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ANTIFUNGAL ACTIVITY OF HOMEOPATHIC DRUGS TREATED SEEDS AGAINST ROOT DECAY PATHOGENS AND IMPROVEMENT OF GROWTH ON CROP PLANTS

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Abstract

Seed treatment with homeopathic drugs considered as an environment-friendly and has positive impacts on plant growth and inhibition of pathogenic fungi (*Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina*). A Field experiment was conducted to investigate the effect of homeopathic drugs and control strategies on the colonization of root infecting fungi. Seed treatment with *Arnica montana* and *Thuja occidentalis* at 75% v/v concentration (prepared from 30C) showed significant effect in the control of root infecting fungi and increased the height and weight of shoot, root, pods, chlorophyll and protein contents, whereas *A. monatana* and *T. occidentalis* at 50% v/v concentration (prepared from 30C) showed maximum growth parameters as well as suppressed the root rot fungi on mung bean, okra, sunflower and mash bean respectively after 180 days of plant growth.

Keywords: Homeopathic drugs, root rot fungi, inhibition, leguminous and non leguminous plants

Introduction

Root infecting fungal pathogens produce rot and wilt diseases in many crops of Pakistan results in yield reduction (Haque & Ghaffar, 1994). Root rot is a major soil borne disease in agricultural field (Kraft & Pfleger, 2001; Mumtaz *et al*., 2011) in which *Fusarium* spp. and *Rhizoctonia solani* are soil inhabiting fungi that produces root decay, wilting, damping off of seedling, cortical rot of roots, chlorosis, early death and rosette (Wallwork, 1996; Summerell *et al.,* 2001; Bentley *et al*., 2006). *Macrophomina phaseolina* exists in soil as sclerotia as a means of survival, which remain dormant for longer period until favorable environment available to produce hyphae (Ammon *et al*., 1974). It is reported that charcoal rot responsible for blockage of xylem vessels ultimately death of seedlings occurs due to defoliation and wilting of plants (Abawi & Corrales, 1990). Numerous methods have been recommended for controlling plant pathogens which includes solarization, fungicide application, cultural practices, antagonist organism and crop rotation to improve the yield of crops (Dubey *et al*., 2011; Choudhary *et al.,* 2004; Rasheed *et al*., 2012). Recently, research on homeopathic drug on plants is recent but it has proven positive effects on human beings (Bonato *et al*., 2006). Due to the production of secondary metabolites, homeopathic drugs involve in biological process of plants considered as environment friendly method (Bonato & Silva, 2003).

Many traditional medicines in use are derived from medicinal plants, organic matter and minerals (Grover *et al*., 2002; Mahmood *et al*., 2011). In the last few years, there has been an expanding growth in the field of herbal medicine and these drugs are gaining fame both in developed and developing countries due to their natural origin and little side effects (Singh, 2011; Walter *et al*., 2011). The World Health Organization (WHO) encourages the use of herbal medicine because they possess great potentials (Amos *et al.,* 2001; Shinwari *et al*., 2012) and also demonstrated the safety and effectiveness of traditional medicine (Anon., 2000). Studies on the use of homeopathic drugs in plants have done recently as they amplify the metabolic process and control disease in plants (Espinoza, 2001) by possessing antifungal activities (Shrivastava & Atri, 1998).

Homeopathic drugs such as *Arnica montana* L. (Asteraceae) is a perennial herbaceous herb and a medicinal important plant widely applied in homeopathy, cosmetics and pharmacy commonly known as wolf's Bane, leopard's bane and mountain arnica (Hultén & Fries, 1986). Main constituent of *Arnica* contains essential oils, terpenoids, flavonoids (quercetin and its derivatives viz., quercetine-3-mono- glucosideo and quercetine-3-glycogalacturonic), sesquiterpene lactones (arnicolide, helenaline and dihydro-helenaline), alcohols (arnidiol, arnilenediol, iso-arnilenediol) and phenolic acids especially pseudoguaianolide sesquiterpenes compose of 0.2-0.8% in the head of *A. montana* flower (Ganzera *et al*., 2008; Gawlik-Dziki *et al*., 2011; Weremczuk-Jeżyna *et al*., 2011) and exhibits anti-inflammatory, anti-septic, anti-sclerotic, anti-oxidant and anti-fungal activities (Ganzera *et al*., 2008; Sugier & Gawlik-Dziki, 2009). Currently, composition of essential oils in rhizomes and roots and new derivatives of chlorogenic acids in *A. montana* flowers have been identified and characterized (Jaiswal & Kuhnert, 2011; Pljevljakušić *et al*., 2012). It has been used as herbal medicine for centuries (Knuesel *et al*., 2002).

Thuja occidentalis (Cupressaceae) known as white cedar or arbor vitae has shown antidiarrheal, antioxidant and antiviral activities (Deb *et al.,* 2007; Nam & Kang, 2005). Important metabolites present in *Thuja occidentalis* contains flavonóides, polysaccharides (Naser *et al*., 2005) essential oils (Svajdlenka *et al.*, 1999) diterpenes, monoterpenes (α-thujone, β-thujone, fenchone), and sequiterpene (Chang *et al*., 2000) polysaccharides as well as flavonoids (kercetin, campherol), tannins and proteins were recognized by phytochemical investigations in ethanolic and methanolic fraction of *T. occidentalis* aerial part used. Several pharmacological studies conducted by Dubey & Batra (2009) for antidiabetic, hepatoprotective, antioxidant, antitumor, hypolipidemic and antiulcerative activities. *Thuja occidentalis* drugs contain 1.4-4.0% essential oil and thujone, which is 60.0%, corresponds to 2.4% present in the whole drug (Hänsel *et al*., 1994). Present research work was conducted to study the antifungal activity of treated seeds with homeopathic drugs against root rot fungi and growth promotion of crop plants.

