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FUNGICIDAL ACITIVITY OF *THUJA OCCIDENTALIS* L. LEAVES AGAINST ROOT ROT FUNGI ON CROP PLANTS

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ABSTRACT

In the present study, we investigated the effect of *T. occidentalis* L. leaves due to its fungicidal activity, therefore leaves extract as well as its whole powder form was taken to control *Fusarium* spp., *Macrophomina phaseolina* and *Rhizoctonia solani*. We found a significant increase in plant growth parameters when 1.0% w/v concentration of leaves extract was used as soil drenching, and seed treatment. Our data also indicated an increase in plant growth parameters when leaves powder was used as soil amendment at 0.5% w/w. We also found a measurable reduction in the colonization of root rot fungi. Although soil amended with 2.5% w/w leaves powder showed complete suppression in the colonization of pathogenic fungi, but failed to improve plant growth seems to be toxic to test plants as it ceased the germination and caused stunted and wilting symptoms were observed.

Key-words: *Thuja occidentalis*, root rot fungi, plant growth

INTRODUCTION

Plant pathogenic fungi, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium* spp. have a broad host range and worldwide distribution (Agrios, 2005). For many years, synthetic fungicides have been used as a quick and effective management strategy for crop protection from pathogenic fungi infestation (Osman and Al-Rehiayam, 2003). However, due to the extensive use of fungicides, there have been negative effects on ecosystems and human health including increased risk of human cancer (Stranger and Scott, 2005). In Western countries, a large number of agrochemicals are banned because of their undesirable attributes such as; extensive degradation period, accumulation in the food chain, high toxicity (Gatto *et al*., 2011). In addition, most of the available agrochemicals can also destroy beneficial organisms (Gatto *et al*., 2011). Thus there is a need to search of alternative control strategies to minimize the reliance on the synthetic fungicides. This can be achieved by exploring crop plants that may contain a rich source of antimicrobial agents (Mahesh and Satish, 2008).

Extracts from a number of plants have been reported to have fungicidal activity against many plant pathogenic fungi. The natural plant products possess anti-microbial activity, biodegradability as well as non- phytotoxicity (Gómez *et al*., 1990; Talibi *et al*., 2012), produce a great deal of secondary metabolites which exhibit antifungal activity (Bennett and Wallsgrove, 1994; Osbourne, 1996) and has been adequately explored against phytopathogenic fungi (Gangadevi *et al*., 2008). Currently, plant extracts has received attention because of their phytochemicals (Jasuja *et al.*, 2012) which exhibit great potential as microbial inhibitors are naturally toxic to fungi and bacteria (Basile *et al.*, 1999). Use of medicinal plants having antifungal properties could be used against plant pathogens in an organic farming system because of their environment friendly mode of disease management (Srivastava *et al*., 1997; Shanmugavalli *et al*., 2009). For instance, *Thuja occidentalis* L., belonging to family Cupressaceae is characteristically coniferous and monoecious tree measuring from 12-21 meters in height (Naser *et al.,* 2005). The tree has given numerous names, including white cedar of the east, arbor vitae, white cedar of the north, white cedar, tree of life, swamp cedar and yellow cedar (Tsiri *et al.*, 2009). *T. occidentalis* leaves have oil and resin which gives the characteristic of intense odor, a sharp taste, including camphoric and balsamic (Farmacopeia, 2011).

Fresh plant contains reducing sugar (2.07%), water soluble minerals (2.11%), essential oil (0.6%), free acid (1.67%), tannic agents (1.31%) and water-soluble polysaccharides (4.9%) (Harnischfeger and Stolze, 1983). The essential oil of fresh leaves contains isothujone (8%), thujone (65%), fenchone (8%), sabines (5%) and α-pinen (2%) as the main monoterpenes (Witte *et al*., 1983). Thujones compounds are known as insect repellant which was confirmed by Hwang *et al*., (1985) which can cause high mortality in root worm larvae in western corn. Thujones also exhibit antimicrobial activity against gram positive bacteria, gram negative bacteria and many fungal strains (Tsiri *et al*., 2009). In addition, flavonoids compound is also present which are known to be synthesized by plants in response to microbial infection (Cowan, 2002). Flavonoid contains amentoflavone that exhibits strong antifungal activity against numerous pathogenic fungi (Jung *et al.*, 2006) and antiviral activity against respiratory syncytial virus (Ma *et al*., 2001). *T. occidentalis* leaves exhibits anticancer, antidiabetic, antitumor, anti-ulcerative, antioxidant, hepatoprotective and hypolipidemic activities (Dubey and Batra, 2009; Madhuri and Pandey, 2009).

In the present study, we investigated the fungicidal potential of *T. occidentalis* leaves in the management of root rot fungi and its effect on the growth of crop plants.

MATERIALS AND METHODS

Collection of plants: Fresh *Thuja occidentalis* L. leaves were collected from trees growing in Karachi University (KU) campus. After collection, the collected leaves were dried under shade at room temperature. Then leaves were grinded into powder form using an electric grinder machine. The seeds powder thus obtained were stored in glass jars for further studies.

Preparation of aqueous extract: For the aqueous extraction, *T. occidentalis* leaves @ 0.5, 1.0, 1.5, 2.0 and 2.5 g were separately soaked in 100 mL sterilized distilled water, respectively, for 48 hours at room temperature (28-34˚C). The extract was separately filtered through two layers of Whatman No. 1 filter paper in 250mL Pyrex flask. Filtrate stored at 4˚C for further study.

