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Crop Protection against Root Rot Fungi by Combined Effect of Homeopathic Drugs and Microbial Antagonists

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Abstract The objective of this research was to reduce economic losses result from root rot fungi by improving plant growth and control the plant pathogenic fungi to a low and safe level. Seeds of okra, sunflower, mung bean and mash bean treated with homeopathic drugs such as *Arnica montana* and *Thuja occidentalis* @ 50 and 75% v/v concentrations (prepared from 30C) along with microbial antagonists namely, *Trichoderma harzianum*, *Paecilomyces variotii*, *Bacillus subtilus* and *Rhizobium meliloti* drenched in soil which enhanced the plant weight and height but also reduced the *Fusarium* spp, *Rhizoctonia solani* and *Macrophomina phaseolina* colonization as compared to alone treatments. Highest number of nodules was observed on mash bean and mung bean seeds when treated with 75% v/v concentrations by both homeopathic drugs and soil were drenched with *R. meliloti*. Off the microbial antagonists used, *T. harzianum* together with treated seeds @ 75% v/v concentrations by both drugs showed better plant productivity and completely suppressed root rot fungi of test plants.

Keywords Homeopathic drugs, Antagonistic fungi and bacteria, Root rot fungi and crop plants

1. Introduction

Root rot diseases caused by soil borne pathogens considered as the most important diseases of many crops. Root rot pathogens including *Fusarium* spp., (produces wilt, root and stem rot diseases on a broad range of plants), *Rhizoctonia solani* Kühn (produces damping off of seedling, wilt, root and seed rot on over 2000 spp of plants), *Macrophomina phaseolina* (Tassi) Goid (produces charcoal rot, root and stem rot, seedling blight on 500 plant species) which decline yield of crop and reduced market values in many worldwide countries (Ghaffar, 1992; Benhamou *et al.*, 1994; El-Mougy, 1995; Wheeler & Rush, 2001; Tanina *et al.,* 2004). Extensively used of pesticides and other chemicals developed concerned of environment and health in finding the substitute technique for disease management of crop plants (Papavizas & Lumsden, 1980).

Homeopathic drugs involves in biological processes of plants due to the secondary metabolites production which act as an environmental friendly without producing toxicity and leaving no residue (Bonato & Silva, 2003). Different formulations have been used to control soil borne pathogens like suspensions of bacteria (Paulitz *et al*., 1992), fungal spores (Harman *et al*., 1980), and powdery preparations of fungal mycelium (Latunde-Dada, 1993). Several studies have been conducted by additions of microbial antagonist in the soil it suppressed the population of soil borne plant pathogens and plant parasitic nematodes (Park, 1989; Saleem *et al.*, 2000). Microbial antagonist such as *Trichoderma* spp., *Paecilomyces lilacinus, Verticillium chlamydosporium, Bacillus* spp., *Stachybotrys atra, Pseudomonas aeruginosa, Gliocladium virens,* have potential to decreased the colonization of root rot pathogens and also improved the plant growth (Rodriguez- Kabana *et al.*, 1984; Lumsden & Locke, 1989; Izhar *et al.*, 1995; Lewis *et al.,* 1996; Bajwa *et al.*, 2003; Abdel-Monaim, 2014). Antagonistic microbes have an ability to control soil borne pathogens by secretion of chitinolytic enzymes and production of mycoparasitism inhibitory compounds (Haram *et al.*, 1996). These mycoparasitism inhibitory compounds might be the reason for the controlling of soil borne pathogens (Bari, 2001). Howell (2003) reported that due to chitinase enzyme it caused breaking of β-glucan, chitin and polysaccharides of fungal cells destroying pathogen. Such microbial antagonists build a protective layer around the roots of plants which prevent the entering of soil borne pathogen (Weller, 1988). Present research was conducted to explore the mutual relationship of homeopathic drugs along with microbial antagonist in the inhibition of root rot fungi.

2. Material and Methods

Collection and population of antagonists: Cultures of *Paecilomyces variotii* (Pv-14), *Trichoderma harzianum* (Th-60), *Bacillus subtilus* (Bs-13) and *Rhizobium melioti* (Rm-19) were obtained from the Karachi University Culture

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Collection (KUCC). Antagonistic fungi (*P. variotii* and *T. harzianum*) were grown on PDA medium whereas antagonistic bacteria (*B. subtilis* and *R. meliloti*) were grown on nutrient broth medium and yeast extract mannitol agar (YEMA) respectively, incubated at 28-30°C for 24-96 hours depending upon population growth. Bacterial colonies (*B. subtilis* and *R. meliloti*) growing on Petri plates were counted and multiplied by the dilution factor which gave CFU/ml of bacteria (Yadav *et al.*, 2010). Fungal population (*P. variotii* and *T. harzianum*) was determined by using serial dilution technique given by Aneja (2001).

