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FUNGICIDAL POTENTIAL OF HOMEOPATHIC PELLETS IN THE INHIBITION OF ROOT ROT FUNGI AND FOR PROMOTION OF CROP PLANTS PRODUCTIVITY

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ABSTRACT: *Root rot pathogens cause heavy economic damages in agricultural field. The aim of this research is to evaluate the fungicidal efficacy of potentised homeopathic pellets (30C) namely Arnica montana and Thuja occidentalis on germination, growth, yield of crop plants and root rot fungi particularly Fusarium spp, Rhizoctonia solani and Macrophomina phaseolina. Homeopathic pellets were found to be effective in inhibiting the mycelial growth of test fungi in vitro experiment. Whereas, investigation in vivo field experiment showed that A. montana and T. occidentalis pellets @ 75% v/w concentration (prepared from 30C) remarkably control the pathogenic fungi and significantly enhanced the growth parameter and yield of crop plants followed by 50% v/w concentration (prepared from 30C) as compared to control. Experiments have shown positive effect in reducing the intensity of disease caused by root rot pathogen and improve the growth of crop plants.*

KEYWORDS: Homeopathic Pellets, Inhibition, Concentrations, Root Rot Fungi, Leguminous and Non Leguminous Crops.

INTRODUCTION

Root rot is a major soil borne disease includes *Aphanomyces euteiches, Rhizoctonia solani, Pythium ultimum* and *Fusarium solani* (Kraft and Pfleger, 2001). *Fusarium* spp considered as the most important soil borne plant pathogens producing economic damage all over the world in agricultural production (Saremi, 2000; Bentley *et al*., 2006). Numerous *Fusarium* pathogens have similar symptoms on infected crops which include cortical rot of roots, root decay, chlorosis, wilting, rosette and early death (Summerell *et al*., 2001). *Fusarium* spp produces insidious disease which attacks valuable crops mainly in humid and semi-humid areas worldwide (Schroeder and Christensen, 1963). *R. solani* attacks broad range of crops and transported in infected soil or either through diseased plants (Wallwork 1996). *Macrophomina phaseolina* (Tassi) Goid. is reported to infect plants on all growth stages (Reuveni *et al*., 1983) which is responsible of producing charcoal rot causing xylem vessels blockage which resulted in wilting and plant defoliation, ultimately death of seedling occurs (Abawi and Pastor-Corrales, 1990) causing 100% yield losses (Bashir and Malik, 1988) depending upon disease severity which may be enhanced due to dry and hot environment (Gaige *et al.*, 2010). Various methods such as solarization, cultural practices and chemical control have been used against pathogenic fungi (Dubey, 2001). Using chemicals injudicious emerge many problems such as development of resistant strains of the pathogens (Deising *et al*. 2008) few of them are carcinogenic and slow biodegradation (Brent and Hollomon, 1998) as well as undesirable changes they impose upon the environment (Arcury and Quandt, 2003).

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Plant pathologist now raised safer and friendly approach to control plant pathogens (Kumbhar *et al*., 2000). From ancient period, products from plants were used to treat different ailments due to their synergistic effects (Rates, 2001). Economically important medicinal plants are rich source of phenol and nitrogen containing compound including vitamins and minerals exhibiting anti-oxidant, anti-mutagenic, anti-carcinogenic, anti-microbial and diuretic properties (Bajpai *et al.,* 2005). Broad range of medicinal plant part extract includes stem, root, flower, twig and fruit used as raw drugs due to medicinal properties (Joshi *et al*., 2009). Plant remedies and their preparations used as medicinal treatments around the globe (Balunas and Kinghorn, 2005) which have been scientifically evaluated with results of valuable drugs (Butler, 2004). It is estimated that world population (more than 65%) relies directly on plants as their main source of medicine (Fabricant and Farnsworth, 2001). Homeopathic drugs used as an alternate technique to produce secondary metabolites which take part in biological processes without producing toxicity in plants hence leaving no residue in an environment (Bonato and Silva, 2003). Homeopathic drug such as, *Arnica montana* (Asteraceae) contains volatile oil, flavonoids, tannins, resins, carotenoids, and triterpenic alcohol (Brinkhaus *et al*., 2006). It exhibit anti-inflammatory, antiseptic, decongestive, anti-fungal and anti-bacterial properties (Conforti *et al*., 1997). It also stimulates the forming of granular tissues and thus accelerating the healing process (Bisset, 1994). Its flowers are used to treat wounds, burns and bruises (Stevinson *et al*., 2003). Lower potencies of *A. montana* used instead of mother tincture for boils, superficial phlebitis, dermatitis, insect bites and also used as a mouthwash for inflamed gums and mouth ulcers (Vermeulen, 1994). *Thuja occidentalis* (Cupressaceae) known as Arbor vitae or white cedar has been used to treat enuresis, bronchial catarrh, cystitis, uterine carcinomas, psoriasis, rheumatism and amenorrhea (Chang *et al*., 2000). Plant extract has shown anti-viral, anti-diarrheal, anti-oxidant activity (Nam and Kang, 2005; Deb *et al*., 2006). Previous reports suggested that mother tincture and its potentized forms are efficient in treating several diseases such as lung cancer, breast cancer, etc (Boericke, 2004; Naser *et al*., 2005). *Thuja* is reported to be an excellent drug for skin diseases like lesion as well as effective against diarrhea (Sunila *et al*., 2011). Frenkel *et al*., 2010 claimed that *Thuja* 30C is an effective remedy against numerous diseases. The drug of *Thuja occidentalis* contains essential oil 1.4-4%, thujone which is 60% corresponds to 2.4% thujone in the whole drug (Hänsel *et al*., 1994). Nowadays, *Thuja* is mainly used in homeopathy as mother tincture or dilution (Homöopathisches Arzneibuch, 2003).