Materials and Methods

Seed treatment: 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 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, while seeds treated with sterilized distilled water served as control.

***In vivo* experiment:** Field experiment was conducted in the screen house of Botany Department at Karachi University with appropriately 2.5×2.5 leveled micro plots. Soil used for the experiment was sandy loam containing; 72% sand, 13% clay and 15% silt (Gee and Bauder, 1986) having 7.6 pH (Brady, 1990), water holding capacity 39% (Keen & Rakzowski, 1922) and total nitrogen 0.82% (Mackenzie & Wallace, 1954). The soil consists of natural infestation having 9-11 sclerotia g^{-1} of *M. phaseolina* (Sheikh & Ghaffar, 1975), *R. solani* 28% (Wilhelm, 1955) and *Fusarium* spp 3800 cfu g^{-1} (Nash & Synder, 1962). Five treated seeds of mash bean, mung bean, sunflower and okra with different concentrations of *A. montana* and *T. occidentalis* at 75 and 50% v/w concentrations respectively, were sown in each plot, whereas seeds treated with sterilized water was regarded as control. Each treatment was replicated thrice. Daily watering the plants and observed the plants till it reached the fruiting stage.

Isolation of root rot fungi: After uprooting plants carefully, growth parameters were recorded. Roots were washed with water to remove soil particles and cut cautiously each root into five pieces. These root pieces after surface sterilization with 1.0% $Ca(OCl)_2$ for 2-5 minutes dried on blotter paper under laminar hood and transferred to poured Petri dishes containing 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 (32-36°C) and the colonization of root rot fungi was recorded from each root segment.

Photosynthetic pigment extraction and estimation: Fresh leaf samples (0.5g) were taken from each treatment of crops and were homogenized in 80% acetone. The whole contents were centrifuged at 10,000 RPM for 10 minutes. The supernatants were then separated and the optical density was read at 662, 645 and 470 nm. Total photosynthetic pigments were calculated and expressed as fresh weight according to the formulas of Lichtenthaler and Wellburn in 1985.

Protein estimation: Soluble protein contents of leaf samples were taken from each treatment of crops and were determined by the method of Bradford in 1976 where Bradford Assay reagent method was used against Bovine Serum Albumin as standards.

Statistical analysis: Data were analyzed by two 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).

Results

Okra seeds when treated with homeopathic drugs showed increment in the shoot length and weight, root length and weight, seeds weight and number as compared to control. Colonization percentage of *R. solani, Fusarium* spp and *M. phaseolina* reduced significantly ($P<0.01$) in both concentrations of drugs. In physiological parameters, 75% v/v concentration of *A. montana* showed the highest amount of total chlorophyll and pigments followed by *T. occidentalis* whereas 50% v/v concentration was found to be maximum. *T. occidentalis* at 75% v/v concentration showed the highest amount of protein as compared to other treatments (Fig.1A). In case of sunflower plants, seeds treated with both concentrations of *A. montana* and *T. occidentalis* improved the plant length and weight, but also increased the flower weight as well as showed maximum inhibition of root rot fungi colonization. Greater total chlorophyll and pigments along with protein content were significantly ($P<0.05$) observed by *A. montana* when 75% v/v concentration was used. However, minimum total pigments and protein was recorded by using 50% v/v concentration of *T. occidentalis* (Fig.1B). However, in mung bean plants, it was striking to notice that slight increase in growth parameters when both concentrations of *A. montana* and *T. occidentalis* were used. Number of nodules, pod number and weight as well as seed weight and numbers were found to be significantly ($P<0.001$) higher in 75% v/v concentration of *T. occidentalis* with the slight difference in *A. montana*. Colonization of *Fusarium* spp., *R. solani* and *M. phaseolina* showed maximum inhibition by all the treatments of homeopathic drugs. Total chlorophyll pigments along with protein content elevated when 75% v/v concentration of both homeopathic drugs were used. However, *T. occidentalis* at 50% v/v concentration showed minimum amount of total chlorophyll and pigments, whereas 50% v/v concentration of *A. montana* showed the less amount of protein as compared to other treatments (Fig.1C). Shoot length and weight, root length and weight and number of nodules in mash bean plants significantly ($P<0.001$) showed better plant growth as compared to control. Pod and seed numbers along with the weight showed greater increase in *T. occidentalis* when 75% v/v concentration used followed by *A. montana*. However, maximum suppression of root rot fungi colonization was recorded by 75% v/v and minimum colonization of pathogenic fungi was noticed by 50% v/v concentration in both homeopathic drugs. In physiological parameters, highest total chlorophyll and pigments were obtained by *A. montana* in both concentrations, but maximum protein content was observed by both drugs used at 75% v/v concentration. While, less protein content was recorded by *A. montana* at 50% v/v concentration (Fig.1D).

Overall results showed that all tested crops of mung bean, mash bean, sunflower and okra when treated with 75% v/v concentration of both homeopathic drugs produced promising effect in controlling the root rot fungi colonization and increase the height and weight of shoot, root and pods as well as total pigments of chlorophyll and protein contents followed by 50% v/v concentration which showed maximum growth parameters as well as suppressed the colonization of root decay pathogens.

