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IN VITRO, EVALUATION OF DIFFERENT CONCENTRATIONS OF HOMEOPATHIC DRUGS IN THE INHIBITION OF ROOT ROT FUNGI

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ABSTRACT

Present research was carried out to investigate the fungicidal effectiveness by using different concentrations of homeopathic drugs in the inhibition of root rot fungi. *Arnica montana* and *Thuja occidentalis* (30C) by using 100% v/v concentration was found to be most effective in 1.5 and 2.0 mL in which no mycelial growth of *F. oxysporum, R. solani* and *M. phaseolina* was observed. However, in 0.5 and 1.0 mL showed highest zone of inhibition. In 75% v/v concentration (prepared from 30C) by using different doses of 0.5, 1.0, 1.5 and 2.0 mL, both homeopathic drugs showed maximum zone of inhibition of pathogenic fungi. Whereas, in case of 50% v/v concentration (prepared from 30C) failed to inhibit root rot fungi when 0.5, 1.0, 1.5 and 2.0 mL were used.

KEYWORDS: Concentrations, Doses, *Arnica montana*, *Thuja occidentalis*, Root rot fungi.

INTRODUCTION

Root rot pathogen includes *Rhizoctonia solani, Pythium ultimum, Sclerotium rolfsii, Fusarium solani* and *Aphanomyces euteiches* (Abdel-Kader *et al*., 2002; Infantin *et al*., 2006) which caused destructive threats to crop production (Fravel, 2005) results in root rot and wilt diseases (Armstrong *et al*., 1976) producing heavy losses in volume and farmer's income (Hafiz, 1986). *Fusarium* spp. considered as a pathogenic soil inhabitant producing economic damage in agriculture throughout the world (Bentley *et al*., 2006). Numerous pathogens of *Fusarium* spp have similar symptoms causing the diseases of root rot, cortical decay of roots, wilting, chlorosis and early death (Summerell *et al*., 2001) on disease plants mostly in humid and semi-humid areas of world (Schroeder and Christensen, 1963). *R. solani* attacks broad range of crops causing the diseases of root rot and damping off on infected crops (Abu-Taleb *et al*., 2011) when it is favored by intermediate moisture and relatively high temperature (Steve, 2001). *M. phaseolina* important plant pathogen found throughout the world (Hoes, 1985) mostly in tropical and sub-tropical regions (Reuveni *et al*., 1983). It is reported to infect plants causing the symptoms like charcoal rot, damping off, wilt and dry rot, leaf and stem blight (Cowan, 1999) and disease severity enhanced due to dry and hot environmental condition (Gaige *et al.*, 2010). Various methods of controlling plant disease have been used against pathogenic fungi (Dubey, 2001) mostly by fungicidal applications (Pérez *et al*., 2004) which increase the growth quality of crop plants (Stephan *et al.*, 1988) but using these chemicals causes undesirable changes they inflict upon the environment (Arcury and Quandt, 2003) and human health risks (Mancini *et al*., 2008). For that reason, environment friendly methods of disease control are needed in plants (El-Mougy *et al*., 2004).

Homeopathic drugs are used as a substitute method for production of secondary metabolites and involves in physiological processes of plants without harming the environment (Bonato and Silva, 2003). Fungicidal effects of homeopathic drugs have been reported earlier (Goswami and Das, 1980; Khanna and Chandra, 1981; Khurana and Gupta, 1981). *Arnica montana* (Asteraceae) important herbal plants generally used in pharmaceutical and cosmetic industry (Bilia *et al.*, 2006) which contains volatile oil, tannins, flavonoids, resins, triterpenic alcohol and carotenoids (Brinkhaus *et al*., 2006; Ganzera *et al.*, 2008; Gawlik-Dziki *et al.,* 2011) exhibiting anti-inflammatory, anti-septic, decongestive, anti-bacterial and anti-fungal properties (Conforti *et al*., 1997; Siedle *et al*, 2004). Flowers of *A. montana* are used to treat wounds, bruises and burns (Stevinson *et al*., 2003). Instead of mother tincture, lower potencies of *A. montana* are used for treating superficial phlebitis, boils, dermatitis, insect bites, swollen gums and mouth ulcers (Vermeulen, 1994). *Thuja occidentalis* (Cupressacae) contains major element of essential oil and mono terpene thujone, an active ingredient used pharmacologically for the production of cough suppressants and nasal decongestants (Food and Agriculture Organization of the United Nations, 1995). The drug of *T. occidentalis* contains essential oil 1.4-4%, thujone which is 60% corresponds to 2.4% thujone in the whole drug (Hänsel *et al*., 1994) and known to have anti-viral, anti-diarrheal, anti-oxidant, anti-bacterial, anti-cancer, anti-HIV, neuro-pharmacological, insecticidal, anti-atheroscelorosis, radio protective, anti-body production and anti-spasmodic activity (Nam and Kang, 2005; Deb *et al*., 2006; Brijesh *et al*., 2012). Homeopathic drug from *Thuja* have been reported for treating skin lesion as well as diarrhea (Sunila *et al*., 2011).