Seed treatment: Seeds of mung bean (*Vigna radiata* (L.) R. Wilczek. cv. NM-2006), okra (*Abelmoschus esculentus* (L.) Moench cv. Arka anamika), sunflower (*Helianthus annuus* L. cv. Hysun-38) and mash bean (*Vigna mungo* (L.) Hepper cv. NM-97) were treated with Dr. Willmar Schwabe homeopathic drugs like *Arnica montana* and *Thuja occidentalis* with the concentrations of 75 and 50% v/v (prepared from 30C) respectively whereas, seeds treated with sterilized water served as control for about 10-15 mins. and dried aseptically.

***In vitro*:** Pots experiment was conducted at the screen house bench of Botany department (Karachi University) in natural sunlight in a randomized design. Soil used for experiment was sandy loam containing; 74% sand, 9% clay and 17% silt (Gee & Bauder, 1986) of pH 7.6 (Brady, 1990), organic carbon 4.10% (Sparks, 1996), water holding capacity 38% (Keen & Rakzowski, 1922) and total nitrogen 0.83% (Mackenzie & Wallace, 1954) were placed in plastic pots containing 300g (8 cm diameter). Soil consist of natural infestation having *R. solani* 27% (Wilhelm, 1955), 8-11 sclerotia g-1 of *M. phaseolina* (Sheikh & Ghaffar, 1975), *Fusarium* spp 3700 cfu g-1 (Nash & Synder, 1962). Five treated seeds of mung bean, mash bean, sunflower and okra with different concentrations of homeopathic drugs were sown in pots respectively. Soil was drenched with 5 ml suspension of five day old cultures of *P. variotii* (24×10^{-6} conidia/ml), *T. harzianum* (72×10^{-7} conidia/ml) and 48h-old cultures of *B. subtilus* (14×10^{-8} cells/ml), *R. meliloti* (22×10^{-8} cells/ml). Treatments were replicated thrice and soil without antagonist suspension and untreated seeds served as control. Regularly water was given till the plants fully grown and then uproots it after one month.

Isolation of root rot fungi: After careful uprooting, growth parameters like plant height, weight and number of nodules were recorded. Roots were cautiously washed in running tap water to remove soil particles and each root was cut into five pieces. These root pieces after surface sterilization with 1% $Ca(OCl)_2$ for 5 mins., dried in blotter paper under laminar hood and transferred on poured potato dextrose agar (PDA) medium supplemented with antibiotics (penicillin @ 100,000 unit/L and streptomycin @ 200 mg/L) to inhibit the growth of bacteria. Incubate the Petri plates for one week at room temperature (27-33°C) and colonization of root rot fungi was recorded from each root segment.

Statistical analysis: Data were analyzed by one way analysis (ANOVA) as per experimental design separately followed by the least significant difference (LSD) test at P = 0.05 according to Sokal & Rohlf (1995).

3. Results

Seeds of okra and sunflower treated with *A. montana* and *T. occidentalis* @ 50 and 75% v/v concentrations along with *T. harzianum* drenched in soil showed significant enhancement in the weight and height of plants but also completely suppressed *Fusarium* spp, *Rhizoctonia solani* and *Macrophomina phaseolina* colonization. When both concentrations of *A. montana* and *T. occidentalis* combined with *P. variotii*, *B. subtilus* and *R. meliloti* respectively, it increased the shoot and root weight. Maximum inhibition of root rot fungi was observed in all treatments except *B. subtilus* which showed complete inhibition of *R. solani* colonization when seeds treated with both homeopathic drugs @ 75% v/v concentration (Table 1.1 and 1.2). Whereas, when mung bean and mash bean seeds treated with *T. occidentalis* and *A. montana* @ 50 and 75% v/v concentrations and soil was drenched with *Trichoderma harzianum*, *Paecilomyces variotii*, *Bacillus subtilus* and *Rhizobium meliloti* improved the growth parameters. *R. meliloti* when used with treated seeds of *A. montana* and *T. occidentalis* @ 50 and 75% v/v concentrations showed greater number of nodules and increased root weight. Root rot fungi were completely inhibited by *T. harzianum* drenched soil along with seed treatment of both concentrations with homeopathic drugs. When soil was drenched with *P. variotii*, *B. subtilus* and *R. meliloti* and seeds treated with *T. occidentalis* and *A. montana* @ 50 and 75% v/v concentrations respectively showed maximum inhibition of *Rhizoctonia solani, Fusarium* spp and *Macrophomina phaseolina* colonization (Table 1.3 and 1.4).