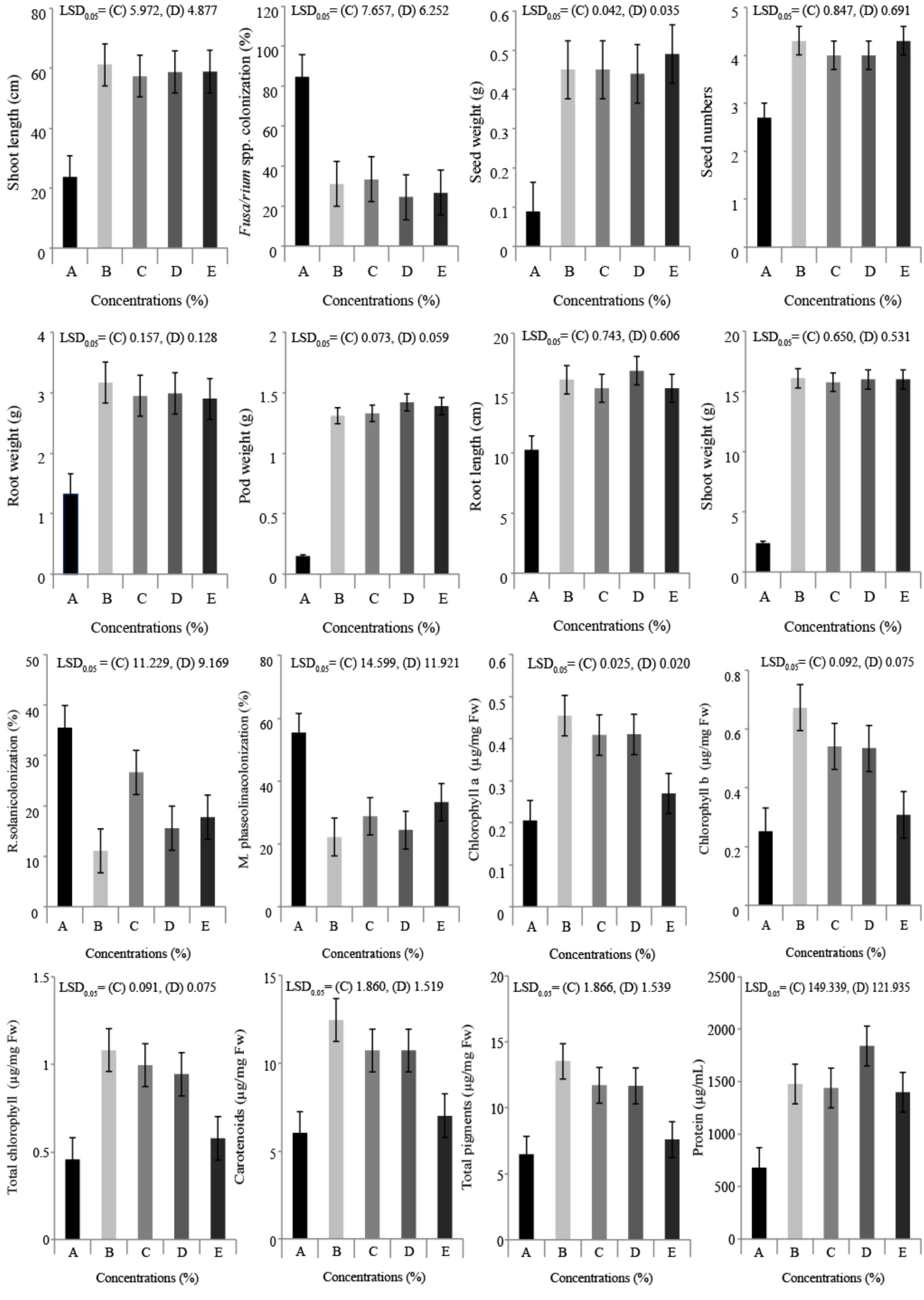

Fig.1A. Effect of seed treatment with *A. montana* and *T. occidentalis* on growth and physiological parameters in the management of root rot fungi on okra

Where; (C) =Concentrations, (D) = Drugs, (%) = Percentage, Fw= Fresh weight A=Control (Sterilized water), B=*A. montana* @ 75%, C=*A. montana* @ 50%, D= *T. occidentalis* @ 75%, E=*T. occidentalis* @ 50% v/v concentrations (Prepared from 30C)