Soil properties: Soil used for this study was collected from an experimental plot in the Botany Department (Karachi University). The sandy loam soil contained; sand 73%, silt 12%, clay, 15%, pH 7.3 with moisture holding capacity (MHC) of 42% (Keen and Raczkowski, 1922). Natural soil infestation contains 6-8 sclerotia/g soil of *M. phaseolina* found by wet sieving technique (Sheikh and Ghaffar, 1975), 15-20% of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 2300 CFU/g soil of *Fusarium* spp., was assessed by soil dilution technique (Nash and Synder, 1962).

Pot experiment:

Soil amendment: *T. occidentalis* leaves powder was amended in the 300g soil at 0.5, 1.0, 1.5, 2.0 and 2.5% w/w respectively. Soil without leaves powder (control) was used for comparison. Pots containing soil were watered daily to allow the decomposition of organic substrate. After one week, four 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 planted in each pot containing various amount of leaves powder.

Seed treatment: Test seeds after surface sterilization at 1% Ca $(OCl)_2$, dried aseptically under laminar flow hood. Surface disinfested seeds were separately treated with different concentrations of *T. occidentalis* aqueous leaves extracts for 5-10 minutes, whereas seeds without any treatments served as control. Four treated seeds of test crops with different concentrations along with the control were separately planted in each pot.

Soil drenching: Twenty milliliter of aqueous *T. occidentalis*, leaves extracts at 0.5, 1.0, 1.5, 2.0, 2.5% w/concentrations, respectively, were separately drenched in each plastic pot containing 300g soil. Then 4 seeds were planted in each pot. Soil drenched with 20 mL sterilized distilled water served as control.

Pots were placed in completely randomized design (CRD) on a greenhouse bench in three replicates per treatment under the natural sunlight. All plants were watered regularly in order to maintain sufficient moisture content required for the growth of test plants. Growth parameters including shoot weight and height, root weight and length were recorded after one month of seed germination.

Determinations colonization of root rot fungi: Plant roots were washed in running tap water, after washing roots were surface sterilized with 1% Ca $(OCl)_2$ for 2-3 minutes, cut into one cm and dried on blotter paper under laminar flow hood. Transferred root pieces (5 root pieces/plate) on Petri plates containing Potato dextrose agar medium supplemented with antibiotics to inhibit the growth of bacteria. Petri plates were incubated at room temperature (27-33˚C) for a week and the colonization of roots by root rot fungi was recorded.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) followed by the least significant difference (LSD) test as P = 0.05 and Duncan’s multiple range test to compare treatment means, using Statistica software according to Sokal and Rohlf (1995).

RESULTS

We found that seeds of mash bean and mung bean treated with *T. occidentalis* leaves extracts @ 2.0 and 2.5% w/v showed a higher growth as compared to other concentrations. Soil amendment and soil drenched with *T. occidentalis* leaves @ 0.5 and 1.0% concentrations effectively increased both the plant height and the weight over 2.5% in both methods. Colonization of root rot pathogens were significantly (P<0.01) reduced at all concentrations (Fig. 1)

