Symposium* (p. 392). APS Press, The American Phytopathology Society, St. Paul, Minnesota, USA.
- Tikhonov, O. I., Nedelko, O. K., & Persestova, T. A. (1976). Methods for pathogenicity tests for seed borne *Macrophomina phaseolina* isolated from different hosts. *Phytopathology*, 88(3), 234-237. <http://dx.doi.org/10.1111/j.1439-0434.1977.tb03973.x>
- Timblin, C. R., & Kahn, M. L. (1984). Lactose inhibits the growth of *Rhizobium meliloti* cells that contain an actively expressed *Escherichia coli* lactose operon. *Journal of Bacteriology*, 158(3), 1204-1207. <http://dx.doi.org/0021-9193/84/061204-04>

- Trinick, M. J. (1982). Host-*Rhizobium* associations. In J. M. Vincent (Ed.), *Nitrogen Fixation in legumes* (pp. 111-122). Academic Press, Sydney.
- Vincent, J. M. (1970). A manual for the practical study of root nodule bacteria. *IBP Handbook No. 15*. Blackwell Scientific Publications Ltd., Oxford.
- Wilhelm, S. (1955). Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathology*, *45*, 180-181.
- Windels, C. E., & Nabben, D. J. (1989). Characterization and Pathogenicity of Anastomosis Groups of *Rhizoctonia solani* isolated from *Beta vulgaris*. *Phytopathology*, *79*(1), 83-88. <http://dx.doi.org/10.1094/Phyto-79-83>
- Yang, S. M., & Owen, D. F. (1982). Symptomology and detection of *Macrophomina phaseolina* in sunflower plants parasitized by *Cylendrocopturus adspersus* larvae. *Phytopathology*, *72*(7), 819-821. <http://dx.doi.org/10.1094/Phyto-72-819>

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).