Results showed that seeds of sunflower, okra, mung bean and mash bean treated with *A. montana* and *T. occidentalis* @ 75% v/v concentration used along with microbial antagonists drenched in soil improved the plant growth followed by 50% v/v concentration respectively. However; off all the microbial antagonist, complete suppression of pathogenic fungi were observed in the roots of crop plants by *T. harzianum* drenched soil and seeds treated with both homeopathic drugs @ 75% v/v concentrations.

Table 1.1. Effect of seed treatment with homeopathic drugs along with soil drenching with microbial antagonist on growth parameters and in the control of root rot fungi on okra (Abelmoschus esculentus (L.) Moench) plants

TREATMENTS	Shoot Length(cm) ± SD	Shoot Weight(g) ± SD	Root Length(cm) ± SD	Root Weight(g) ± SD	*Fusarium spp* colonization (%) ± SD	*Rhizoctonia solani* colonization (%) ± SD	*Macrophomina phaseolina* colonization (%) ± SD
Control	13.20±0.87	0.35±0.03	9.67±1.36	0.147±0.03	88.89±3.84	55.56±10.18	62.22±7.70
Seed treatment							
Arnica montana @ 75%	18.60±0.53	0.99±0.04	14.73±0.76	0.327±0.05	24.45±3.85	22.22±3.85	33.32±13.33
Arnica montana @ 50%	16.30±0.62	0.84±0.05	13.27±0.61	0.26±0.02	35.54±10.19	37.76±7.72	35.54±10.18
Thuja occidentalis @ 75%	18.30±0.62	0.95±0.03	15.20±0.40	0.27±0.021	22.22±3.85	26.66±6.65	28.88±3.83
Thuja occidentalis @ 50%	16.07±0.61	0.86±0.037	14.27±0.76	0.24±0.02	33.33±6.66	28.88±3.83	39.99±6.68
Soil drenching							
Trichoderma harzianum	16.80±0.60	1.046±0.03	14.40±0.92	0.347±0.031	22.22±3.85	15.55±3.85	20.00±6.67
Paecilmyces variotii	16.70±0.40	0.89±0.03	12.13±0.83	0.27±0.026	44.45±3.85	33.32±6.67	44.43±10.20
Bacillus subtilus	16.53±0.94	0.88±0.06	12.67±0.50	0.25±0.031	60.00±6.67	24.43±10.17	62.20±7.70
Rhizobium meliloti	15.20±0.40	0.81±0.03	12.37±0.51	0.26±0.02	46.67±6.67	44.44±10.18	60.00±6.67
Combined effect							
T. harzianum + *A. montana* @ 75%	26.07±0.42	2.37±0.15	15.93±0.61	0.82±0.03	0.00±0.00	0.00±0.00	0.00±0.00
T. harzianum + *A. montana* @50%	24.40±1.44	2.28±0.04	15.20±0.40	0.65±0.083	11.11±3.84	0.00±0.00	0.00±0.00
T. harzianum + *T. occidentalis* @75%	28.73±1.33	3.18±0.04	16.93±0.81	0.88±0.04	0.00±0.00	0.00±0.00	0.00±0.00
T. harzianum + *T. occidenatlis* @50%	25.70±1.04	2.51±0.14	15.87±0.31	0.71±0.03	8.87±3.86	0.00±0.00	6.67±0.00
P. variotii + *A. montana* @ 75%	24.37±1.56	1.04±0.032	14.73±0.50	0.387±0.03	24.45±3.85	15.55±3.85	17.78±7.70
P. variotii + *A. montana* @ 50%	23.20±0.87	1.06±0.01	15.67±0.50	0.347±0.03	33.32±6.66	22.22±7.70	26.66±6.65
P. variotii + *T. occidentalis* @ 75%	26.08±0.81	1.12±0.02	16.27±0.50	0.45±0.03	20.0±6.67	11.11±3.84	8.88±3.83
P. variotii + *T. occidentalis* @ 50%	24.20±0.87	1.07±0.021	15.10±0.36	0.36±0.03	22.22±3.85	15.55±3.85	24.45±3.85
B. subtilus + *A. montana* @ 75%	25.67±0.91	1.16±0.02	15.40±1.11	0.39±0.03	17.78±7.702	0.00±0.00	24.43±10.17
B. subtilus + *A. montana* @ 50%	22.33±0.50	1.11±0.04	12.63±0.87	0.31±0.03	19.97±6.67	13.32±6.66	26.65±6.65
B. subtilus + *T. occidentalis* @ 75%	25.27±0.42	1.15±0.03	14.87±0.305	0.37±0.04	11.11±3.85	0.00±0.00	22.18±7.70
B. subtilus + *T. occidentalis* @ 50%	23.87±0.70	1.097±0.021	13.60±0.80	0.367±0.064	26.63±6.65	8.89±3.84	28.83±3.87
R. meliloti + *A. montana* @ 75%	25.60±1.11	0.987±0.07	14.87±0.702	0.37±0.05	24.45±3.85	17.78±7.70	26.65±6.65
R. meliloti + *A. montana* @ 50%	24.0±0.35	0.940±0.02	13.30±0.36	0.31±0.03	31.09±3.83	26.65±6.65	35.54±10.18
R. meliloti + *T. occidentalis* @ 75%	25.80±0.60	1.017±0.055	14.43±0.32	0.36±0.02	20.0±6.67	11.11±3.84	22.22±7.70
R. meliloti + *T. occidentalis* @ 50%	22.20±0.40	0.89±0.042	13.00±0.72	0.25±0.03	35.547±10.188	17.78±3.85	35.53±3.87
$LSD_{0.05}$=	1.357	0.089	1.124	0.061	9.278	9.275	11.503
Probability level (P<) =	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Where: ±SD=Standard deviation