Keeping in view the drawback of using of chemical control against plant diseases, the use of homeopathic drugs is now gaining importance in the control of plant pathogen. Present research work was to study the fungicidal potential of homeopathic pellets in the inhibition of root rot fungi and for promotion of crop plants productivity.

MATERIALS AND METHODS

Pellets Preparation with Homeopathic Drugs

Dr. Willmar Schwabe homeopathic drugs like *Arnica montana* and *Thuja occidentalis* (30C) were purchased from medicinal market of Karachi. Slight modification of preparation of pellet was made on the method given by Tariq and Dawar (2011). *A. montana* and *Thuja occidentalis* with the concentrations of 100%, whereas 75 and 50% v/w (prepared from 30C) were mixed with pyrophyllite (hydrous aluminum silicate-$AlSi_2O_5OH$) respectively and were prepared by

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using multiple pellet sampler. Each pellet contains 1ml drug in 150mg. Sterilized distilled water and absolute alcohol (MERCK) mixed with pyrophyllite served as control. Homeopathic pellets and non-treated pellets were dried aseptically under the laminar air flow hood.

In Vitro Experiment

Homeopathic pellets with the concentrations of 100, 75 and 50% v/w of *A. montana and T. occidentalis* were placed at the corner of the poured Potato Dextrose Agar (PDA) Petri plates (90mm dia. x 15mm H) respectively. Whereas, sterilized distilled water and absolute alcohol pellets regarded as control. At the other end of the Petri plate, test fungi namely *F. oxysporum*, *R. solani* and *M. phaseolina* were inoculated respectively in each Petri plate. Each root infecting fungus replicated thrice and plates were incubated for one week at room temperature (25-32˚C). The percent growth inhibition over control was determined according to the formula given by Pinto *et al.*, 1998.

In Vivo Experiment

Field experiment was conducted at the screen house of Botany department (Karachi University) from August 2014 to February 2015 with appropriately leveled 2.5×2.5 micro plots. Soil consist of natural infestation having *R. solani* 26% (Wilhelm, 1955), 7-9 sclerotia g-1 of *M. phaseolina* (Sheikh and Ghaffar, 1975), *Fusarium* spp 3800 cfu g^{-1} (Nash and Synder, 1962). Soil used for the experiment was sandy loam containing sand (73%), clay (12%) and silt (13%) of pH 7.6, electrical conductivity (EC) 0.63 $ds.m^{-1}$, Na+ ion 7.3 $\mu g.g^{-1}$, K+ ion 0.9 $\mu g.g^{-1}$ and organic matter 1.1 %, amended with six homeopathic pellets (900mg) of *A. montana* and *T. occidentalis* of 75 and 50% v/w concentrations (prepared from 30C) separately in each plot. Plot with non-treated pellet was regarded as control. Each treatment was replicated thrice. Water the plots for 2-3 days to facilitate decomposition of pellets, so it spread and evenly distributed throughout the soil. Five 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 sown in 4 ft furrows which after sowing covered with soil. Daily water and observed the plants till it reached the fruiting stage. Experiment was completed after 180 days.

Isolation of Fungi from Roots

After uprooting the plants, growth parameters were recorded. Roots were carefully washed in running tap water and cut into five pieces. These root pieces after surface sterilization with 1% $Ca(OCl)_2$, for 5-10 mins, 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 plate for one week at room temperature (27-33˚C) and colonization of root rot fungi was recorded from each root segment by the formula given by Ajmal *et al*., (2001).

Statistical Analysis

Data were analyzed to two and three way analysis (ANOVA) as per experimental design separately followed by the least significant difference (LSD) test at P = 0.05 as given by Gomez and Gomez (1984).

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RESULTS

In vitro experiment, homeopathic pellets of *A. montana* and *T. occidentalis* (100, 75 and 50% v/w conc.) were observed for the inhibition of root infecting fungi such as *F. oxysporum*, *M. phaesolina* and *R. solani*. *T. occidentalis* and *A. montana* pellets (100% v/w conc.) showed highest zone of inhibition and effective results in the inhibition of test fungi. However, *T. occidentalis* pellets (75% v/w conc.) showed significant suppression of *R. solani* mycelium followed by *F. oxysporum* and *M. phaseolina* but *A. montana* (75% v/w conc.) showed greater zone of inhibition in the mycelial growth of *F. oxysporum* and maximum inhibition in *R. solani* and *M. phaseolina*. Both *A. montana* and *T. occidentalis* pellets (50% v/w conc.) showed minimum inhibition of test fungi. Results showed that *T. occidentalis* pellets in all concentrations found to be best for the inhibition of root rot fungi followed by *A. montana* pellets as compared to control which was failed in controlling the test fungi (Fig.1A and Table 1B).

