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FUNGICIDAL EFFECTS OF HOMEOPATHIC DRUGS IN THE CONTROL OF ROOT ROT FUNGI AND GROWTH OF LEGUMINOUS AND NON LEGUMINOUS CROPS

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ABSTRACT

Homeopathic drugs due to fungicidal potential are used as substitute technique in reducing the incidence of root rot fungi like *Rhizoctonia solani, Fusarium* spp and *Macrophomina phaseolina*. Homeopathic drugs such as *Arnica montana* and *Thuja occidentalis* with 100, 75 and 50% v/v concentrations were used to investigate growth parameters and for the control of root rot fungi by using soil drenching and seed treatment methods. Results showed that pure homeopathic concentration (100% v/v) not only enhanced plant growth but also completely inhibited the incidence of root rot fungi followed by 75 and 50% v/v concentrations which also improved plant growth and showed maximum inhibition in root colonization of both leguminous viz., mung (*Vigna radiata* (L.) R.Wilczek) and mash (*Vigna mungo* (L.) Hepper) and non-leguminous viz., sunflower (*Helianthus annuus* L.) and okra (*Abelmoschus esculentus* (L.) Moench) plants.

Keywords: Homeopathic drugs**,** Control, Root rot fungi, Leguminous and non-leguminous crops.

INTRODUCTION

In Pakistan, soil borne root infecting fungi attack many crops which immensely decrease yield (Ehtesham-ul-Haque and Ghaffar, 1994). Root infection caused by soil borne root infecting fungi results in root rot and wilt diseases (Armstrong *et al*., 1976). *Fusarium* spp. are soil inhabiting pathogen producing wilt disease causes severe economic losses to large variety of crop plants (Larkin and Fravel, 1998). *Macrophomina phaseolina* (Tassi) Goid, causes severe damages to more than 500 different monocotyledonous and dicotyledonous plant species in many regions of the world (Das *et al*., 2008; Ma *et al*., 2010). *M. phaseolina* infects all stages of plant growth and are initiated by soil, seed and plant residues (Reuveni *et al*., 1983). It exists in soil as sclerotia which can remain dormant for longer periods and under appropriate environment produces hyphae which infect host plant roots (Ammon *et al*., 1974). Pathogen produces charcoal rot and results in seedling death due to blockage of xylem vessels. Formation of reddish brown lesions on roots and stems due to the dark mycelium viz., black micro-sclerotia resulted in wilting and defoliation of plants (Abawi and Pastor-Corrales, 1990). It may possibly result into 100% yield damage (Bashir and Malik, 1988) depending upon disease severity enhanced due to dry and hot weather conditions (Gaige *et al*., 2010).*Rhizoctonia solani* Kühn is a soil inhabiting fungus exists as active mycelium known to cause seed rot, wilt, damping off of seedling and root rot around 2000 plant species. (Parmeter, 1970) of which 63 hosts have been reported from Pakistan (Mirza and Qureshi, 1978; Ghaffar, 1988). A large number of disease control practices like fungicide application, antagonist organisms and crop rotation have been adopted to improve the growth and enhanced the yield of crops (Pineda, 2001; Choudhary *et al.,* 2004; Stephan *et al.,* 1988). Homeopathic drugs used as alternate techniques for the production of secondary metabolites and take part in biological processes of plants without producing toxicity and act as environmental friendly leaving no residue (Bonato and Silva, 2003). The scientific work of homeopathic drug has been proved positive effects in human being but in plant it is recent (Bonato *et al*., 2006).

Homeopathic drug such as *Thuja occidentalis* (Cupressacae) grown in Europe which has been used to treat enuresis, bronchial catarrh, uterine carcinomas, amenorrhea, psoriasis, cystitis and rheumatism (Chang *et al*., 2000). The oil of eastern cedar leaves (*T. occidentalis*) was investigated by Von Rudloff (1962). Cedar leaf oil can be obtained by steam or hydro distillation of the foliage used in the production of insecticides, perfumes, deodorants and soaps (Duke, 1985; Kamden and Hanover, 1993).The major element of the oil is mono terpene thujone, an active ingredient used pharmacologically for the production of nasal decongestants and cough suppressants and also used in making perfumes, shoe polishes and soaps (Food and Agriculture Organization of the United Nations, 1995). *Arnica montana* (Asteraceae) grows mostly on the Central Europe and East. It is considered as the important herbal plants mostly used in pharmaceutical and cosmetic industry (Bilia *et al.*, 2006). It's active constituents identified in flower, leaves and roots contains sesquiterpene lactones (arnicolide, helenaline and dihydro-helenaline), alcohols (arnidiol, arnilenediol, iso-arnilenediol), flavonoids (quercetin and its derivatives viz., quercetine-3-mono-