Table 1.2. Effect of seed treatment with homeopathic drugs along with soil drenching with microbial antagonist on growth parameters and in the control of root rot fungi on sunflower (Helianthus annuus L.) plants

TREATMENTS	Shoot Length(cm) ± SD	Shoot Weight(g) ± SD	Root Length(cm) ± SD	Root Weight(g) ± SD	*Fusarium spp* colonization (%) ± SD	*Rhizoctonia solani* colonization (%) ± SD	*Macrophomina phaseolina* colonization (%) ± SD
Control	9.37±1.43	0.32±0.06	8.20±1.25	0.14±0.03	86.67±6.66	71.11±13.88	77.78±7.70
Seed treatment							
Arnica montana @ 75%	18.07±1.10	0.99±0.03	11.43±0.96	0.21±0.06	39.99±6.69	26.65±6.65	24.44±10.18
Arnica montana @ 50%	14.96±0.57	0.89±0.03	13.53±1.81	0.20±0.025	48.88±3.83	37.77±3.87	42.21±7.72
Thuja occidentalis @ 75%	17.87±0.30	1.00±0.03	14.53±1.21	0.27±0.02	26.66±6.65	20.00±6.67	24.45±3.85
Thuja occidentalis @ 50%	15.80±0.60	0.92±0.025	13.53±0.70	0.22±0.04	35.54±10.19	33.34±11.54	37.76±7.71
Soil drenching							
Trichoderma harzianum	17.27±0.76	0.97±0.01	12.80±0.40	0.26±0.02	22.22±3.85	15.55±3.85	17.78±7.70
Paecilmyces variotii	16.40±0.53	0.75±0.04	12.60±0.92	0.22±0.038	40.00±6.67	17.78±10.18	28.88±3.83
Bacillus subtilus	12.87±0.64	0.67±0.05	9.93±0.42	0.16±0.035	60.00±6.67	19.99±6.69	51.11±3.84
Rhizobium meliloti	15.13±0.95	0.58±0.04	10.47±0.94	0.17±0.012	64.44±10.18	37.76±7.72	60.00±6.67
Combined effect							
T. harzianum + *A. montana* @ 75%	25.27±0.42	1.48±0.07	14.20±0.40	0.45±0.031	0.00±0.00	0.00±0.00	0.00±0.00
T. harzianum + *A. montana* @50%	24.87±0.31	1.25±0.02	13.60±0.80	0.40±0.053	17.78±7.70	0.00±0.00	8.89±3.84
T. harzianum + *T. occidentalis* @75%	23.96±1.19	1.46±0.02	14.20±1.25	0.47±0.05	0.00±0.00	0.00±0.00	0.00±0.00
T. harzianum + *T. occidenatlis* @50%	22.26±0.61	1.29±0.03	12.27±0.76	0.39±0.03	11.11±3.85	0.00±0.00	15.55±3.85
P. variotii + *A. montana* @ 75%	24.93±0.61	1.19±0.06	14.73±0.61	0.40±0.053	22.22±7.70	8.89±3.84	17.77±7.70
P. variotii + *A. montana* @ 50%	24.07±1.94	1.06±0.02	14.67±1.01	0.31±0.083	31.09±3.83	15.55±3.85	22.22±10.18
P. variotii + *T. occidentalis* @ 75%	25.80±0.60	1.16±0.015	14.70±0.50	0.43±0.05	17.78±3.85	6.67±0.00	11.11±3.84
P. variotii + *T. occidentalis* @ 50%	23.67±1.10	1.07±0.05	14.33±0.64	0.32±0.036	24.45±3.85	13.33±6.66	24.44±10.18
B. subtilus + *A. montana* @ 75%	24.80±0.72	1.06±0.015	14.80±0.40	0.29±0.021	17.77±7.70	0.00±0.00	22.22±7.70
B. subtilus + *A. montana* @ 50%	23.00±0.53	0.94±0.02	14.40±0.53	0.24±0.02	40.00±6.67	11.11±3.84	20.00±6.67
B. subtilus + *T. occidentalis* @ 75%	25.2±0.87	1.09±0.03	14.00±0.72	0.33±0.031	17.77±3.85	6.67±0.00	13.33±6.66
B. subtilus + *T. occidentalis* @ 50%	24.53±0.83	1.037±0.015	13.87±0.31	0.32±0.04	20.00±6.67	15.55±3.85	24.43±10.17
R. meliloti + *A. montana* @ 75%	23.40±1.22	1.00±0.049	14.07±0.42	0.27±0.01	33.32±6.67	22.22±7.70	24.45±3.85
R. meliloti + *A. montana* @ 50%	20.27±0.76	0.91±0.03	14.17±0.85	0.23±0.01	44.44±10.18	26.65±6.65	33.32±6.66
R. meliloti + *T. occidentalis* @ 75%	23.67±0.90	1.01±0.03	15.20±0.40	0.30±0.021	31.09±3.83	8.89±3.84	19.99±13.32
R. meliloti + *T. occidentalis* @ 50%	21.13±1.14	0.98±0.035	13.67±0.50	0.24±0.02	44.45±3.85	15.55±3.85	37.76±7.72
$LSD_{0.05}$=	1.481	0.061	1.358	0.062	10.256	9.781	11.777
Probability level (P<) =	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Where: ±SD=Standard deviation