In vivo experiment, *T. occidentalis* and *A. montana* pellets (75 and 50% v/v conc.) amended in soil for the control of root rot fungi. *T. occidentalis* pellets (75% v/w conc.) showed greater length and weight of shoot, root, number of nodules as well as weight of seeds in mash bean plants. *A. montana* pellets (75% v/w conc.) showed enhancement in growth parameters and also significantly suppressed ($P<0.001$) root rot fungi such as *F. oxysporum*, *M. phaesolina* and *R. solani*. Both *T. occidentalis* and *A. montana* (50% v/w conc.) pellets increase the growth as well as yield of mash bean plants (Fig.2A). In case of mung bean, shoot length and weight, root length and weight, number of nodules and weight of seeds were increased but also showed significant inhibition ($P<0.001$) of root infecting fungi when *T. occidentalis* and *A. montana* pellets (75% v/w conc.) were amended in the soil. However, *T. occidentalis* (50% v/w conc.) showed maximum inhibition of pathogenic fungi followed by *A. montana* (50% v/w conc.) pellets as compared to control (Fig. 2B). In okra plant, length and fresh weight of plants significantly ($P<0.001$) improved the growth as well as increased the weight of seed when *A. montana* and *T. occidentalis* (75% v/w conc.) pellets were used. It also significantly suppressed the root rot fungi ($P<0.001$). When *A. montana* and *T. occidentalis* (50% v/w conc.) pellets were amended in soil, it showed maximum increased in the growth productivity as well as maximum suppression of colonization percentage of *Fusarium* spp., *R. solani* and *M. phaseolina* (Fig. 2C). Whereas in sunflower plants, it showed significant ($P<0.001$) increased in the growth and yield but also suppressed the root rot fungi when soil was amended with the pellets of *A. montana* and *T. occidentalis* (75% v/w conc.) respectively. However with the application of *T. occidenalis* and *A. montana* (50% v/w conc.) pellets in the soil showed maximum fresh length and weight of shoot, root and flower. Results showed that homeopathic pellets of *T. occidentalis* followed by *A. montana* (75% v/w conc.) showed greater growth productivity and maximum suppression of root rot fungi (Fig. 2D).

DISCUSSION

Homeopathic pellets of *A. montana* and *T. occidentalis* (30C) reduced the disease infection and increased the growth of crop plants. It was reported that when combined parts pellets of *R. mucronata* and *A. marina*, when applied to soil it enhanced plant length, weight and yield in both mung bean and okra but also effective in controlling *M. javanica* infection (Tariq and Dawar, 2015). Alginate pellets @ 1 and 10/250 g mixed in soil significantly reduced the *M.*

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phaseolina on mung bean and chickpea (Ghaffar, 1995). Earlier reports claimed that potencies of *Thuja* 30C and 200C found to be effective against *Aspergillus flavus* causing cutaneous aspergillosis whereas, *Thuja* 50M effective against *Aspergillus niger* to cause otomycosis in human (Gupta, 2002). It was reported that all potencies of Thuja (Q, 30C, 200C, 1M, 10M, 50M) showed significant inhibition against *Bipolaris* spp., followed by *Curvularia* spp., *Exserohilum* spp. and *Aspergillus flavus* (Asha *et al*., 2014). Similarly, it was reported that homeopathic drugs namely *Thuja* and *Natrum muriaticum* were significantly effective on *Fusarium* spp (Hussain *et al*., 2000). *Kali iodatum* (149CH) and *Thuja occidentalis* (87CH) when applied in pre and post-harvest conditions, it significantly control the tomato fruit rot caused by *Fusarium roseum* (Khanna and Chandra, 1992). *Thuja* contains thujeine which is essential oil having antimicrobial activity. Extracts of *Thuja occidentalis* showed antifungal activity against *A. parasiticus, Saccharomyces cereviciae Macrophomina, F. solani, Candida albicans* and *Trichophyton rubrum* (Jahan *et al*., 2010). Dawar *et al*., (2007) reported that *Eucalyptus* sp., parts in aqueous 5% concentration were found to be effective in the control of *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Dawar *et al.*, (2008) also observed that *Curcuma longa and Myristica fragrans* in 5% concentration were effective in the growth reduction of *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*. *In vitro*, *Thuja occidentalis* showed significant result against *Aspergillus flavus* in 30 M and 200 M, whereas in 50 M found promising result against *Aspergillus niger* (Gupta and Srivastava, 2002).Similar results were also obtained by Singh and Gupta (1981, 1985) which proved antiviral effectiveness of homeopathic drugs against animal and plant viruses. Saxena *et al*., (1988) inhibited twenty two genera of fungi on okra seeds when treated with *Thuja ocidentalis*. *Arnica montana* and *Thuja occidentalis* (30C) by using seed treatment and soil drenching methods showed significant suppression of *Rhizoctonia solani*, *Fusarium* spp and *Macrophomina phaseolina* but also promote plant growth at 100% followed by 75 and 50% v/v concentrations (prepared from 30C) on leguminous and non leguminous crops (Hanif and Dawar, 2015). *Arnica montana* of 3, 6 and 12 CH potencies were used to improve and increase plant growth. (Bonfim *et al*., 2008). Application of amendments in the soil provides nutrients and energy which promote the growth of plants (Muchovej and Pacovsky, 1997). Several organic amendments found to inhibit soil borne phytopathogens by releasing toxic compound like phenols directly and enhancing micro-organisms in soil indirectly (Rodriguez-Kabana, 1986; Ali *et al*., 2001). Hence, based on findings of the present research, *T. occidentalis* and *A. montana* pellets amended in the soil released fungicidal compounds which reduced the growth of root rot fungi infection and enhanced the plant growth productivity.