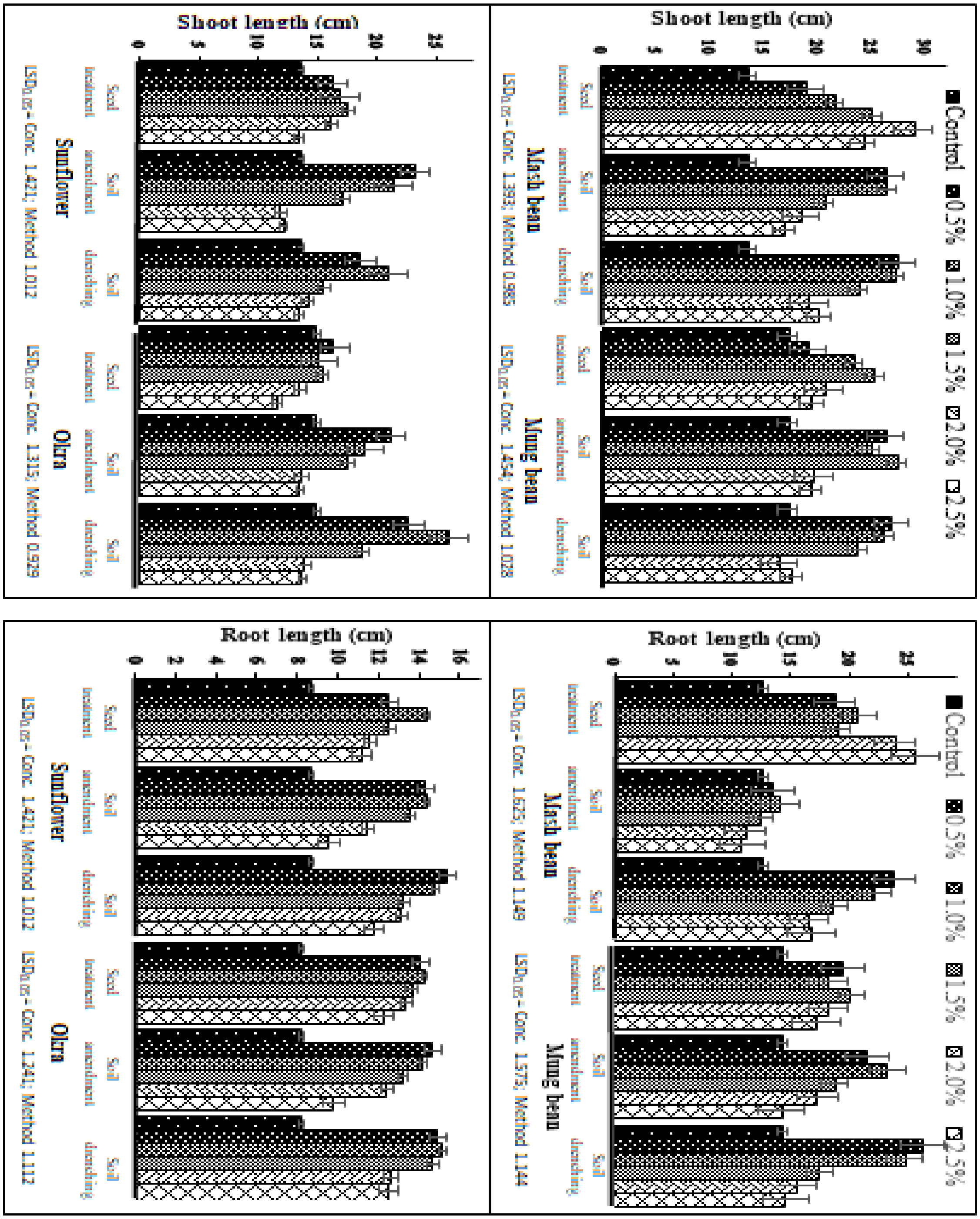

Fig 1. Effect of seed treatment, soil amendment and soil drenching with *T. occidentalis* leaves on growth parameters of crop plants.

When seed treatment method used, we found a complete inhibition of root rot colonization in both mash bean and mung bean crops. When the soil was drenched at 2.5% w/v (P<0.001) with *T. occidentalis* aqueous leaves extracts, we also found a complete suppression of *R. solani* colonization in mash bean plants that were planted in soil amended with 2.5% w/w leaves powder. Both seed treatments and soil drenched with *T. occidentalis* leaves extracts used @ 0.5, 1.0 and 1.5% w/v concentrations (P<0.001) enhanced the growth parameters in both sunflower and okra plants (Fig. 2).