The present research is carried out *In vitro* to study the effective doses by using different concentrations of homeopathic drugs in the inhibition of root rot fungi.

MATERIALS AND METHODS

Homeopathic drugs such as *Arnica montana* and *Thuja occidentalis* (30C) were purchased from market of Karachi. 100% v/v used as a pure form while 75 and 50% v/v concentrations were prepared from 30C potency. Different doses (0.5, 1.0, 1.5 and 2.0 mL) were poured in PDA medium supplemented with antibiotics (streptomycin and penicillin). Plates were rotated gently so it evenly spread throughout the Petri plates. Then the disc of 5mm of root rot fungi namely, *M. phaseolina*, *F. oxysporum* and *R. solani* were placed in the centre respectively and treatments were replicate thrice. The sterilized water and absolute alcohol (MERCK) served as control. Plates were incubated for one week at room temperature (28-33ºC). The percent growth inhibition of fungi over control was determined according to the formula given by Pinto *et al.* (1998).

Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) was set at $p<0.005$ and Duncan's multiple range test was employed to compare treatment means as proposed by Sokal and Rohlf (1995) using "Statistica" software.

RESULTS

In vitro experiment, concentrations of *A. montana* and *T. occidentalis* (100, 75 and 50% v/v conc.) were tested by using different doses to observed the inhibition of *F. oxysporum*, *M. phaesolina* and *R. solani*. *A. montana* and *T. occidentalis* @ 100% v/v concentration showed complete zone of inhibition of root rot fungi ($p<0.001$) when used at dose of 2.0 and 1.5 mL. However, highest zone of inhibition was observed in case of *M. phaseolina* when *T. occidentalis* used at 1.0 mL. Maximum zone of inhibition of *M. phaesolina*, *F. oxysporum* and *R. solani* were recorded when both homeopathic drugs were used at 0.5 mL. Whereas, in case of 75% v/v concentration when a dose of 2.0 mL used by both homeopathic drugs showed greater zone of inhibition of test fungi. Drugs of *A. montana* and *T. occidentalis* showed significant inhibition ($p<0.001$) against *R. solani*, *F. oxysporum* and *M. phaseolina* when 1.5 and 1.0 mL used but least inhibition were recorded by *F. oxysporum* and *M. phaseolina* at 0.5 mL dose. *A. montana* and *T. occidentalis* @ 50% v/v concentrations were not effective when different test doses were used. Similarly, sterilized water and absolute alcohol showed no zone of inhibition against the growth of root rot fungi (Table 1 and Fig. 1).