CONCLUSIONS

Used of the homeopathic pellets had shown positive effect in reducing the intensity of root rot pathogen attack in the field and enhanced the growth of plant. Therefore, it is suggested that it should be applied on large scale as it is cheap, easily available, nonhazardous and environment friendly.

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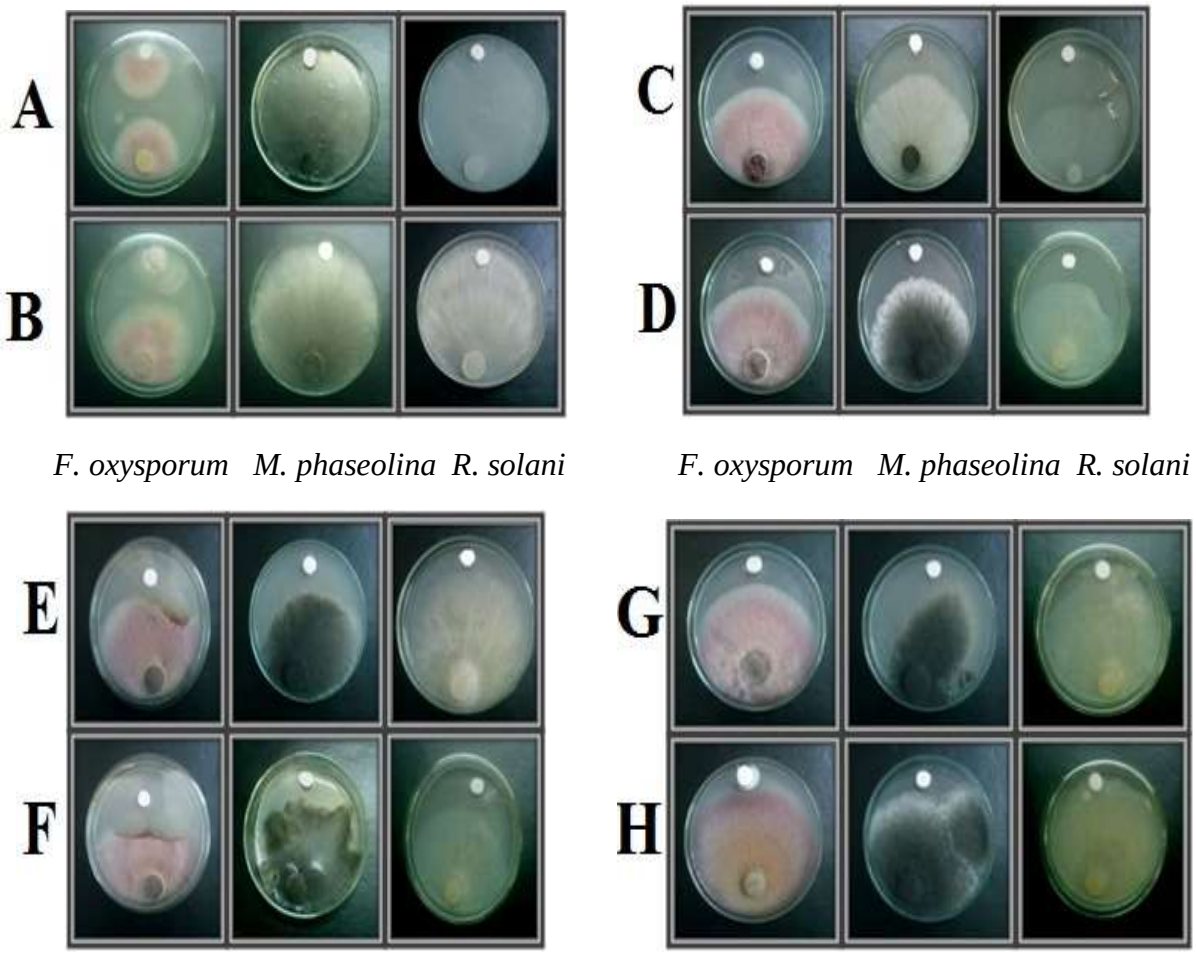
Fig.1A. Effect of Arnica montana and Thuja occidentalis pellets (30C) with different concentrations in the inhibition of root rot fungi



F. oxysporum *M. phaseolina* *R. solani* *F. oxysporum* *M. phaseolina* *R. solani*

F. oxysporum *M. phaseolina* *R. solani* *F. oxysporum* *M. phaseolina* *R. solani*

Whereas, **A**= Control (Absolute Alcohol) **B**= Control (Sterilized water), **C**= A@100%, **D**=T@100%, **E**=A@75%, **F**=T@75%, **G**=A@50%, **H**=T@50% v/w concentrations.