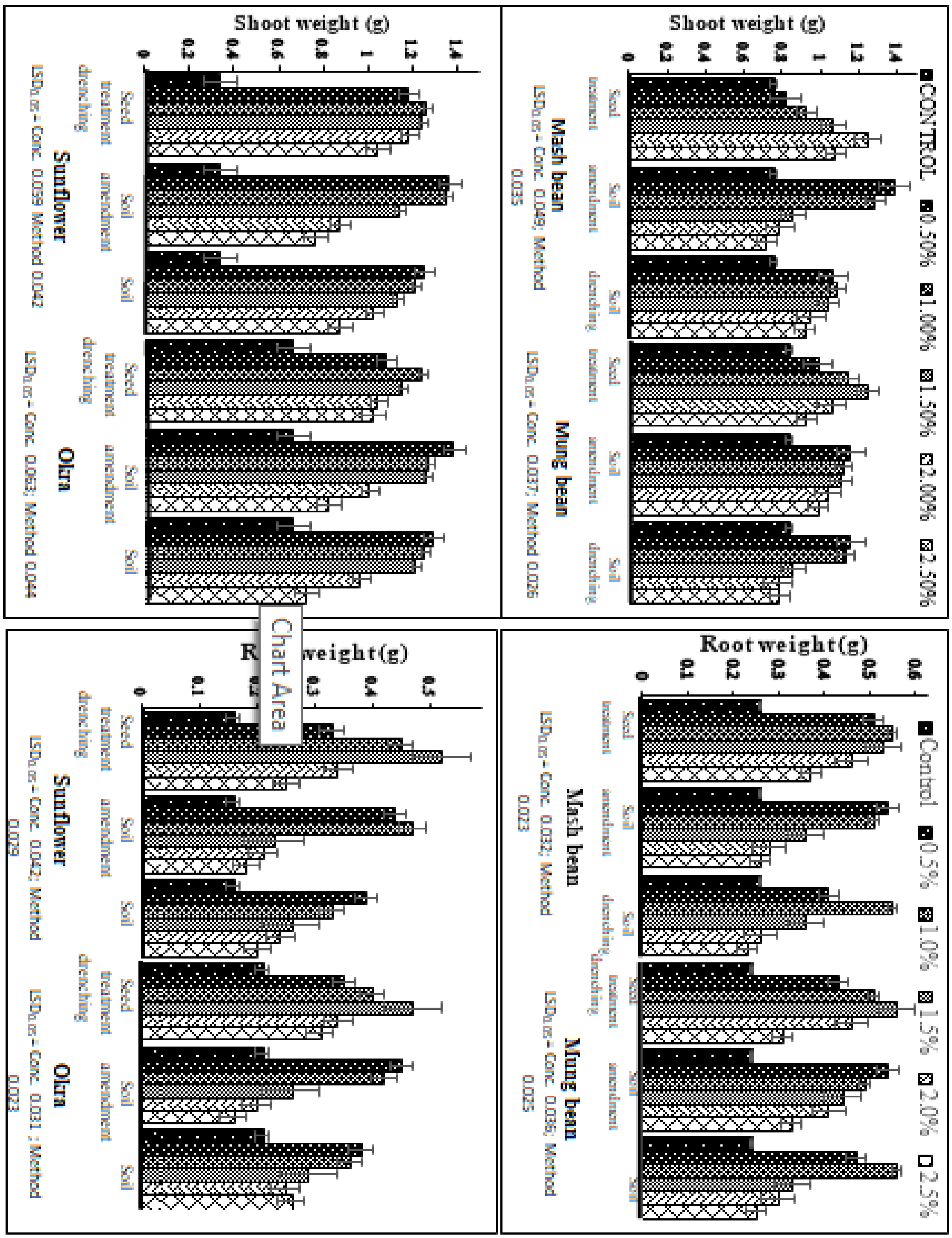

Fig 2. Effect of seed treatment, soil amendment and soil drenching with *T. occidentalis* leaves on growth parameters of crop plants.

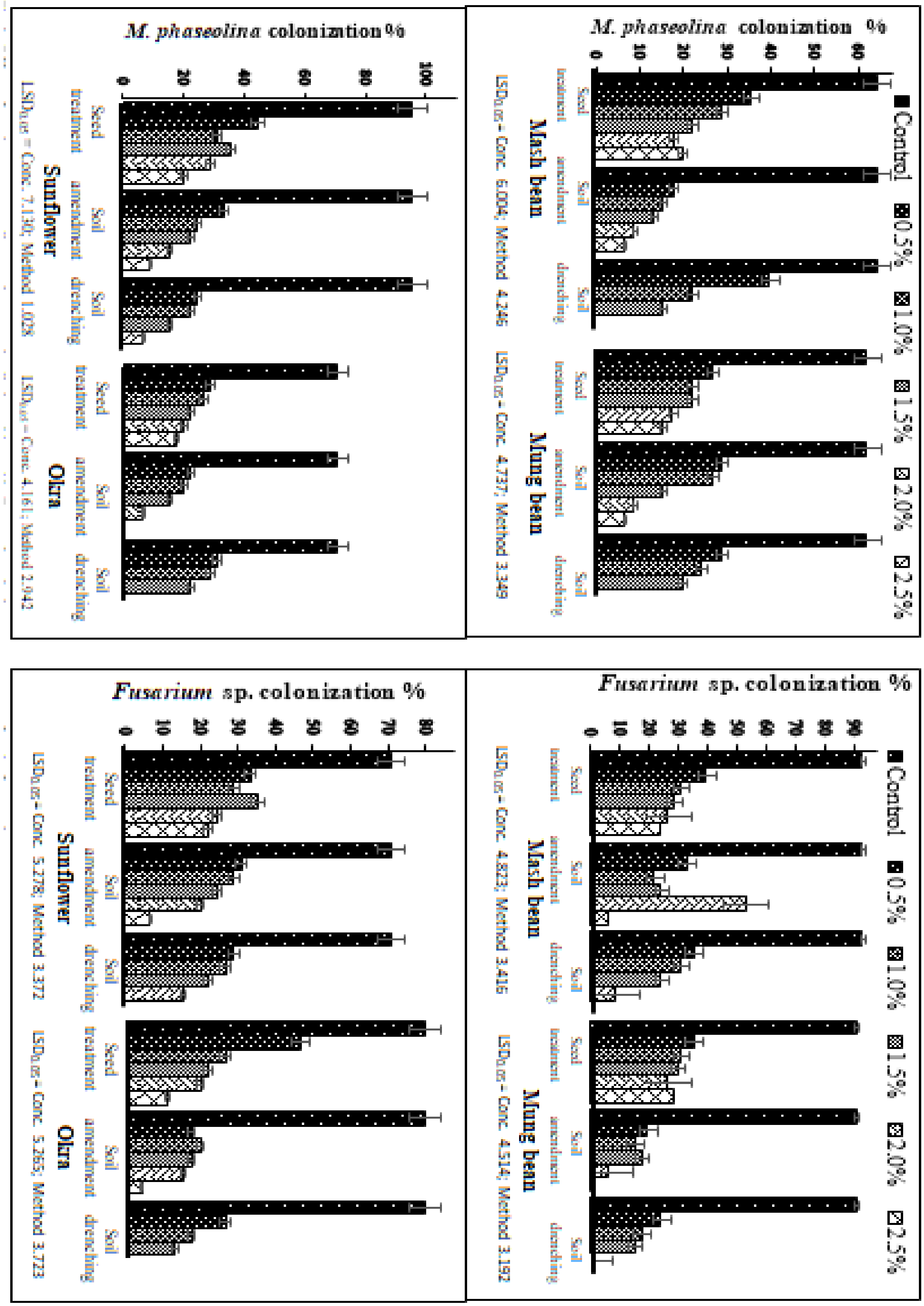

Fig 3. Effect of seed treatment, soil amendment and soil drenching with *T. occidentalis* leaves on root rot fungi of crop plants.