Table 1.3. Effect of seed treatment with homeopathic drugs along with soil drenching with microbial antagonist on growth parameters and in the control of root rot fungi on mash bean (*Vigna mungo* (L.) Hepper) plants

TREATMENTS	Shoot Length(cm) ± SD	Shoot Weight(g) ± SD	Root Length(cm) ± SD	Root Weight(g) ± SD	# of Nodules ± SD	*Fusarium spp* colonization (%)± SD	*Rhizoctonia solani* colonization (%) ± SD	*Macrophomina phaseolina* colonization (%) ± SD
Control	13.57±1.15	0.29±0.05	14.20±0.60	0.14±0.02	9.00±3.61	95.55±3.85	73.33±6.67	80.00±6.67
Seed treatment								
Arnica montana @ 75%	18.17±0.71	0.83±0.076	17.87±1.14	0.29±0.03	17.67±1.53	44.44±3.85	33.32±6.67	28.88±3.83
Arnica montana @ 50%	17.40±0.53	0.73±0.05	16.60±1.31	0.24±0.02	15.67±2.08	48.88±3.83	42.21±7.72	37.76±7.72
Thuja occidentalis @ 75%	20.13±0.83	0.95±0.03	21.07±1.94	0.36±0.02	20.33±1.53	37.76±7.72	22.22±3.85	33.32±6.67
Thuja occidentalis @ 50%	18.90±0.50	0.83±0.05	16.87±1.81	0.29±0.026	19.67±1.53	39.99±6.69	35.53±3.87	42.21±7.72
Soil drenching								
Trichoderma harzianum	19.40±1.11	0.94±0.03	19.43±1.62	0.31±0.044	21.00±2.00	24.43±10.17	15.55±3.85	15.56±10.18
Paecilmyces variotii	18.47±0.31	0.82±0.04	17.80±0.72	0.31±0.042	18.00±2.00	44.43±10.20	35.56±10.18	46.67±6.67
Bacillus subtilus	17.10±0.62	0.68±0.06	15.13±0.70	0.21±0.03	15.33±1.53	55.57±10.18	15.56±10.18	44.45±3.85
Rhizobium meliloti	15.20±0.87	0.64±0.053	15.20±2.09	0.34±0.035	26.00±2.00	68.89±3.85	44.43±10.20	68.89±13.88
Combined effect								
T. harzianum + *A. montana* @ 75%	27.80±1.25	1.22±0.04	26.67±0.64	0.58±0.12	27.00±1.00	0.00±0.00	0.00±0.00	0.00±0.00
T. harzianum + *A. montana* @50%	24.90±1.73	1.05±0.026	26.10±1.75	0.44±0.053	23.67±1.53	11.11±3.85	6.67±6.66	8.89±3.85
T. harzianum + *T. occidentalis* @75%	27.97±0.81	1.21±0.03	25.80±2.75	0.63±0.03	24.33±2.08	0.00±0.00	0.00±0.00	0.00±0.00
T. harzianum + *T. occidenatlis* @50%	23.80±1.40	1.15±0.03	25.73±2.72	0.52±0.072	24.67±2.08	15.53±3.87	8.89±3.85	13.33±0.00