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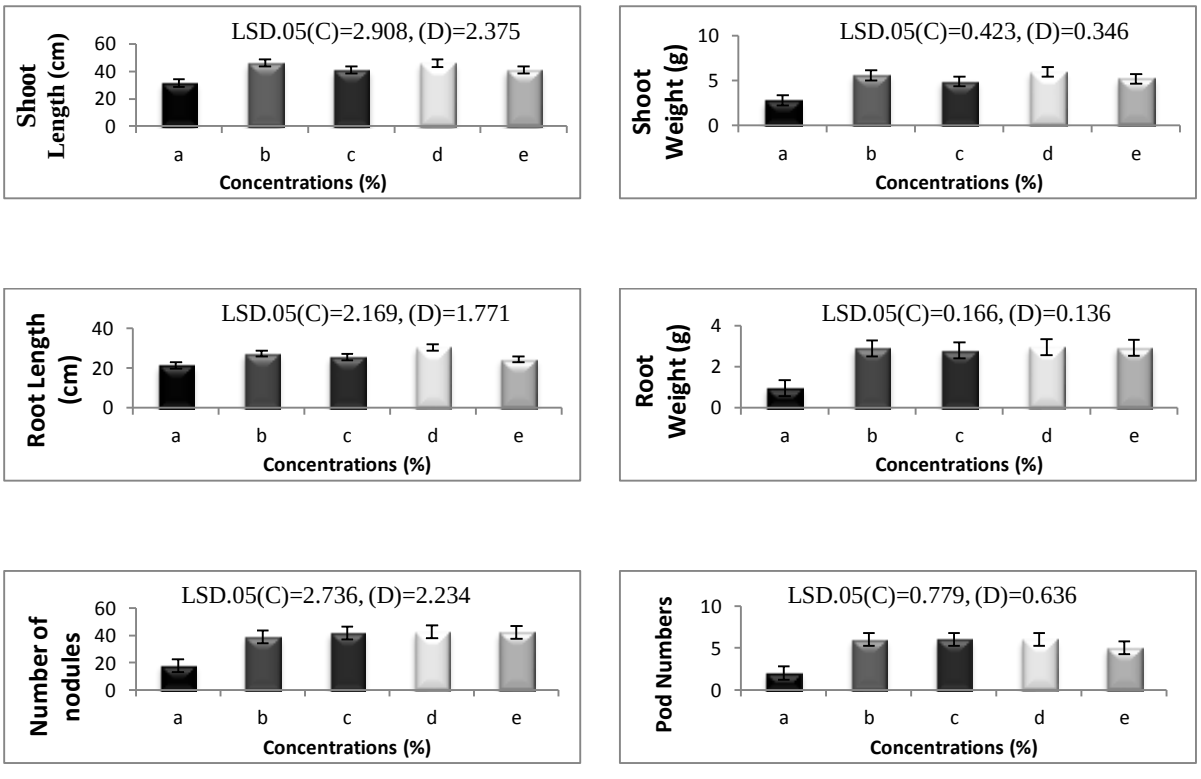
Table 1B: In vitro, growth inhibitions of root rot fungi by different concentrations of homeopathic pellets

Homeopathic drugs	Concentrations/ Growth inhibition											
	Fusarium oxysporum				*Rhizoctonia solani*				*Macrophomina phaseolina*			
	C ±SD	50% ±SD	75% ±SD	100 % ±SD	C ±SD	50% ±SD	75% ±SD	100 % ±SD	C ±SD	50% ±SD	75% ±SD	100 % ±SD
Arnica montana	0.0 ±0.0	39.6 ±3.05	65.8 ±6.08	69.7 ±3.21	0.0 ±0.0	31.4 ±3.21	55.6 ±1.53	77.3 ±3.05	0.0 ±0.0	34.9 ±2.52	49.0 ±4.73	59.2 ±4.93
Thuja occidentalis	0.0 ±0.0	37.6 ±4.00	60.35 ±1.53	71.4 ±1.53	0.0 ±0.0	36.5 ±5.29	61.2 ±3.0	70.94 ±3.05	0.0 ±0.0	40.3 ±3.21	58.0 ±3.21	71.4 ±2.08
LSD.05(Conc.) =	3.805				3.389				3.822			
LSD.05(Drug) =	2.691				2.396				2.702			

where; Conc. = Concentration and SD= ± Standard deviation ***=P<0.001; **=P<0.01; *=P<0.05; ns=non-significant

Fig.2A. Effect of Arnica montana and Thuja occidentalis pellets on growth parameters and control of root rot fungi on mash bean (Vigna mungo (L.) Hepper cv. NM-97) plants.