The highest growth was noticed, when the soil was amended with 0.5% w/w leaves powder as compared to 2.0 and 2.5% w/w. In all three methods, 2.5% leaves extracts ($P<0.05$), did not improve the plant growth, but rather completely suppressed root rot colonization (Fig. 3). Greater inhibition of *R. solani*, *M. phaseolina* and *Fusarium* spp. colonization ($P<0.01$) was recorded in seed treatment, soil drenching and soil amendment methods, when *T. occidentalis* leaves used @ 1.0%, followed by 0.5% concentration which not only increased the plant weight and height but also recorded maximum control of pathogenic fungi. When *T. occidentalis* leaves powder amended @ 2.5% w/w in soil, it completely inhibited the *R. solani* colonization observed in sunflower plants, but in case of okra it suppressed both *R. solani* and *M. phaseolina* colonization (Fig. 4).

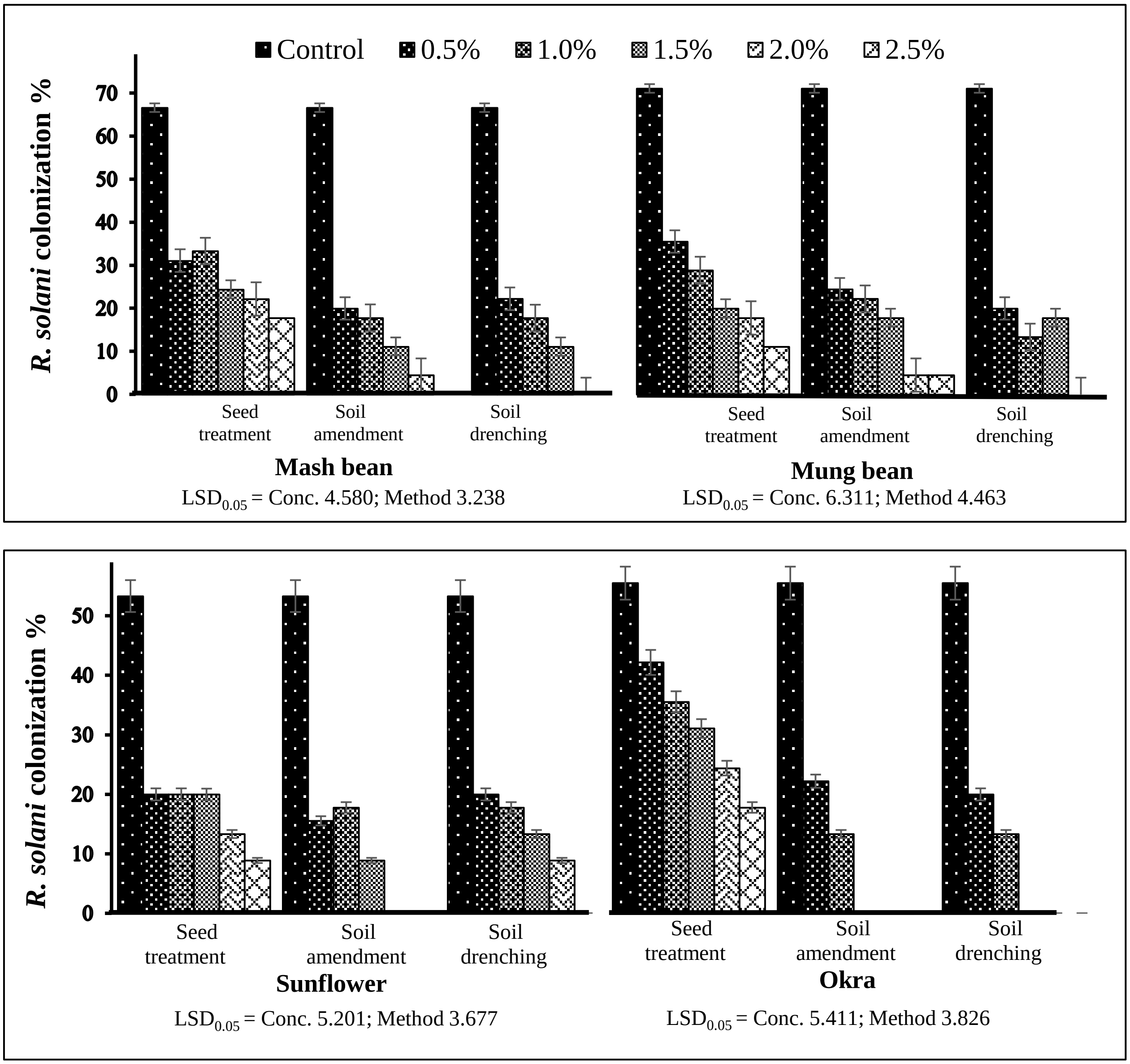

Fig 4. Effect of seed treatment, soil amendment and soil drenching with *T. occidentalis* leaves on root rot fungi of crop plants.

DISCUSSION

Application of *Thuja occidentalis* leaves used @ 1.0% in seed treatment, soil drenching and soil amendment not only enhanced the plant weight and height but also showed maximum inhibition in the colonization of root rot fungi in both leguminous and non-leguminous crops. Ikram and Dawar (2012) also found a similar result. They used

Aerva javanica leaves powder at 1% w/w mixed with soil and noticed significant inhibition of *R.solani, M. phaseolina* and *Fusarium* spp. colonization.