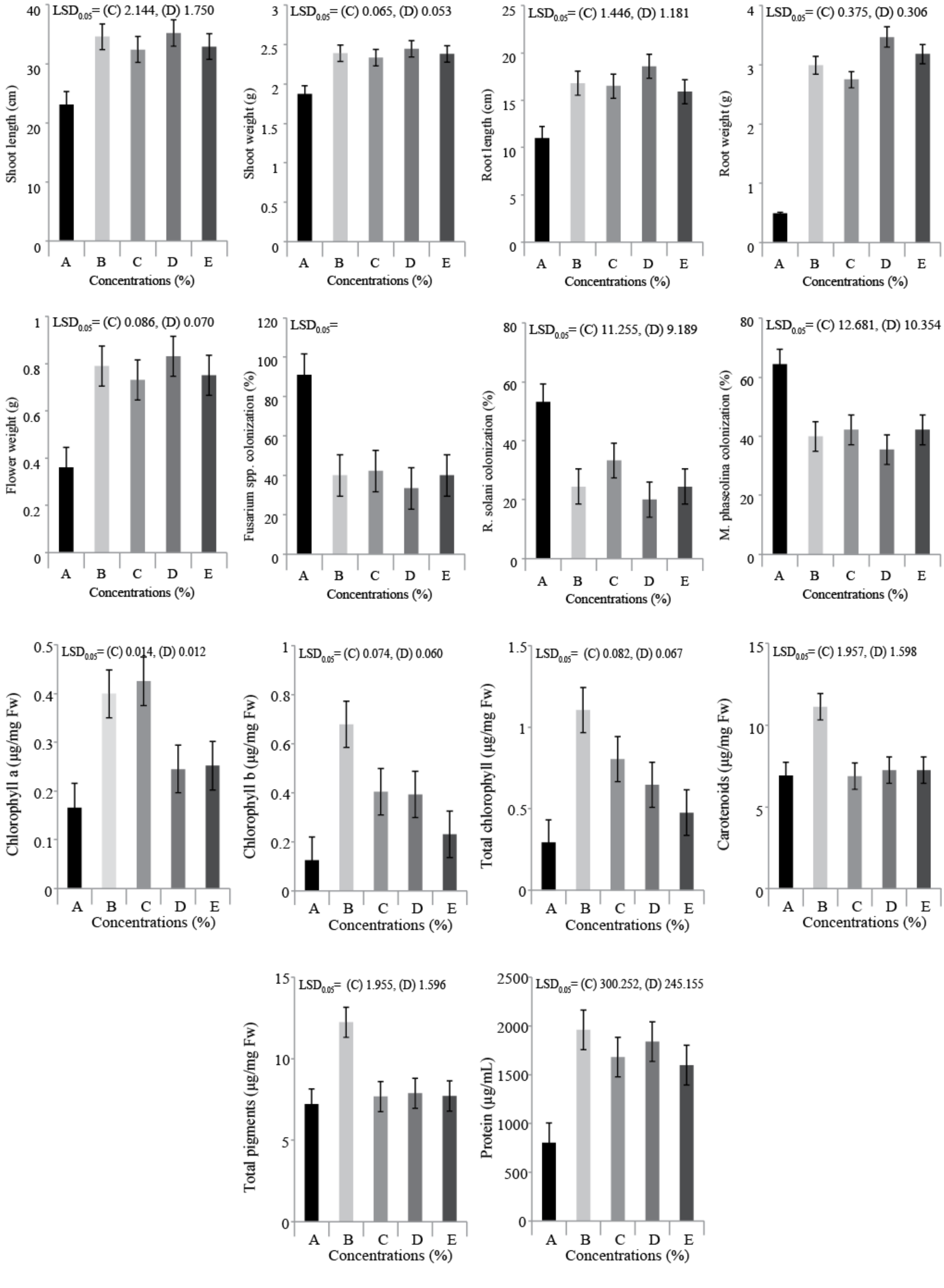

Fig.1B. Effect of seed treatment with *A. montana* and *T. occidentalis* on growth and physiological parameters in the management of root rot fungi on sunflower

Where; (C) =Concentrations, (D) = Drugs, (%) = Percentage, Fw= Fresh weight A=Control (Sterilized water), B=*A. montana* @ 75%, C=*A. montana* @ 50%, D= *T. occidentalis* @ 75%, E=*T. occidentalis* @ 50% v/v concentrations (Prepared from 30C)