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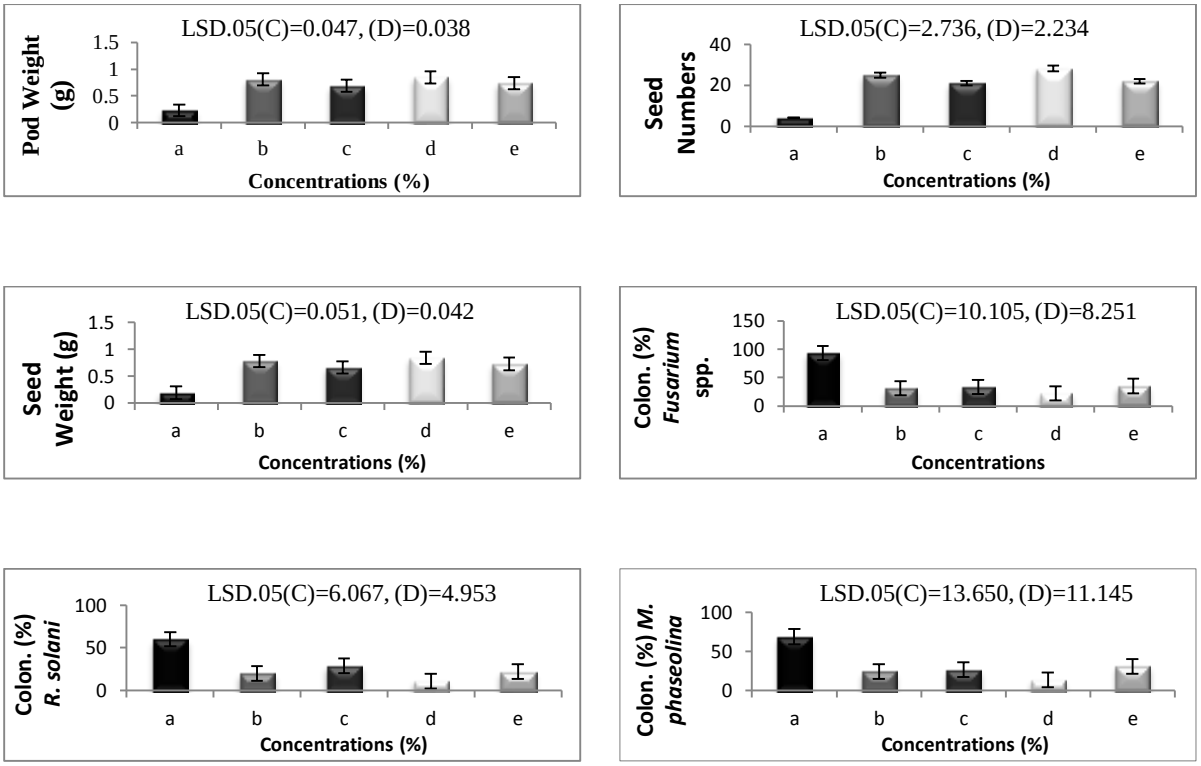
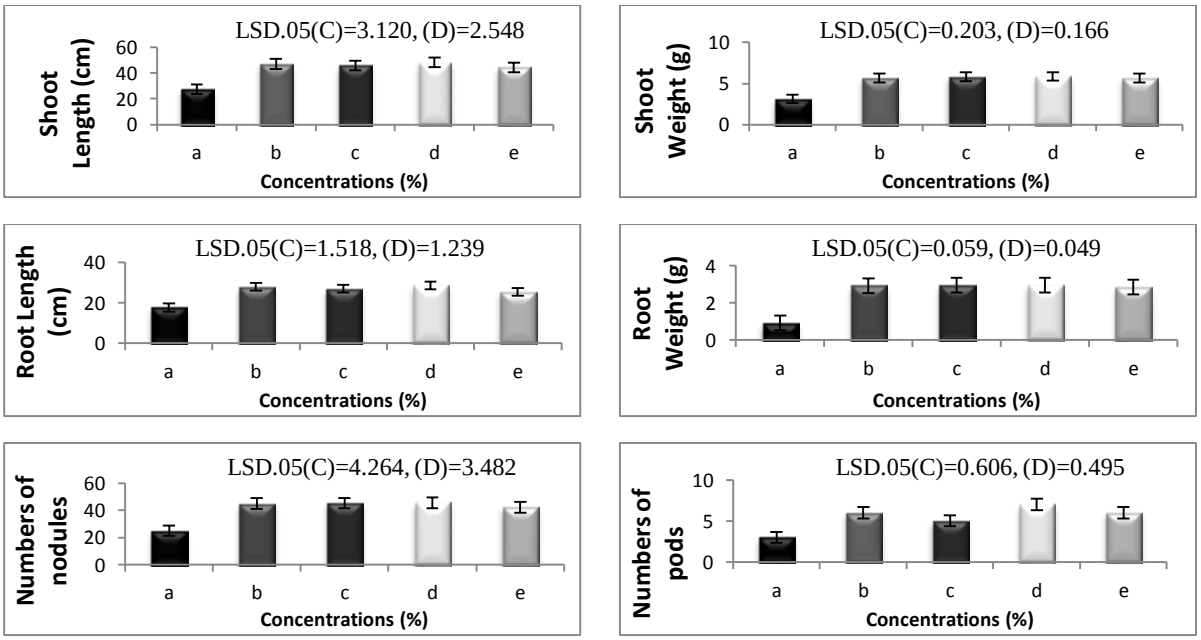


Where; (C) =Concentrations, (D) = Drugs, Colon. (%) = Colonization percentage, a=Control, b=A@75%, c=A@50%,d=T@75%, e=T@50% v/w concentrations (Prepared from 30C).

Fig.2B. Effect of Arnica montana and Thuja occidentalis pellets (30C) on growth parameters and control of root rot fungi on mung bean (Vigna radiata (L.) R. Wilczek. cv. NM-2006) plants.