Organic amendments not only improve crop productivity, but also suppress soil borne diseases caused by plant pathogenic fungi and nematodes (Stone *et al.,* 2003; Alam, 1990; Mital and Gowswami, 2001). Addition of organic amendments such as; animal manure, green manure, sawdust, compost, wood chips, grass clippings, sphagnum peat, wood ash and many others are commonly used in farmlands conditions suitable for improving plant growth (Hadar *et al.*, 1992; Muchovej and Pacovsky, 1997). Tariq *et al*. (2006) and (2008) reported that when *Avicennia marina* and *Rhizophora mucronata* applied to the soil as organic amendment at different ratios not only increased the plant weight and height but also controlled plant parasitic nematode and root rot fungi infestation on different crops. Application of soil amendment with plant powder inhibits plant pathogens by releasing toxic substances like phenols (Ali *et al*., 2001; Shaukat *et al.,* 2001). Seed treatment inhibit the fungi residing either on the surface of the seed or maybe inside the seed but also protect from the pathogen that are inhabited in the soil causes seed rot, damping off and root rot diseases (Martha *et al.,* 2003).

In previous studies, okra and mash bean seeds treated with aqueous extracts of *Sida pakistanica* and *Senna holosericea* @ 25, 50 and 100% w/v concentrations have shown reduced colonization of root rot fungi (Emmanual *et al.,* 2010). In addition, *In vitro* study, the alcoholic extracts of *T. occcidentalis* twigs have not only shown antibacterial activity against *Citrobacter, Escherichia coli, Staphylococcus aureus, Shigella flexenari, Yersinia aldovae* and *Pseudomonas aeruginosa* but have also shown antifungal activity against *Trichophyton rubrum, Macrophomina* spp*., Fusarium solani, Saccharomyces cereviciae, Candida albicans* and *Aspergillus parasiticus* (Jahan *et al.,* 2010). Both aqueous and alcoholic extracts of *T. occcidentalis* twigs exhibited antioxidants and anti-inflammatory activity (Dubey and Batra, 2009). *T. occidentalis* leaves contain carotenoid compounds as a secondary metabolite (Krinsky 1979). Carotenoids are an effective inhibitor of various diseases such as heart and cancer disease (Kotikova *et al.,* 2009). Chaudhary *et al*. (2015) reported that carotenoid present in *T. occidentalis* leaves have potent antibacterial activity against *Bacillus subtilis, Bacillus megaterium, Bacillus amyloliqui faciens, Proteus vulgaris* and *Salmonella typhi*. Aqueous extract of leaves, bark, fruits and stem of *Eucalyptus* sp., used @ 5% w/v were found to inhibit *M. phaseolina, R. solani* and *Fusarium* sp. Infestation on mung bean and chick pea (Dawar *et al.,* 2007). Soil drenching has been shown as an effective method of controlling root rot diseases reported by Abdel-Kader *et al*., (2012) studied with different bio-agents such as; *Trichoderma harzianum, Trichoderma viride, Bacillus subtilis, Pseudomonas flourescens* and *Sacchromyces serivisae* and drenched into the soil, showed their efficacy in reducing root rot incidence of post-emergence growth stage of Cucumber, Cantaloupe, Tomato and Pepper.

We suggest that low concentration of soil drenching and soil amended with *T. occidentalis* leaves powder may decrease the colonization of fungal pathogens inhabiting in the soil. However, seed treatment is the best method either used at low or high concentration protects seeds from external attack of the pathogens considered as biological control and eco-friendly. We found *T. occidentalis* leaves can be best used as a fungicide against root rot fungi and may also improve plant growth.