glucosideo and quercetine-3-glycogalacturonic), carotenoids, essential oil, tannins, chlorogenic acids and phenolic acid compounds (Ganzera *et al.*, 2008; Gawlik-Dziki *et al.*, 2011; Weremczuk-jezyna *et al*., 2011; Macedo *et al*, 2004; Bucay, 1995). These compounds are responsible for anti-inflammatory properties (Siedle *et al*, 2004).The present research is to study the fungicidal effectiveness of homeopathic drugs in reducing the incidence of root rot fungi on crop plants.

MATERIALS AND METHODS

Homeopathic drugs like *Arnica montana* (30) and *Thuja occidentalis* (30) were purchased from medicinal market of Karachi. Soil was obtained from the Department of Botany, Karachi University campus which had sandy loam having pH 7.8 with moisture holding capacity (MHC) of 27% (Keen and Raczkowski, 1922) and total nitrogen 0.7% (Mackenzie and Wallace, 1954) was determined electrometrically. Soil had natural infestation of 8-9 sclerotia g^{-1} of *M. phaseolina* measured by wet sieving dilution technique (Shiekh and Ghaffar, 1975), 28% colonization of *R. solani* estimated on sorghum seeds which used as bait (Wilhelm, 1955) and 3600 cfu g^{-1} *Fusarium* spp., evaluate by soil dilution technique (Nash and Synder, 1962). Soil was sieved through 2mm sieve to remove stone particles and transferred in plastic pots containing 300 g (8 cm diameter). In seed treatment method, leguminous seeds viz., mung and mash and non-leguminous seeds viz., okra and sunflower were treated with *Arnica montana* and *Thuja occidentalis* of 100, 75 and 50% v/v concentrations separately and dried aseptically. Treated seeds were then sown in soil. Treatments were replicated thrice and the pots without untreated seeds were served as control. Whereas, in soil drenching method, 20 ml *Arnica montana* and *Thuja occidentalis* of 100, 75 and 50% v/v concentrations transferred in soil respectively. Five seeds of leguminous plants such as mung and mash bean and non-leguminous plants such as okra and sunflower were sown in each pot separately of different concentrations. Non drenched soil and non treated seeds served as control. Each treatment replicated thrice and randomized on a greenhouse bench for one month of growth and watered daily. After one month of growth, plants were uprooted and growth parameters in terms of shoot length shoot weight, root length, root weight and nodules per plant were recorded. Roots were washed and cut into five pieces. These root piece after surface sterilization with 1% $Ca(OCl)_2$, 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 for one week at room temperature (25-30^oC) and colonization of root rot fungi was recorded from each root segment. Data were analyzed by using (ANOVA) followed by the least significant difference (LSD) test at P = 0.05 as given by Gomez and Gomez (1984).

RESULTS

Seeds and soil treated with homeopathic drugs showed 100% germination of mung (*Vigna radiata* (L.) R. Wilzeck), mash (*Vigna mungo* (L.) Hepper), sunflower (*Helianthus annuus* L.) and okra (*Abelmoschus esculentus* (L.) Moench) plants. In mash bean, significant ($P \leq 0.001$) enhancement in growth parameters was observed at 100, 75 and 50% v/v concentrations respectively by *A. monatana* and *T. occidentalis.* Pure concentration (100% v/v) of both drugs showed complete inhibition of root rot fungi by both methods. *Thuja* @ 75% v/v ($P \leq 0.001$) showed complete inhibition of *R. solani* and *M. phaseolina* in both methods (Fig.1). There was significant increased($P \leq 0.001$) in growth parameters of mung bean and complete inhibition of root infecting fungi observed when *Arnica* and *Thuja* used @ 100% v/v($P \leq 0.001$) respectively treated by both methods. In seed method, *Thuja* @ 75% v/v ($P \leq 0.001$) suppressed *R. solani* colonization (Fig.2). In non-leguminous plants viz., sunflower and okra both showed significant growth ($P \leq 0.001$) and complete inhibition of root rot fungi was observed by pure concentrations when both drugs applied by using both methods. In soil drenching method, colonization of *R. solani* was completely inhibited when soil was treated with *Arnica* and *Thuja* @ 75 and 50% ($P \leq 0.001$) v/v concentrations respectively (Fig.3 and Fig.4).