P. variotii + *A. montana* @ 75%	27.13±0.83	1.12±0.03	24.30±0.44	0.55±0.03	25.00±2.65	31.09±3.83	20.00±6.67	26.64±11.53
P. variotii + *A. montana* @ 50%	25.97±0.67	0.94±0.02	22.77±1.45	0.51±0.025	19.33±1.53	37.77±3.87	31.11±7.69	22.22±7.70
P. variotii + *T. occidentalis* @ 75%	28.60±0.20	1.08±0.035	25.53±0.93	0.59±0.03	25.00±1.00	24.45±3.85	15.56±10.18	13.33±6.67
P. variotii + *T. occidentalis* @ 50%	26.83±0.51	1.02±0.015	22.43±1.00	0.53±0.01	21.67±1.53	28.88±3.83	22.22±3.85	17.78±7.70
B. subtilus + *A. montana* @ 75%	23.60±1.11	0.92±0.04	21.07±0.42	0.32±0.04	22.00±2.65	35.53±3.87	8.89±3.85	20.00±6.67
B. subtilus + *A. montana* @ 50%	21.43±0.78	0.77±0.03	19.87±0.83	0.26±0.025	19.67±3.21	42.21±7.72	15.55±3.85	24.45±3.85
B. subtilus + *T. occidentalis* @ 75%	24.73±0.60	0.89±0.03	22.27±0.42	0.38±0.015	24.00±1.00	26.66±6.65	0.00±0.00	17.78±7.70
B. subtilus + *T. occidentalis* @ 50%	22.57±0.87	0.82±0.02	20.23±0.93	0.34±0.015	20.33±1.53	35.53±3.87	11.11±3.85	22.22±3.85
R. meliloti + *A. montana* @ 75%	25.90±1.71	0.96±0.025	24.50±2.07	0.71±0.035	37.67±1.53	22.22±7.70	15.55±3.85	31.10±3.84
R. meliloti + *A. montana* @ 50%	26.13±0.83	0.87±0.05	22.53±1.70	0.59±0.03	33.66±1.53	28.87±7.69	26.67±6.65	35.54±10.19
R. meliloti + *T. occidentalis* @ 75%	27.00±0.26	1.04±0.015	24.93±0.42	0.69±0.042	37.33±2.08	15.55±3.85	8.89±3.85	26.66±6.65
R. meliloti + *T. occidentalis* @ 50%	25.23±0.40	0.84±0.02	22.10±1.77	0.58±0.053	35.33±4.16	24.45±3.85	15.55±3.85	28.88±3.83
$LSD_{0.05}$=	1.502	0.002	2.396	0.069	3.445	9.536	9.944	11.362
Probability level (P<) =	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Where: ±SD=Standard deviation

Table 1.4. Effect of seed treatment with homeopathic drugs along with soil drenching with microbial antagonist on growth parameters and in the control of root rot fungi on mung bean (*Vigna radiata* (L.) R. Wilczek.) plants