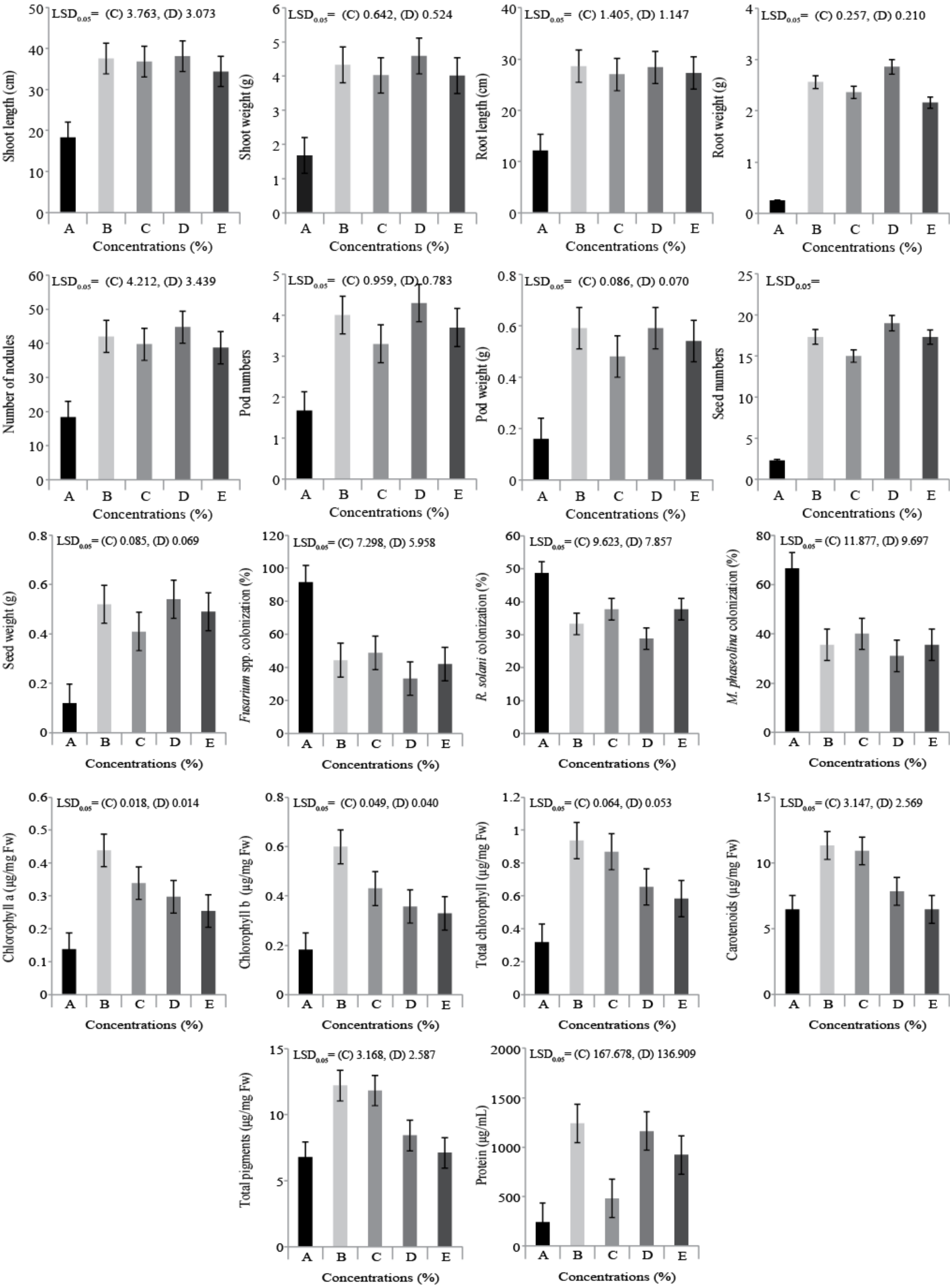

Fig.1C. Effect of seed treatment with *A. montana* and *T. occidentalis* on growth and physiological parameters in the management of root rot fungi on mung bean

Where; (C) =Concentrations, (D) = Drugs, (%) = Percentage, Fw= Fresh weight A=Control (Sterilized water), B=*A. montana* @ 75%, C=*A. montana* @ 50%, D= *T. occidentalis* @ 75%, E=*T. occidentalis* @ 50% v/v concentrations (Prepared from 30C)

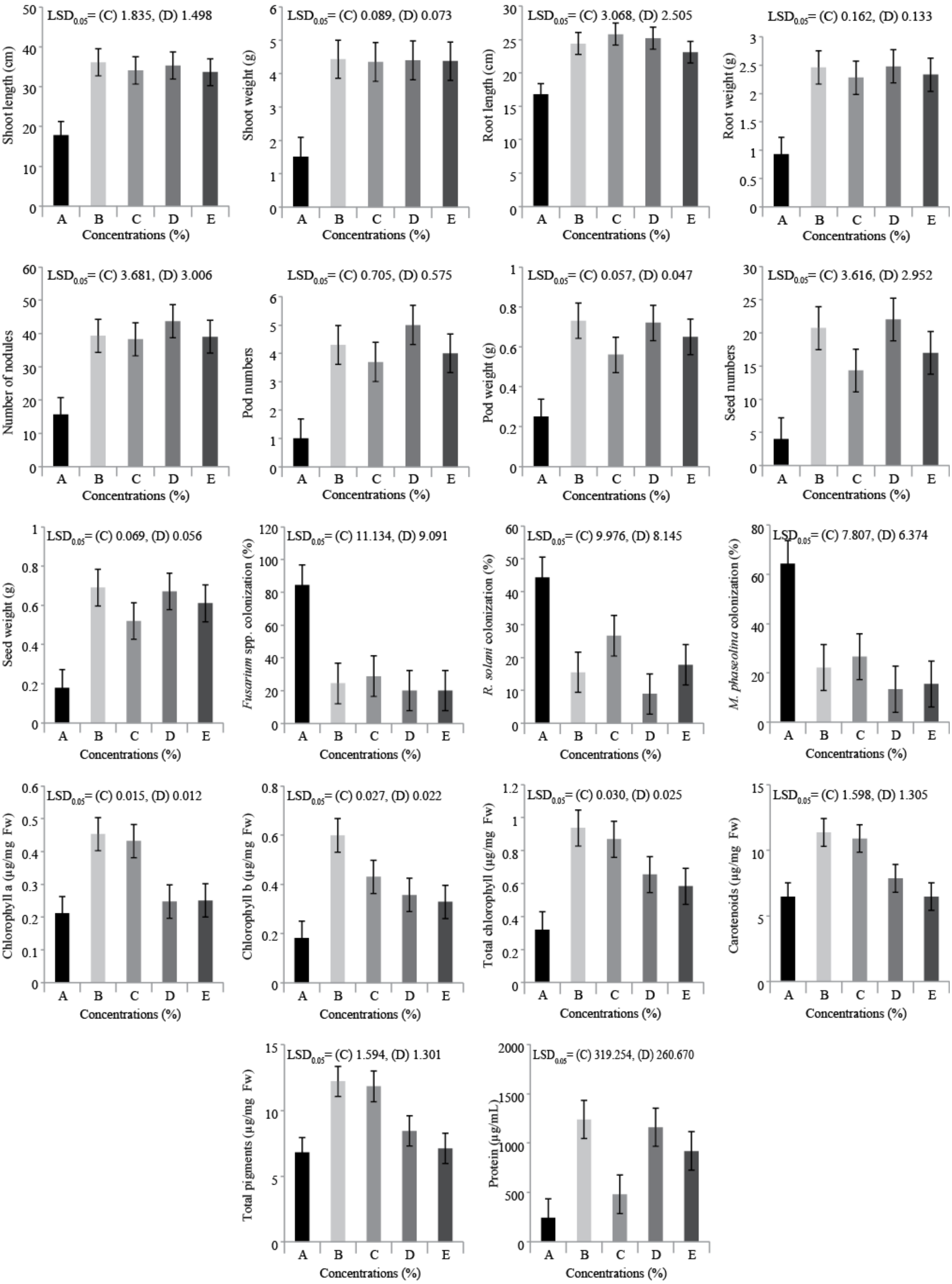

Fig.1D. Effect of seed treatment with *A. montana* and *T. occidentalis* on growth and physiological parameters in the management of root rot fungi on mash bean

Where; (C) =Concentrations, (D) = Drugs, (%) = Percentage, Fw= Fresh weight A=Control (Sterilized water), B=*A. montana* @ 75%, C=*A. montana* @ 50%, D= *T. occidentalis* @ 75%, E=*T. occidentalis* @ 50% v/v concentrations (Prepared from 30C)