DISCUSSION

Observations showed that *A. montana* and *T. occidentalis* (30Q) when used in 100% v/v concentration not only improved plant growth but showed complete inhibition of root rot fungi like *R. solani, M. phaseolina* and *Fusarium* spp. followed by 75 and 50% v/v concentrations which also enhanced plant growth and showed maximum inhibition in both leguminous and non-leguminous plants by using seed treatment and soil drenching methods, respectively. Seed dressing improved yield and reduce economic losses by suppressing pathogenic fungi (Martha *et al*., 2003).

There are several reports where soil amendment with oil cakes viz., neem and cotton cake showed significant results of controlling root rot fungi (Ehtesham-ul-haque *et al.,* 1995). Organic amendment also used in soil which not only suppressed plant pathogens but increased the activity of bio-control agents (Sitaramaiah, 1990).

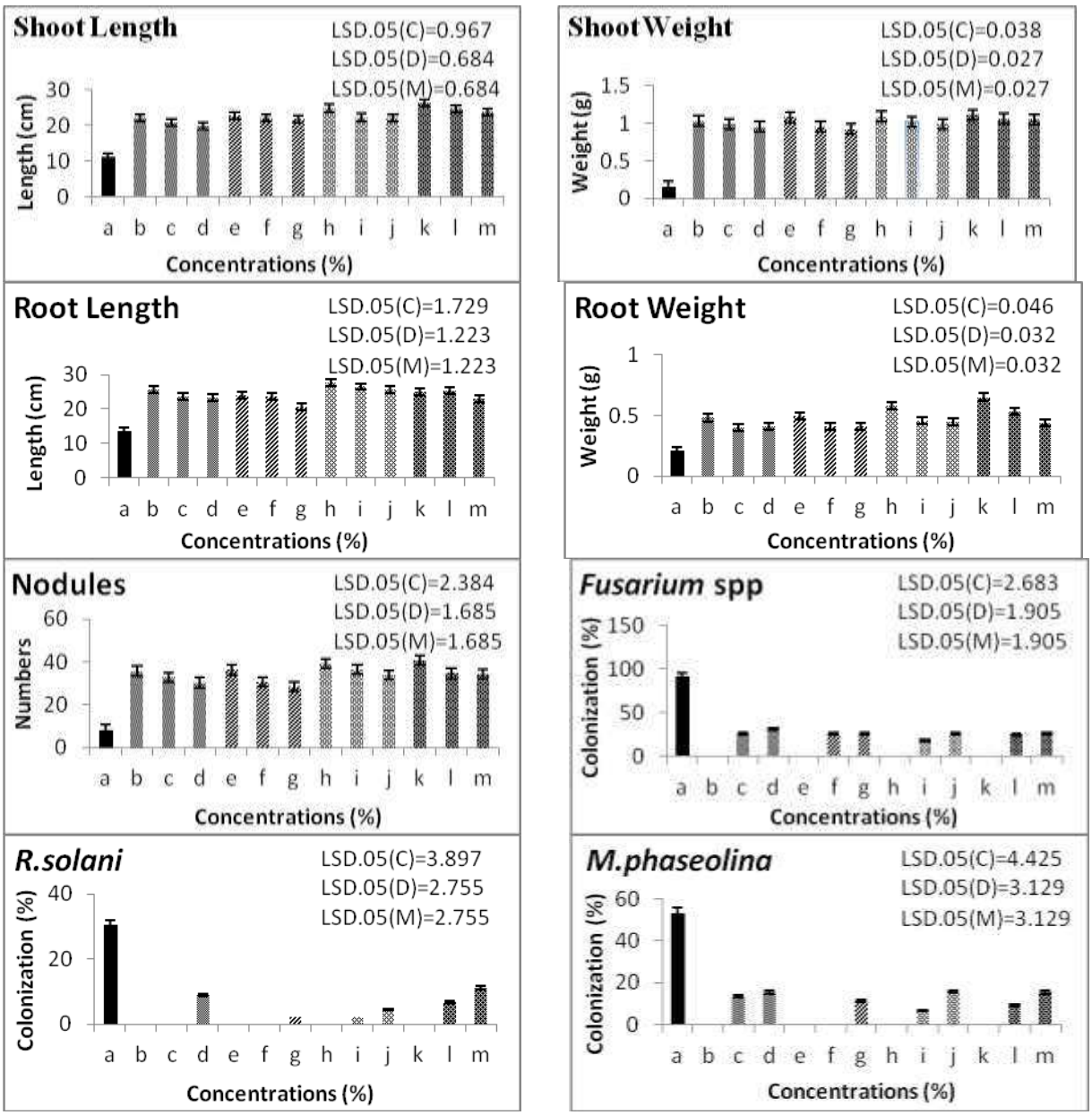

Fig.1. Effect of seed treatment and soil drenching with *Arnica montana* and *Thuja occidentalis* on growth parameters and control of root rot fungi on mash bean (*Vigna mungo* (L.) Hepper) plants.
Where; a=Control; (C) =Concentrations, (D) = Drugs, (M) = Methods
(Seed treatment) b=A@100%, c=A@75%, d=A@50%,e=T@100%, f=T@75%, g=T@50%
(Soil drenching) h=A@100%, i=A@75%, j=A@50%, k=T@100%, l=T@75%, m=T@50%

Similarly, antagonist micro-organisms when used as seed treatment or soil drenching promoted plant growth (Zaki and Ghaffar, 1987; Ehtesham-ul-haque *et al.,* 1990). *Arnica montana* exhibited anti-bacterial, anti-septic, anti-fungal, anti-oxidant and anti-sclerotic activities (Ganzera *et al.,* 2008; Sugier and Gawlik-Dziki *et al.,* 2009). In ancient times extract of *A. montana* flowers used as herbal medicines for external injury and rheumatic joint and muscle complaints (Willuhn and Wichtl, 2004). Currently, *Arnica montana* with the potencies of 3, 6 and 12 CH used to enhance and improve the growth of plants (Bonfim *et al*., 2008). Plant extracts of *Thuja occidentalis* has

been reported for anti-diarrheal and anti-viral activity (Deb *et al*., 2007). Plant also proposed for its sedative activity (Aziz *et al*., 2014).