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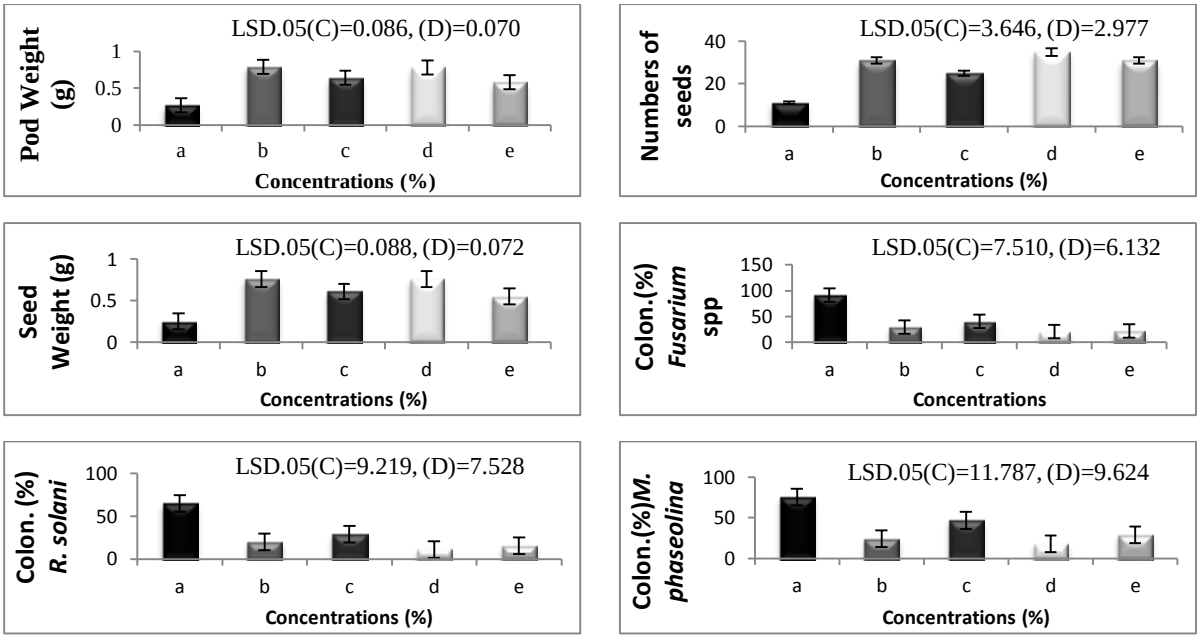
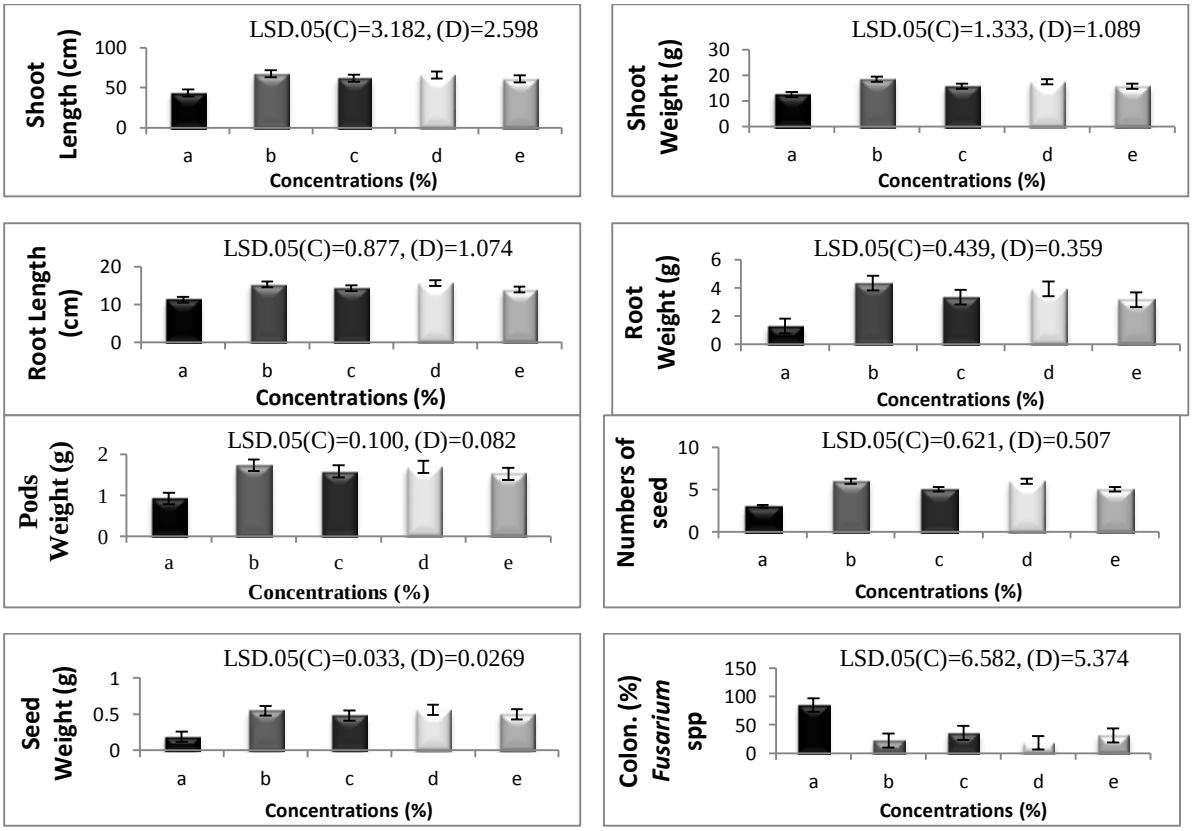


Where; (C) =Concentrations, (D) = Drugs, Colon. (%) = Colonization percentage, a=Control, b=A@75%, c=A@50%,d=T@75%, e=T@50% v/w concentrations (Prepared from 30C).