Discussion

Seed treatment with *Arnica montana* and *Thuja occidentalis* @ 75% v/v concentration (prepared from 30C) showed significant effect in the control of root infecting fungi and increased the height and weight of shoot, root and pods as well as chlorophyll and protein contents, whereas *A. monatana* and *T. occidentalis* @ 50% v/v concentration (prepared from 30C) showed maximum plant growth as well as suppressed the root rot fungi after 180 days of tested plant growth. Extracts of *Thuja occidentalis* showed sedative, antidiarrheal and antiviral properties (Deb *et al*., 2007; Aziz *et al*., 2014). Using 3, 6 and 12CH potency of *Arnica montana* had shown improvement in plant growth (Bonfim *et al*., 2008). Seeds treatment with homeopathic drugs and soil amendment with fertilizers found to be effective in the inhibition of root rot fungi and improved the growth of crop plants (Hanif & Dawar, 2015). Seed dressing inhibits the infection caused by pathogenic fungi present either superficially or penetrates inside the seed (Martha *et al*., 2003). When homeopathic pellets applied in the field, it enhanced growth parameters along with the greater suppression of *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* (Hanif *et al*., 2015). Asha *et al*., 2014 reported that by using all potencies of *Thuja* (Q, 30C, 200C, 1M, 10M and 50M) showed an effective suppression against *Bipolaris* spp., followed by *Curvularia* spp., *Exserohilum* spp. and *Aspergillus flavus*. Homeopathic drugs such as *Thuja* and *Natrum muriaticum* significantly control *Fusarium* spp. (Hussain *et al*., 2000). Frenkel *et al*., (2010) claimed highly potent form of *Thuja* was successful therapy against several diseases which includes lung and breast cancer (Sunila *et al*., 2011) also against diarrhea (Gaskin, 2005). *T. occidentalis* methanolic extracts at 10% v/v concentrations showed significant inhibition of *Aspergillus* sp., *Microsporium* sp., *Penicillium* sp. and *Fusarium* sp. (Nam & Kang, 2005). Bee and Atri in 2012 reported that using the potencies of *A. montana* (3C, 6C, 12C, 30C, 200C, 1M and 10M) showed remarkable reduction in aflatoxin, G_1 production by *Aspergillus parasiticus* (strain # = MTCC-411) in groundnuts under *in vitro* and *in vivo* conditions. Gupta and Srivastava in 2002 proved that using 30 and 200C potency of *T. occidentalis* was found to be effective against *Aspergillus flavus* whereas, higher dilution 1M, 10M and 50M was not effectual which contradicts theory of high dilution and energy as it is controversial aspect of homeopathic dilution that trituration and succussion increases remedy power which disqualify the concept in case of fungi study. Homeopathic drugs possess antifungal (Shrivastava & Atri, 1998) as well as antiviral properties (Singh & Gupta, 1985). Homeopathic drugs are inexpensive and eco-friendly (Toledo *et al*., 2011). For that reason, research on the herbal drugs needs to be increased (Benzie & Wachtel-Galor, 2011). Present results in field experiment suggested that seed treatment with *A. montana* and *T. occidentalis* showed better growth of plants as well as significantly suppressed the root rot fungi.

References

Abawi, G.S. and M.A.P. Corrales. 1990. Root rots of beans in Latin America and Africa: diagnosis, research methodologies and 129 management strategies. *CIAT*, pp. 114.

Ammon, V., T.D. Wyllie and M.F. Brow. 1974. An ultra-structural investigation of pathological alterations induced by *Macrophomina phaseolina* (Tassi) Goid. in seedlings of soybean, *Glycine max* (L.). Merril, *Physiol. Plant Pathol*., 4: 1-4

Amos, S., E. Kolawole, P. Akah, C. Wambebe and K. Gamaniel. 2001. Behavioral effects of the aqueous extract of *Guiera senegalensis* in mice and rats. *Phytomedicine*, 8(5): 356-361.

Anonymous. 2000. World Health Organization. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine, pp.1-20.

Asha, R., P. Nisha, K. Suneer, A. Mythili, H.A. Shafeeq, S.K. Panneer, P. Manikandan and C.S. Shobana. 2014. *In vitro* activity of various potencies of homeopathic drug *Thuja* against molds involved in mycotic keratitis. *Int. J. Pharm. & Pharmaceutical Sci.,* 6(10):555-559.

Aziz, A., I.A. Khan and S.H. Munawar. 2014. Pharmacological evaluation of sedative activity of methanolic extract of *Thuja occidentalis* in mice. *Int. J. Adv. Biol. & Biomedical Res.*, 2(1): 202-210.

Bee, S. and D.C. Atri. 2012. Control of aflatoxin G_1 production in groundnuts by homeopathic drugs. *Int. J. Pharm. Bio. Sci*., 3(4): 896-901.

Bentley, A.R., M.G. Cromey, R. Farrokhi-Nejad, J.F. Leslie, B.A. Summerell and L.W. Burgess. 2006. *Fusarium* crown and root rot pathogens associated with wheat and grass stem bases on the South Island of New Zealand. *Aust. Plant Pathol.,* 35(5): 495-502.

Benzie, I.F.F. and S. Wachtel-Galor. 2011. *Herbal medicine: Biomolecular and clinical aspects*. Second edition. CRC Press. pp.1-9.

Bonato, C.M. and E.P. Silva.2003. Effect of the homeopathic solution *Sulphur* on the growth and productivity of radish. Acta Scientiarum. *Agron*., 25: 259-263.