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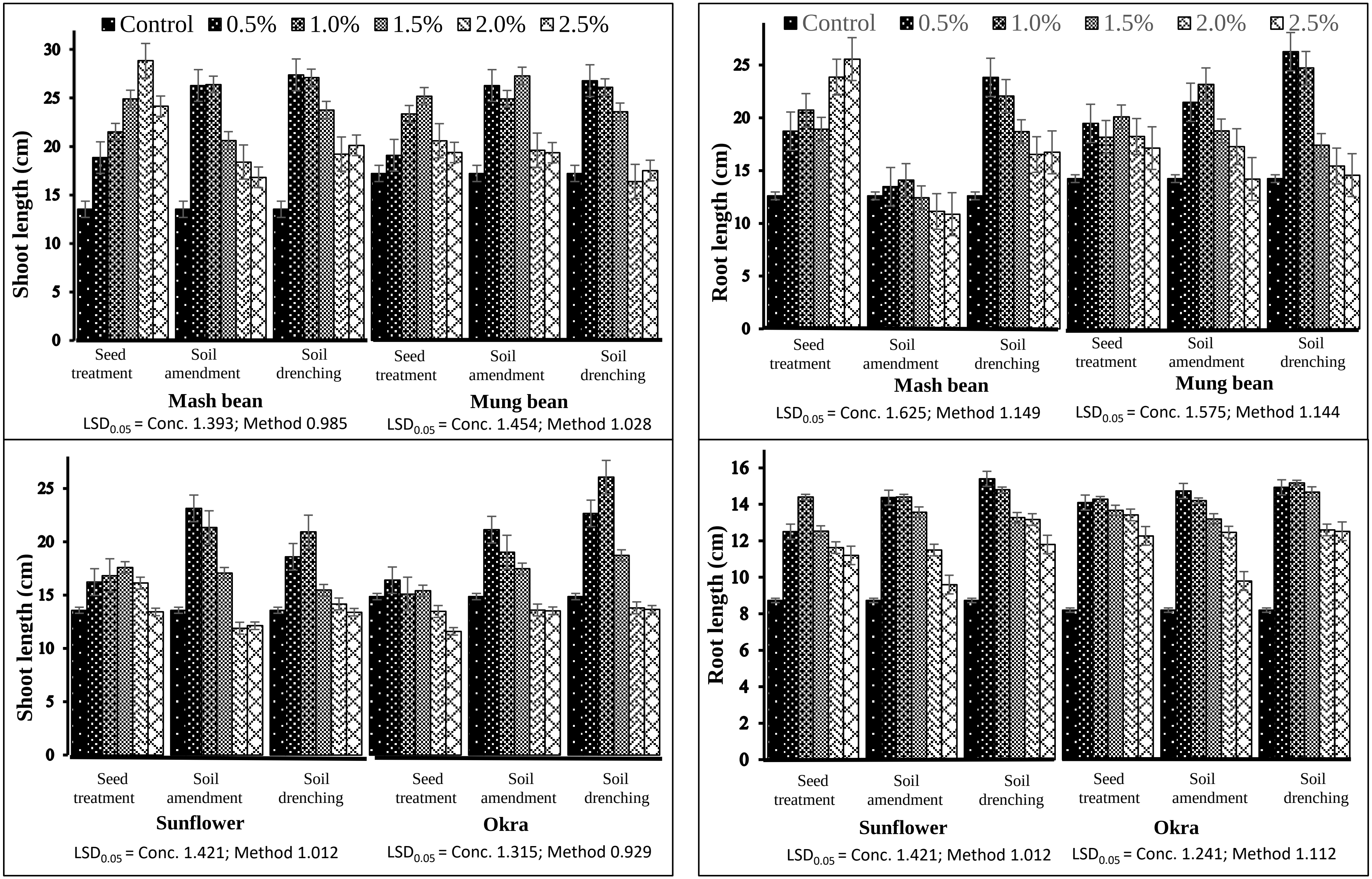

Fig 1. Effect of seed treatment, soil amendment and soil drenching with *T. occidentalis* leaves on growth parameters of crop plants.