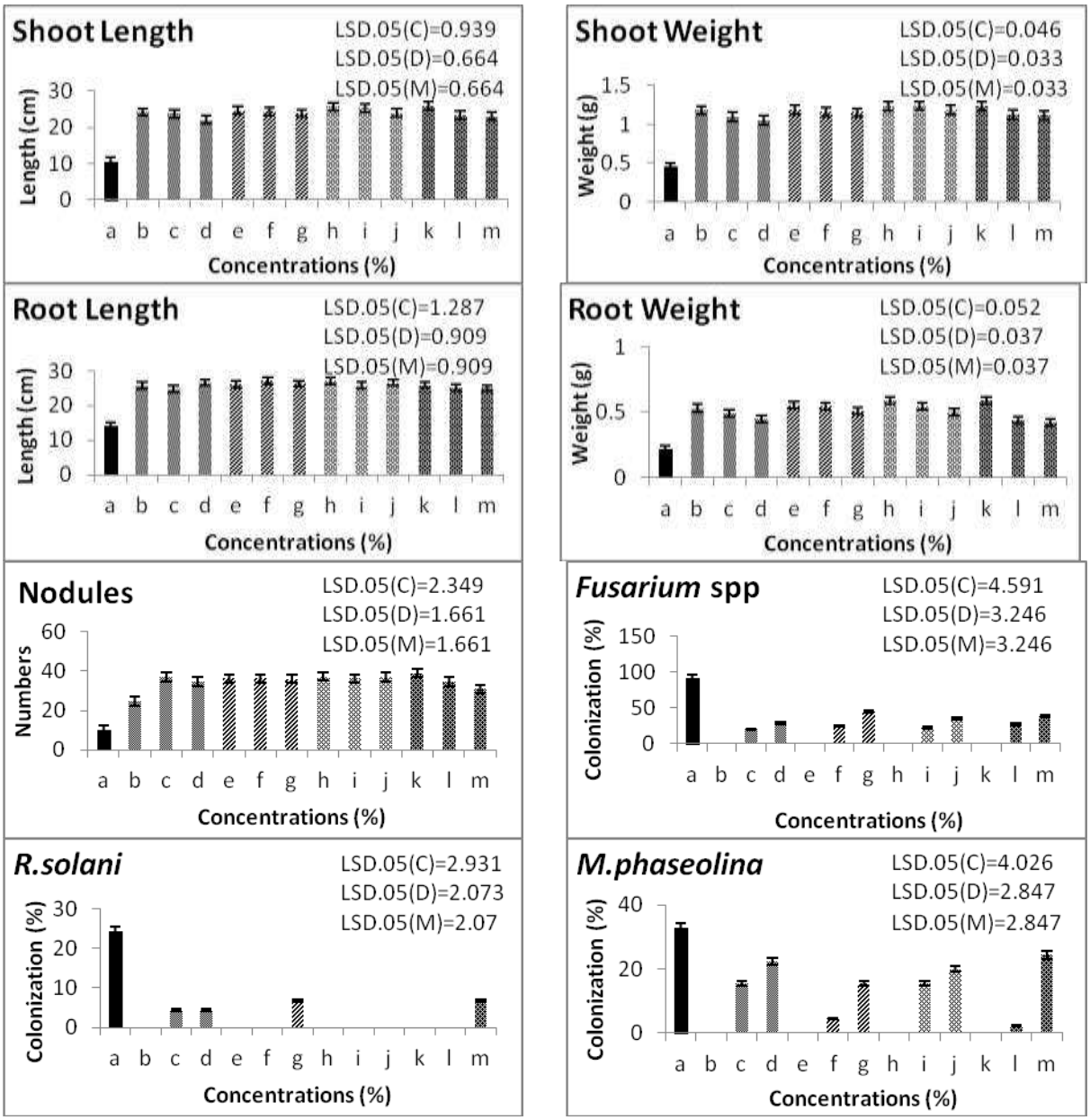

Fig.2. Effect of seed treatment and soil drenching with *Arnica montana* and *Thuja occidentalis* on growth parameters and control of root rot fungi on mung bean (*Vigna radiata* (L.) R.Wilczek) plants.
Where; a=Control; (C) =Concentrations, (D) = Drugs, (M) = Methods
(Seed treatment) b=A@100%, c=A@75%, d=A@50%,e=T@100%, f=T@75%, g=T@50%
(Soil drenching) h=A@100%, i=A@75%, j=A@50%, k=T@100%, l=T@75%, m=T@50%

Earlier reports of *Thuja occidentalis* recommended that mother tincture and its highly potent forms are useful in treating several diseases viz., lung, breast cancer, etc (Boericke, 2004; Sunila *et al*., 2011). *T. occidentalis* also reported for treating skin diseases such as lesion and also effective against diarrhea (Gaskin, 2005). Frenkel *et al*., (2010) claimed highly potent form of *Thuja* 30 CH as an effective therapy against several diseases. Methanol extract of *Thuja occidentalis* with the concentration of 10% v/v found significant inhibiting of *Fusarium* sp., *Aspergillus* sp. *Microsporium* sp. and *Penicillium* sp. (Nam and Kang, 2005). The extracts of the plant showed anti-viral and anti-diarrheal activity (Deb *et al.,* 2007). *T. occidentalis* with 30M and 200M control *Aspergillus flavus*, where *Thuja*

50M showed effectiveness against *Aspergillus niger* (Gupta and Srivastava, 2002). Similar reports were given by Singh *et al*., (1980) and Singh and Gupta in 1985 to verify anti-viral effects of homoeopathic drugs against animal and plant viruses.In the similar way, Hafez and Abdel-Salam (2004) examined the chemical composition of volatile constituents and anti-microbial activity of *T. occidentalis*. Studies on pharmacological, pharmaceutical and clinical properties of *T. occidentalis* were made by Naser *et al*., (2005). Homeopathic drugs are cheap and eco-friendly used in very low doses (Toledo *et al*., 2011). Homoeopathic drugs could fulfill the guarantee as they possess antifungal properties (Sinha and Singh, 1983; Shrivastava and Atri, 1998). Therefore, research on the herbal products needs to be enhanced (Benzie and Watchtel-Galor, 2011). Present research showed that *A. montana* and *T. occidentalis* found to be most effective in the inhibition of root rot fungi and increases the growth of crop plants.