Fig.2C. Effect of *Arnica montana* and *Thuja occidentalis* pellets (30C) on growth parameters and control of root rot fungi on okra (*Abelmoschus esculentus* (L.) Moench cv. *Arka anamika*) plants.



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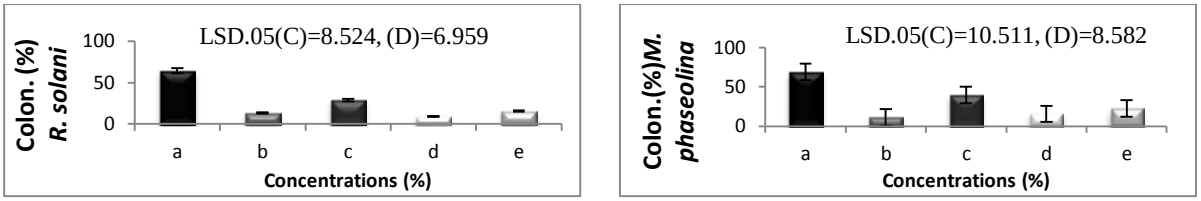
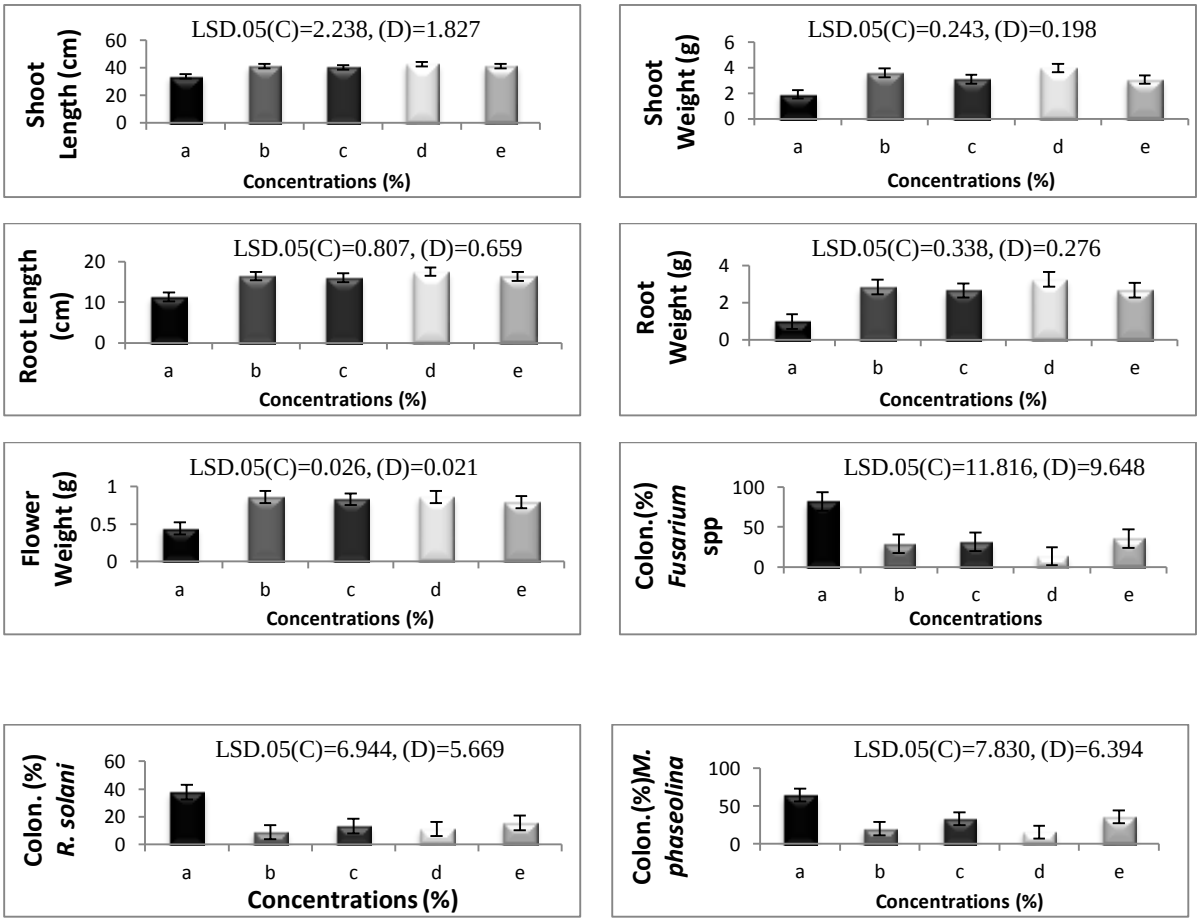


Where; (C) =Concentrations, (D) = Drugs, Colon. (%) = Colonization percentage, a=Control, b=A@75%, c=A@50%,d=T@75%, e=T@50% v/w concentrations (Prepared from 30C).

Fig.2D. Effect of *Arnica montana* and *Thuja occidentalis* pellets (30C) on growth parameters and control of root rot fungi on sunflower (*Helianthus annus* L. cv. Hysun-38) plants.



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

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