TREATMENTS	Shoot Length(cm) ± SD	Shoot Weight(g) ± SD	Root Length(cm) ± SD	Root Weight(g) ± SD	# of Nodules ± SD	*Fusarium spp* colonization (%)± SD	*Rhizoctonia solani* colonization (%) ± SD	*Macrophomina phaseolina* colonization (%) ± SD
Control	14.20±0.60	0.34±0.02	11.43±2.73	0.17±0.021	11.00±3.61	88.89±3.85	64.44±10.18	80.00±6.67
Seed treatment								
Arnica montana @ 75%	17.37±0.21	0.86±0.03	21.60±0.80	0.26±0.02	26.00±2.00	31.09±3.83	24.45±3.85	26.66±6.65
Arnica montana @ 50%	17.03±0.20	0.81±0.026	19.97±0.38	0.25±0.015	20.67±1.53	44.45±3.85	33.30±0.00	39.99±6.69
Thuja occidentalis @ 75%	18.13±0.31	0.94±0.021	23.33±0.50	0.30±0.026	20.67±3.06	28.88±3.83	22.22±3.85	28.88±3.83
Thuja occidentalis @ 50%	17.70±0.56	0.90±0.021	19.93±1.40	0.27±0.01	25.33±1.53	35.53±3.87	39.99±6.69	42.22±3.85
Soil drenching								
Trichoderma harzianum	22.20±0.72	0.97±0.01	20.50±0.75	0.32±0.02	24.33±1.53	20.00±6.67	15.55±3.85	31.09±3.83
Paecilmyces variotii	20.40±0.80	0.86±0.015	19.03±0.57	0.33±0.01	18.00±1.00	53.33±6.67	33.32±6.67	48.88±16.79
Bacillus subtilus	19.73±0.50	0.85±0.04	18.00±0.26	0.24±0.015	17.33±2.52	62.22±7.70	48.89±3.85	68.89±3.85
Rhizobium meliloti	18.83±0.49	0.79±0.03	18.50±0.20	0.29±0.02	28.00±1.00	64.44±10.18	55.56±10.18	75.56±10.18
Combined effect								
T. harzianum + *A. montana* @ 75%	27.77±0.47	1.20±0.026	24.97±2.18	0.63±0.015	25.67±2.52	0.00±0.00	0.00±0.00	0.00±0.00
T. harzianum + *A. montana* @50%	26.63±0.74	1.14±0.055	25.23±2.25	0.59±0.036	22.33±1.53	15.55±3.85	0.00±0.00	20.00±6.67
T. harzianum + *T. occidentalis* @75%	29.26±0.42	1.29±0.046	27.13±0.80	0.64±0.02	27.00±2.00	0.00±0.00	0.00±0.00	0.00±0.00
T. harzianum + *T. occidenatlis* @50%	27.76±0.45	1.22±0.02	26.23±0.45	0.57±0.02	24.33±1.53	11.11±3.85	4.45±3.85	13.33±6.67
P. variotii + *A. montana* @ 75%	28.37±1.47	1.12±0.035	25.30±1.05	0.65±0.07	31.33±3.06	24.43±10.17	17.78±3.85	31.09±3.83
P. variotii + *A. montana* @ 50%	26.18±0.61	1.01±0.031	21.93±1.67	0.51±0.064	26.33±1.53	28.88±3.83	22.22±10.18	46.67±6.67
P. variotii + *T. occidentalis* @ 75%	28.17±0.85	1.15±0.03	26.20±0.60	0.61±0.03	26.33±2.08	20.00±6.67	15.56±10.18	24.45±3.85
P. variotii + *T. occidentalis* @ 50%	25.40±1.74	0.98±0.045	23.47±0.95	0.53±0.062	21.67±1.53	31.11±10.18	28.89±16.78	35.53±3.87
B. subtilus + *A. montana* @ 75%	24.30±1.41	0.88±0.087	24.77±2.10	0.46±0.053	26.67±1.53	20.00±6.67	17.78±3.85	28.88±3.83
B. subtilus + *A. montana* @ 50%	25.76±0.55	0.94±0.072	23.83±1.24	0.43±0.0152	20.33±2.08	22.22±7.70	24.45±3.85	35.53±3.87
B. subtilus + *T. occidentalis* @ 75%	26.33±0.50	0.97±0.015	26.50±2.13	0.47±0.01	26.00±2.00	13.33±6.66	11.11±3.84	20.00±6.67
B. subtilus + *T. occidentalis* @ 50%	25.63±0.49	0.98±0.0058	21.43±0.97	0.42±0.015	21.67±1.53	31.11±3.85	15.55±3.85	33.32±6.67
R. meliloti + *A. montana* @ 75%	26.90±0.78	0.89±0.021	25.37±3.03	0.54±0.02	39.00±3.61	31.09±3.84	28.88±3.83	37.76±7.72
R. meliloti + *A. montana* @ 50%	25.80±0.40	0.84±0.02	23.76±1.12	0.47±0.05	35.33±3.05	42.21±7.72	42.22±3.85	48.87±7.68
R. meliloti + *T. occidentalis* @ 75%	27.27±0.50	0.95±0.03	25.30±1.42	0.56±0.025	40.67±4.16	26.67±6.67	31.09±3.83	24.45±3.85
R. meliloti + *T. occidentalis* @ 50%	26.13±0.30	0.90±0.02	22.97±1.39	0.46±0.015	36.33±1.53	33.32±6.67	37.76±7.72	39.99±6.69
$LSD_{0.05}$=	1.221	0.059	2.389	0.053	3.739	10.099	10.565	10.641
Probability level (P<) =	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Where: ±SD=Standard deviation