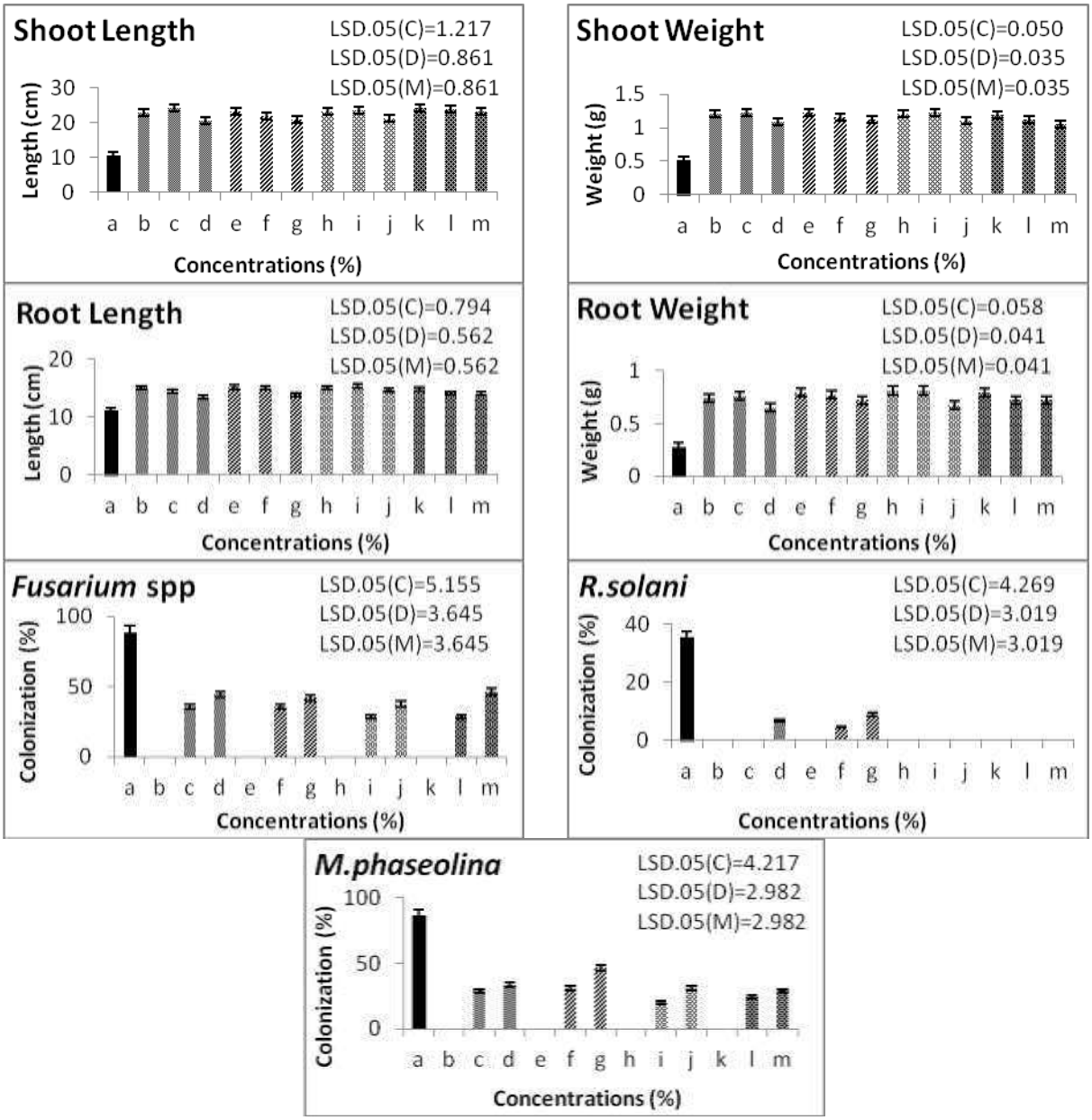

Fig.3. Effect of seed treatment and soil drenching with *Arnica montana* and *Thuja occidentalis* on growth parameters and control of root rot fungi on sunflower (*Helianthus annuus* L.) plants.
Where; a=Control; (C) =Concentrations, (D) = Drugs, (M) = Methods
(Seed treatment) b=A@100%, c=A@75%, d=A@50%,e=T@100%, f=T@75%, g=T@50%
(Soil drenching) h=A@100%, i=A@75%, j=A@50%, k=T@100%, l=T@75%, m=T@50%