Bonato, C.M., E.G. Viotto, J.H.R. Hara, A.T. Mizote and J.A.O. Cisneros. 2006. The application of the homeopathic drugs Lachesis and Isotherapic Virus in the growth and infection control for SCMV in Sorghum (*Sorghum bicolor* (L.) Moench. *Cultura Homeopática Arquivos da Escola de Homeopatia*, 16:51.

Bonfim, F.P.G., E.R. Martins, R.G.R. Dores, C.K.R. Barbosa, V.W.D. Casali and I.C.G. Honorio. 2008. Use of homeopathic ´*Arnica montana* for the issuance of roots of *Rosmarinus officinalis* L. and *Lippia alba* (Mill) N.E. *Int. J. High Dilution Res.*, 7: 72-76.

Bradford, M.M. 1976. A rapid and sensitive mehod for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.*, 72:248-254.

Brady, N.C. 1990. *The Nature and Properties of Soils*. 10th edition. Macmillan pub. Company. New York.

Chang, L.C., L.L. Song, E.J. Park, L. Luyengi, K.J. Lee, N.R. Farnsworth, J.M. Pezzuto and A.D. Kinghorn. 2000. Bioactive constituents of *Thuja occidentalis*. *J. Natural Prod.*, 63(9): 1235-1238.

Choudhary, C.S., S.N. Singh and S.M. Prasad. 2004. *In vitro* effectiveness of chemicals to control *Macrophomina phaseolina* (Tassi.) Goid, causing stem and root rot of sesame. *J. Appl. Biol.*, 14: 46-47.

Deb, L., S.K. Dubey, A.K. Jain, A. Jain, G.S. Pandian and S.P. Rout. 2007. Anti diarrhoeal activity of *Thuja occidentalis* Linn ethanol extract on experimental animal, *Indian Drugs*, 44(4):319-321.

Dubey, S.C., A.S. Dukare, R. Prasanna, L. Nain, V. Chaudhary, R. Singh and A.K. Saxena. 2011. Evaluating novel microbe amended composts as biocontrol agents in tomato. *Crop Protection*, 30(4): 436-442.

Dubey, S.K. and A. Batra. 2009. Antioxidant activity of *Thuja occidentalis* Linn. *Asian J. Pharm. & Clinical Res.*, 2: 73-76.

Espinoza, F.J.R. 2001. Agrohomeopatia: una opcion ecologica para el campo mexicano. *La homeopatia de Mexico*, 70: 110-116.

Frenkel, M., B.M Mishra, S. Sen, P. Yang, A. Pawlus, L. Vence, A. Leblanc, L. Cohen and P. Banerji. 2010. Cytotoxic effects of ultra-diluted remedies on breast cancer cells. *Int. J. Oncol*., 36(2): 395-403.

Ganzera, M., C. Egger, C. Zidorn and H. Stuppner. 2008. Quantitative analysis of flavonoids and phenolic acids in *Arnica montana* L. by micellar electrokinetic capillary chromatography. *Analytica Chimica Acta*, 614(2): 196-200.

Gaskin, A. 2005. *Comparative study on Kent's materiamedica*. Delhi: B. Jain Publishers.pp. 205.

Gawlik-Dziki, U., M. Świeca, D. Sugier and J. Cichocka. 2011. Comparison of *in vitro* lipoxygenase, xanthine oxidase inhibitory and antioxidant activity of *Arnica montana* and *Arnica chamissonis* tinctures. *Acta Scientiarum Polonorum, Hortorum Cultus*, 10(3): 15-27.

Gee, G.W. and J.M. Bauder. 1986. Particle-size analysis. In: *Methods of Soil Analysis*, Part I, *American Society of Agronomy*, Madison, WI, USA. pp. 383-411.

Grover, J.K., S. Yadav and V. Vats. 2002. Medicinal plants of India with antidiabetic potential. *J. Ethnopharmacol*, 81: 81-100.

Gupta, G. and A.K. Srivastava. 2002. *In vitro* activity of *Thuja occidentalis* Linn. against human pathogenic Aspergilla. *The Homeopathic Heritage*, 27(1): 5-12.

Hanif, A. and S. Dawar. 2015. Use of homeopathic drugs in combination with fertilizers for the control of root rot fungi. *Pak. J. Bot.,* 47(6):2455-2462.

Hanif, A., S. Dawar, M. Tariq and F. Imtiaz. 2015. Fungicidal potential of homeopathic pellets in the inhibition of root rot fungi and for promotion of crop plants productivity. *Europ. J. Biol. & Med. Sci. Res.*, 3(6): 26-39.

Hänsel, R., R. Keller, H. Rimpler and G. Schneider. 1994. Hagers Handbuchder Pharmazeutischen Praxis (Eds.): P. Drogen - Z(Thuja), 5th edn. Springer Verlag. *Berlin*. p. 955-966.

Haque, E.S. and A. Ghaffar. 1994. New records of root infecting fungi from Pakistan. *Pak. J. Phytopathol*., 6: 50-57.

Hultén E. and M. Fries. 1986. *Atlas of North European Vascular Plants*: North of the Tropic of Cancer, vol. 1, Koeltz Scientific Books, Königstein, Germany.

Hussain, S.Z., R.J. Anandam and A.S. Rao. 2000. Effect of different fungicides and homeopathic drugs on seed borne fungi of sunflower (*Helianthus annuus* L.). *Indian J. Plant Prot.,* 28(2): 148-151.

Jaiswal, R. and N. Kuhnert. 2011. Identification and characterization of two new derivatives of chlorogenic acids in arnica (*Arnica montana* L.) flowers by high-performance liquid chromatography/tandem mass spectrometry. *J. Agri. & Food Chem.*, 59(8): 4033-4039.

Keen, B.A. and H. Rakzowski. 1922. The relation between clay content and certain physical properties of soil. *J. Agric. Sci.,* 11: 441-449.