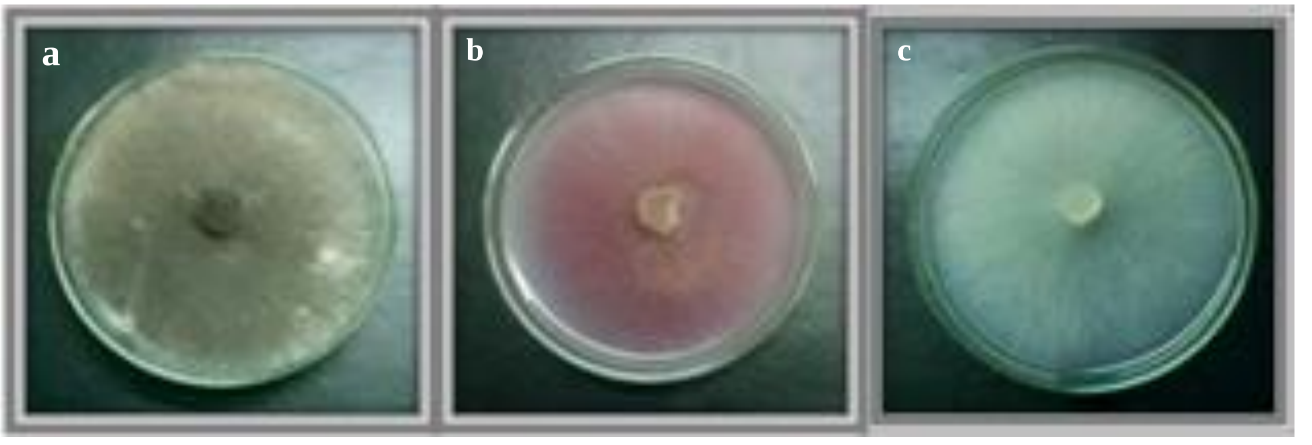
It was found that of all the doses, 2.0 and 1.5 mL were found to be best in suppressing the mycelial growth of *F. oxysporum*, *M. phaesolina* and *R. solani* when *A. montana* and *T. occidentalis* used at 100% v/v concentration.

Table 1. Evaluation of different doses by using *A. montana* and *T. occidentalis* (30C) concentrations in the inhibition of root rot fungi.

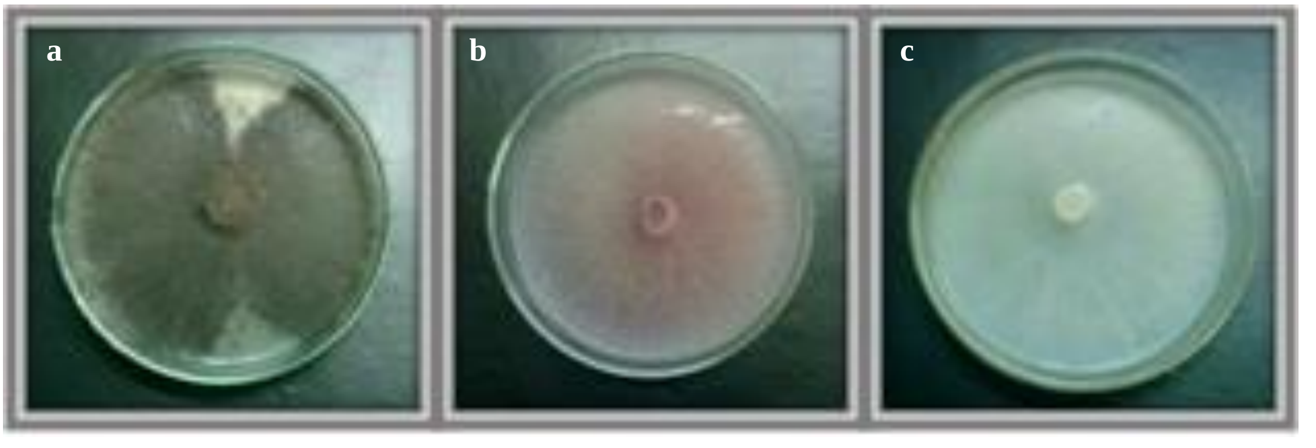
Homeopathic drugs	Doses (mL)	Concentrations/ Growth inhibition (MIC)											
		Fusarium oxysporum **(%)**				***Rhizoctonia solani*** **(%)**				***Macrophomina phaseolina*** **(%)**			
		Control	**A**	**B**	**C**	**Control**	**A**	**B**	**C**	**Control**	**A**	**B**	**C**
A. monatana	0.5	0.0 ± 0.0	57.78 ± 3.00	17.78 ± 1.00	0.0 ± 0.0	0.0 ± 0.0	48.89 ± 3.00	22.22 ± 1.53	0.0 ± 0.0	0.0 ± 0.0	68.89 ± 1.53	15.56 ± 1.53	0.0 ± 0.0
A. monatana	1.0	0.0 ± 0.0	60.00 ± 2.52	28.89 ± 1.53	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	26.67 ± 1.53	0.0 ± 0.0	0.0 ± 0.0	73.00 ± 2.00	35.56 ± 1.00	0.0 ± 0.0
A. monatana	1.5	0.0 ± 0.0	100 ± 0.0	46.67 ± 1.00	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	46.67 ± 2.00	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	42.22 ± 1.53	0.0 ± 0.0
A. monatana	2.0	0.0 ± 0.0	100 ± 0.0	62.22 ± 1.53	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	51.11 ± 1.00	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	55.56 ± 2.08	0.0 ± 0.0
T. occidentalis	0.5	0.0 ± 0.0	60.00 ± 1.15	24.44 ± 2.00	0.0 ± 0.0	0.0 ± 0.0	53.33 ± 1.53	17.78 ± 1.53	0.0 ± 0.0	0.0 ± 0.0	62.22 ± 1.00	13.33 ± 1.00	0.0 ± 0.0
T. occidentalis	1.0	0.0 ± 0.0	64.44 ± 1.53	33.33 ± 2.65	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	26.67 ± 1.00	0.0 ± 0.0	0.0 ± 0.0	80.00 ± 1.53	44.44 ± 2.08	0.0 ± 0.0
T. occidentalis	1.5	0.0 ± 0.0	100 ± 0.0	66.67 ± 1.00	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	37.78 ± 2.65	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	55.56 ± 2.08	0.0 ± 0.0
T. occidentalis	2.0	0.0 ± 0.0	100 ± 0..0	75.56 ± 3.00	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	49.62 ± 1.53	0.0 ± 0.0	0.0 ± 0.0	100 ± 0.0	73.33 ± 1.53	0.0 ± 0.0
$LSD_{0.05}$ (Dose) =		0.69				0.59				0.57			
(Concentration) =		0.69				0.59				0.57			
(Drug) =		0.49				0.42				0.99			