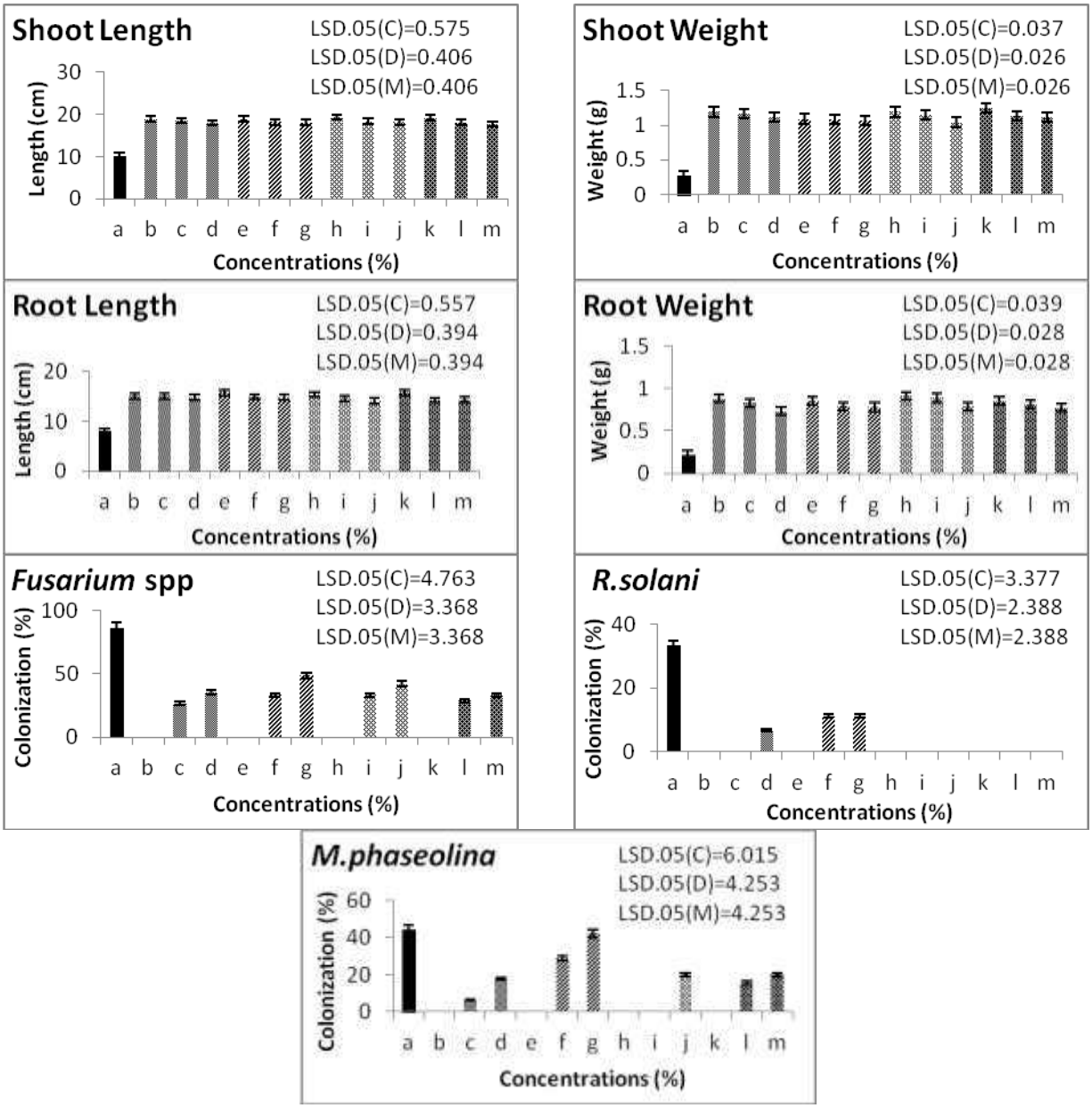

Fig.4. Effect of seed treatment and soil drenching with *Arnica montana* and *Thuja occidentalis* on growth parameters and control of root rot fungi on okra (*Abelmoschus esculentus* (L.) Moench) plants.
Where; a=Control; (C) =Concentrations, (D) = Drugs, (M) = Methods
(Seed treatment) b=A@100%, c=A@75%, d=A@50%,e=T@100%, f=T@75%, g=T@50%
(Soil drenching) h=A@100%, i=A@75%, j=A@50%, k=T@100%, l=T@75%, m=T@50%

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