4. Discussion

Seed treatment with *T. occidentalis* and *A. montana* (30C) and soil drenching with antagonistic organism showed significant results in controlling the root rot fungi and enhancement of plant growth. *T. occidentalis* contain an active ingredient such as mono terpene thujone used in the production of insecticides, pharmacologically, soaps, deodorants and perfumes (Kamden & Hanover, 1993). *T. occidentalis* extracts contain antiviral, antidiarrheal and sedative activities (Deb *et al*., 2007; Aziz *et al*., 2014). *T. occidentalis* (30 and 200M) inhibit *A. flavus* whereas 50M inhibit *A. niger* (Gupta & Srivastava, 2002). *Arnica montana* contain active constituents recognized in flower, leaves and roots having alcohols, flavonoids, sesquiterpene lactones, carotenoids, essential oil, tannins, phenolic and chlorogenic acids compounds (Gawlik-Dziki *et al.,* 2011; Weremczuk-jezyna *et al*., 2011). *A. montana* exhibited antioxidant, antibacterial, antiseptic, antisclerotic and antifungal activities (Ganzera *et al.,* 2008; Sugier & Gawlik-Dziki *et al.*, 2009). *A. montana* (3, 6, and 12CH) improved the growth of plants (Bonfim *et al*., 2008). Hanif and Dawar (2015) reported that by using 100, 75 and 50% v/v concentrations of *T. occidentalis* and *A. montana* (30C) used as a seed treatment and soil drenching methods inhibit the root rot fungi and improved the growth of non leguminous and leguminous plants. *A. montana* and *T. occidentalis* (30C) pellets used *in vitro* and *in vivo* experiments showed positive results in suppressing root rot fungi (Hanif *et al*., 2015). Use of antagonistic bacteria applied as soil drenching or seed dressing showed significant inhibition of root rot pathogens on crop plants (Zaki & Ghaffar, 1987; Ehtesham-ul-Haque *et al*., 1990; Shahzad & Ghaffar, 1992). Besides fungal microbial antagonists, antagonistic bacteria also reduced the colonization of root rot fungi. *Rhizobium* spp., not only control soil borne pathogens but produce beneficial effect on plants by improving nutrient and water uptake of plants (Seuk Bae *et al*., 2000). Soil inoculated with the *Bacillus cereus, Bacillus subtilus* and *Trichoderma* spp. suppressed seedling infection and with the increase of dose it increase antagonist population (Bankole & Adebanjo, 1998). *Paecilomyces* produces peptidal antibiotic (P-168) which reduced root infecting fungi to a larger extent (Isogai *et al*., 1980). Soil application with *Trichoderma viride* and neem oil cake showed significant results in controlling the root knot nematode and *Fusarium* spp. in chick-pea which increased the yield (Pandey *et al*., 2005). Soil amended with *A. javanica* stem and leaves powder @ 1.0% w/w and seeds dressing with *T. harzianum*, *P. aeruginosa* and *A. niger* showed maximum plant growth and suppressed root rot fungi on cowpea and mung bean plants (Ikram & Dawar, 2015). Soil application and seed treatment with *T. harzianum, T. viride* and *G. virens* against *R. bataticola* found to be most effective in suppressing root rot disease but also improved germination, plant growth and yield of chick-pea (Dubey *et al.,* 2011). Seed treatment with *Bacillus subtilis* and *Gliocladium virens* reduced the colonization of *Fusarium* spp in cotton (Zhang *et al.,* 1996). Bio-priming of peanut, chickpea, okra and sunflower seeds with *T. harzianum, R. meliloti* and *Bacillus* sp. conidial/cell suspensions at five, ten and twenty minutes significantly suppressed *Macrophomina phaseolina* and *Fusarium* spp (Rafi & Dawar, 2014). Plant growth promoting *Rhizobacteria* improve plant growth through direct stimulation in the plant either producing growth regulators or suppressing pathogens (Raaijimaker *et al*., 2002; Weller *et al*., 2002). Seed treated with *A. montana* and *T. occidentalis* @ 75 and 50% v/v concentrations and soil drenching with *T. harzianum* showed excellent results in the control of root rot fungi followed by *P. variotii*, *B. subtilus* and *R. meliloti* respectively which was also reported by Abdel Kader *et al*. (2012) in which *T. harzianum* used alone or in combination significantly suppressed the soil borne pathogens as compared to *Pseudomonas flourescens*, *Sacchromyces cerevisiae* and *Bacillus subtilis*. Application of *T. occidentalis* (30C) as a seed treatment with 0.1% concentration increased the weight and length of shoot and root of cereal plants (wheat and millet) but also reduced the root infecting fungi (Dawar *et al*., 2015). Similar results were also obtained by Panda *et al. (*2013) and Baumgartner *et al*. (2004) which found that higher concentrations of drugs is stronger in action in case of pea plants. Homeopathic drugs used in very low doses and found to be environmental friendly and cheap (Toledo *et al*., 2011). Homoeopathic drugs fulfill the promise due to antifungal properties (Sinha & Singh, 1983; Shrivastava & Atri, 1998) and for that reason study on the homeopathic drugs needs to be enhanced (Benzie & Watchtel-Galor, 2011).

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