Where; MIC=Minimum inhibitory concentration, ± Standard deviation and Concentration of drug: **A**=100% v/v, **B**= 75% v/v, **C**= 50% v/v

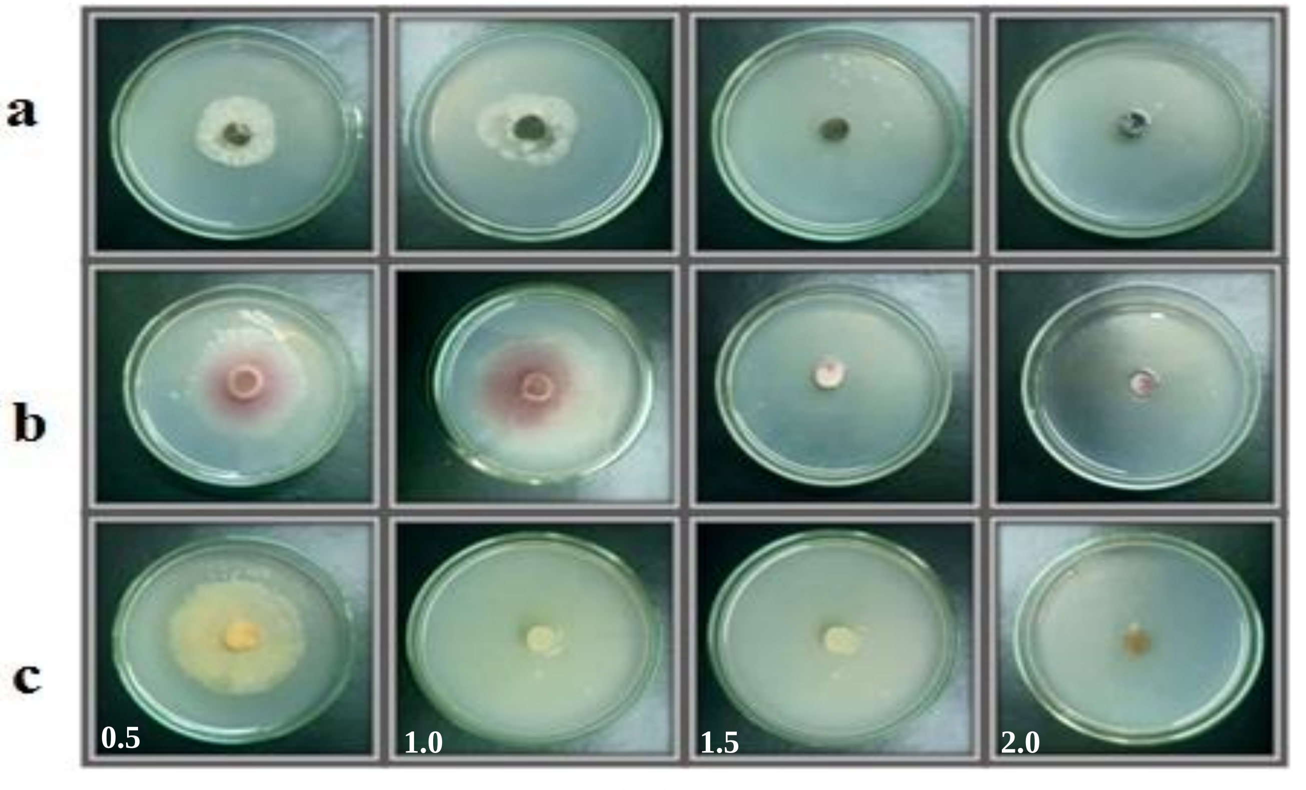
Control (Sterilized water)