Knuesel, O., M. Weber and A. Suter. 2002. *Arnica montana* gel in osteoarthritis of the knee: An open, multicenter clinical trial. *Advances in Therapy, 19(5): 209-218.*

Kraft, J.M. and F.L. Pfleger. 2001. *Compendium of pea diseases and pests*. 2nd ed. The APS Press, St. Paul, MN, USA. pp.110.

Lichtenthaler, H.K. and A.R. Wellburn. 1985. Determination of total carotenoids and chlorophylls A and B of Leaf in Different Solvents, *Biol. Soc. Trans*., 11: 591-592.

Mackenzie, H.A. and H.S. Wallace. 1954. The Kjeldahl determination of nitrogen: A critical study of digestion conditions, temperature, Catalyst and oxidizing agents. *Aust. J. Chem*., 7: 55-70.

Mahmood, A., R.N. Malik, Z.K. Shinwari and A. Mahmood.2011. Ethnobotanical survey of plants from Neelum, Azad Jammu & Kashmir, Pakistan. *Pak. J. Bot*., 43(SI): 105-110.

Martha, M., J. Riesselman, D. Mathre, B. Jhonston and S. Blodgett. 2003. *Manual of small seed grain treatment guide.* pp: 55.

Mumtaz, A.S. Dur-E-Nayab, M.J. Iqbal and Z.K. Shinwari. 2011. Probing genetic diversity to characterize red rot resistance in sugarcane. *Pak. J. Bot*., 43(5): 2513-2518.

Nam, S.H. and M.Y. Kang. 2005. Anti-oxidant activity of medicinal plants, pharmaceutical biotechnology, *Medicinal and Aromatic Plant Abstracts*, 42(6): 409-415.

Naser, B., C. Bodinet, M. Tegtmeier and U. Lindequist. 2005. *Thuja occidentalis* (Arbor vitae): A Review of its pharmaceutical, pharmacological and clinical properties. *Evidence-based complementary and alternative medicine*, 2 (1): 69-78.

Nash, S.M. and W.C. Synder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot, *Fusarium* in field soils. *Phytopath*. 52: 567-572.

Pljevljakušić, D., D. Rančić, M. Ristić, L. Vujisić, D. Radanović and Z. Dajić-Stevanović. 2012. Rhizome and root yield of the cultivated *Arnica montana* L., chemical composition and histochemical localization of essential oil. *Ind. Crops & Products,* 39(1): 177-189.

Rasheed, A., A. S. Mumtaz and Z. K. Shinwari. 2012. Genetic characterization of novel *Lr* Gene stack in spring wheat variety Chakwal86 and its effectiveness against leaf rust in rain fed areas of Pakistan. *Pak. J. Bot*., 44(2): 507-510.

Sheikh, A.H. and A. Ghaffar. 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton field. *Pak. J. Bot*., 7: 13-17.

Shinwari, Z.K., S.A. Gilani and A.L. Khan. 2012. Biodiversity loss, emerging infectious diseases and impact on human and crops. *Pak. J. Bot*., 44(SI): 137-142.

Shrivastava, J. and D.C. Atri. 1998. Effect of the homoeopathic drugs on the production of aflatoxin B1 by *A. flavus. J. Phytol. Res.*, 11(1): 45-49.

Singh, L.M. and G. Gupta. 1985. Antiviral efficacy of Homoeopathic drugs against animal viruses. *The British Homoeopathic Journal*, 74(3):168-174.

Singh, L.W. 2011. Traditional medicinal plants of Manipur as anti-diabetics. *J. Med. Plants Res.*, 5(5): 677-687.

Sokal, R.R. and F.J. Rohlf. 1995. *Biometry: The Principles and practices of Statistics in Biological Research. Freeman*, New York, pp. 887.

Sugier, D. and U. Gawlik-Dziki. 2009. The influence of foliar fertilization on yielding and quality of mountain arnica (*Arnica montana* L.) and chamisso arnica (*Arnica chamissonis* var. foliosa). *Annales UMCS, Agricultura,* 64(3): 129-139.

Summerell, B.A., J.F. Leslie, D. Backhouse, W.L. Bryden and L.W. Burgess. 2001. *Fusarium*: Paul E. Nelson Memorial Symposium. APS Press. *The American Phytopathology Society*. St. Paul-Minnesota. U.S.A. p.392.

Sunila, E.S., T.P Hamsa and G. Kuttan. 2011. Effect of *Thuja occidentalis* and its polysaccharide on cell-mediated immune responses and cytokine levels of metastatic tumor-bearing animals. *Pharm. Biol*., 49(10): 1065-1073.

Svajdlenka, E., P. Mártonfi, I. Tomasko, D. Grancai and M. Nagy. 1999. Essential oil composition of *Thuja occidentalis* L. samples from Slovalia. *J. Essential Oil Res.*, 11: 532-536.

Toledo, M., J. Stangarlin, C. Bonato. 2011. Homeopathy for the control of plant pathogens, *Physiol.*, pp.19-21.

Wallwork, H.1996. *Cereal root and crown diseases*. Perth, Australia: Kondinin Group. pp. 14-16.

Walter C., Z.K. Shinwari, I. Afzal and R.N. Malik. 2011. Antibacterial activity in herbal products used in Pakistan. *Pak. J. Bot*., 43(SI): 155-162.

Weremczuk-Jeżyna, I., H. Wysokińska and D. Kalemba. 2011. Constituents of the essential oil from hairy roots and plant roots of *Arnica montana* L. *J. Essential Oil Res.*, 23(1): 91-97.

Wilhelm, S. 1955. Longevity of the *Verticillium wilt* fungus in the laboratory and field. *Phytopathology,* 45: 180-181.

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