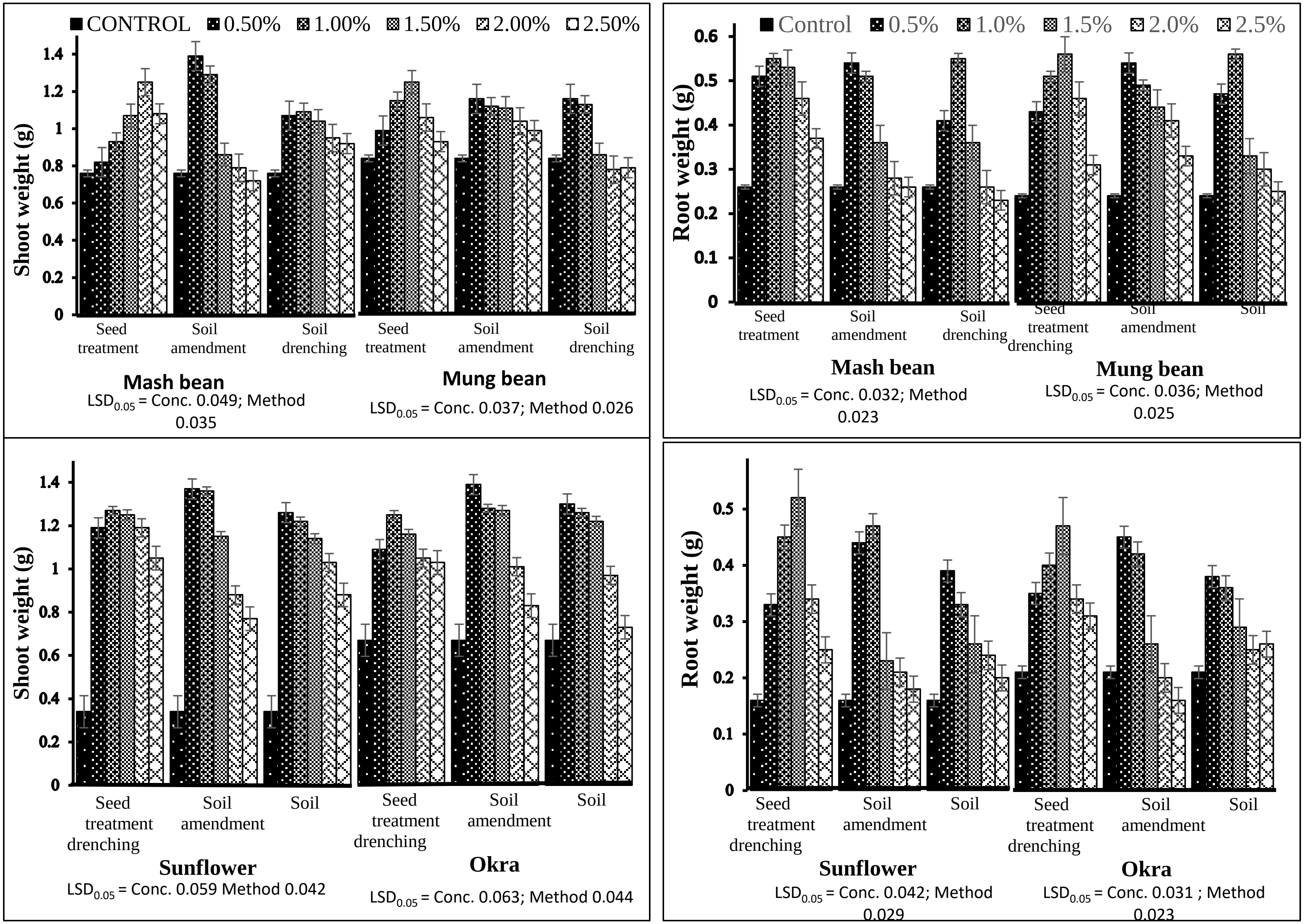

Fig 2. Effect of seed treatment, soil amendment and soil drenching with *T. occidentalis* leaves on growth parameters of crop plants.

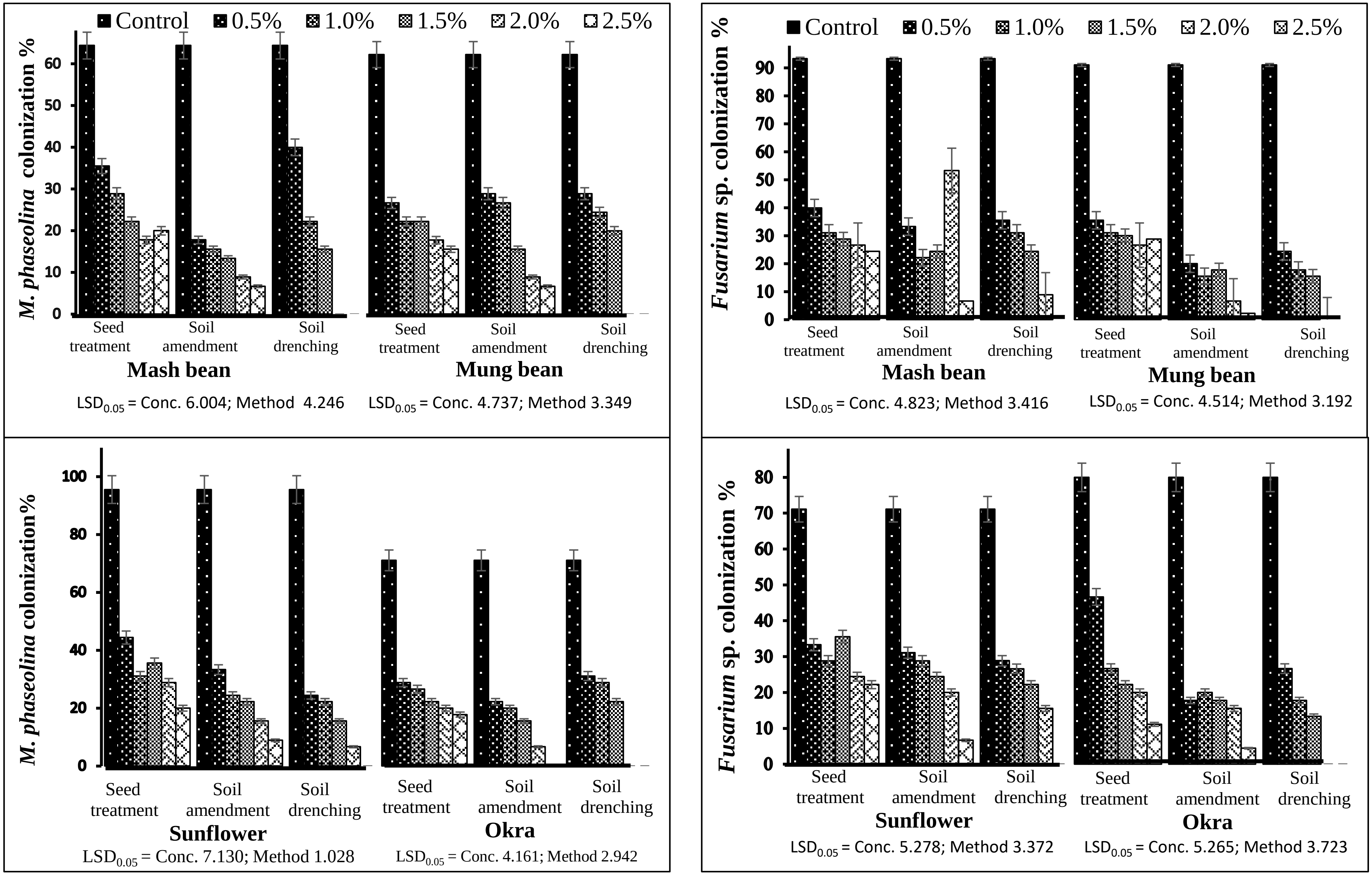

Fig 3. Effect of seed treatment, soil amendment and soil drenching with *T. occidentalis* leaves on root rot fungi of crop plants.