Control (Absolute alcohol)



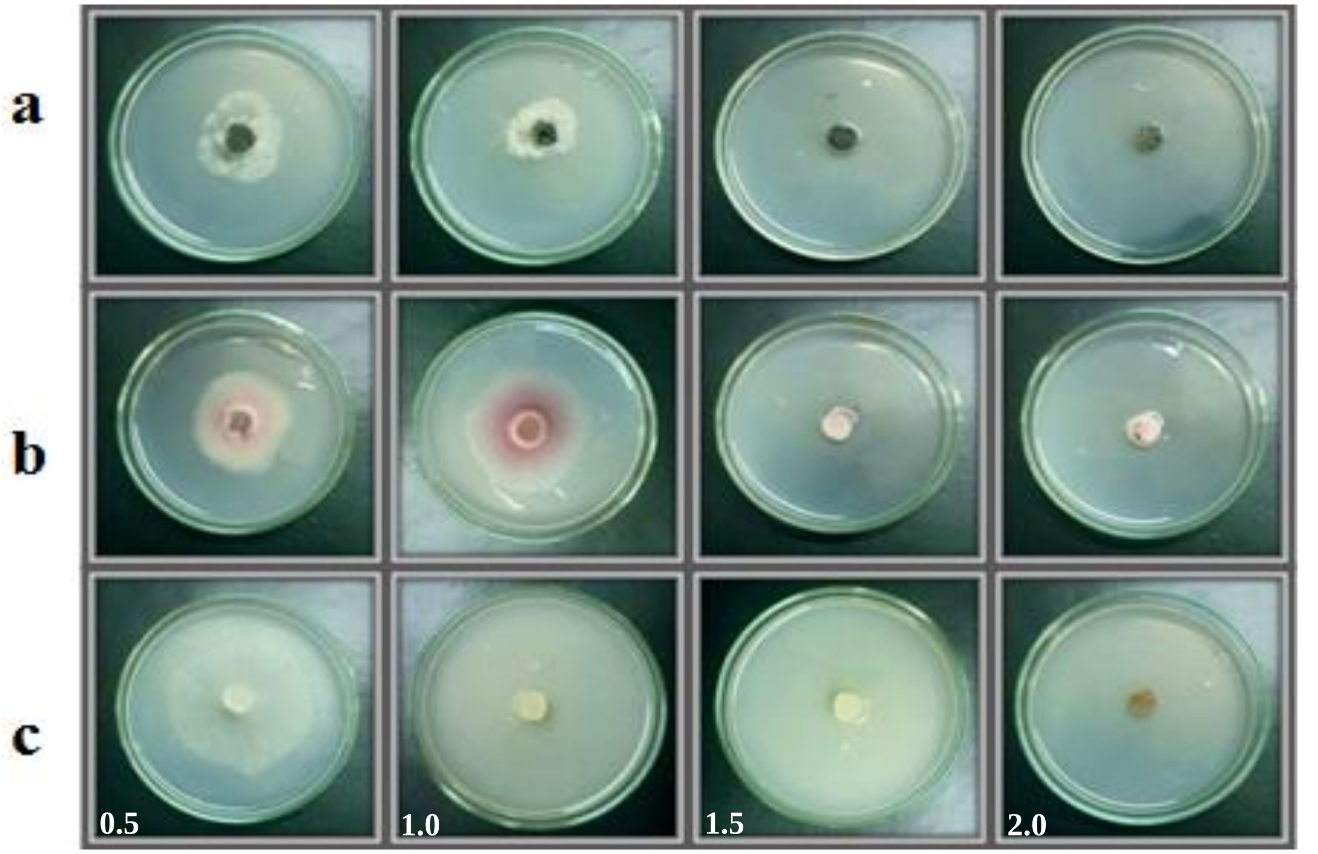
***A. montana* @ 100% v/v conc.**



(mL)

Fig. 1. Cont'd.

***T. occidentalis* @100% v/v conc.**

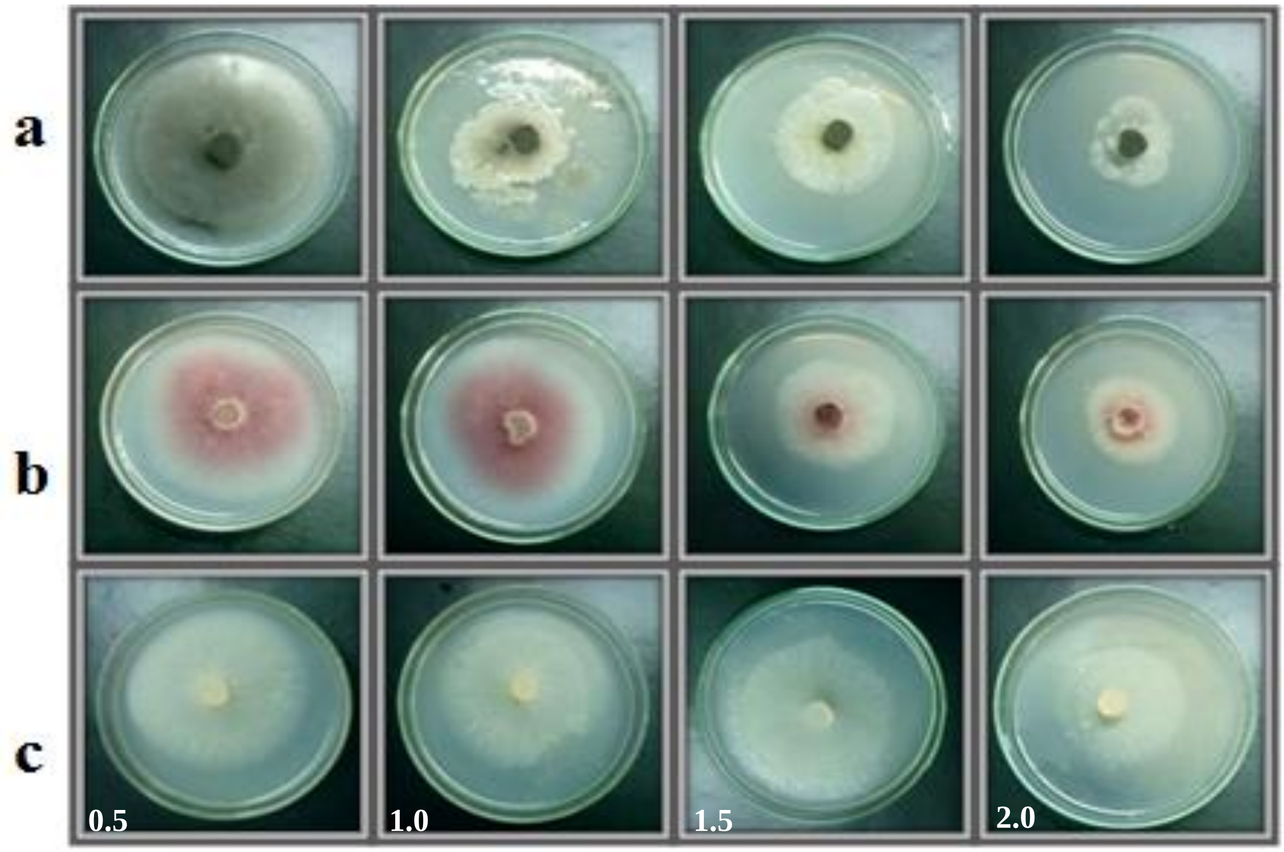


(mL)

***A. montana* @ 75% v/v conc.**

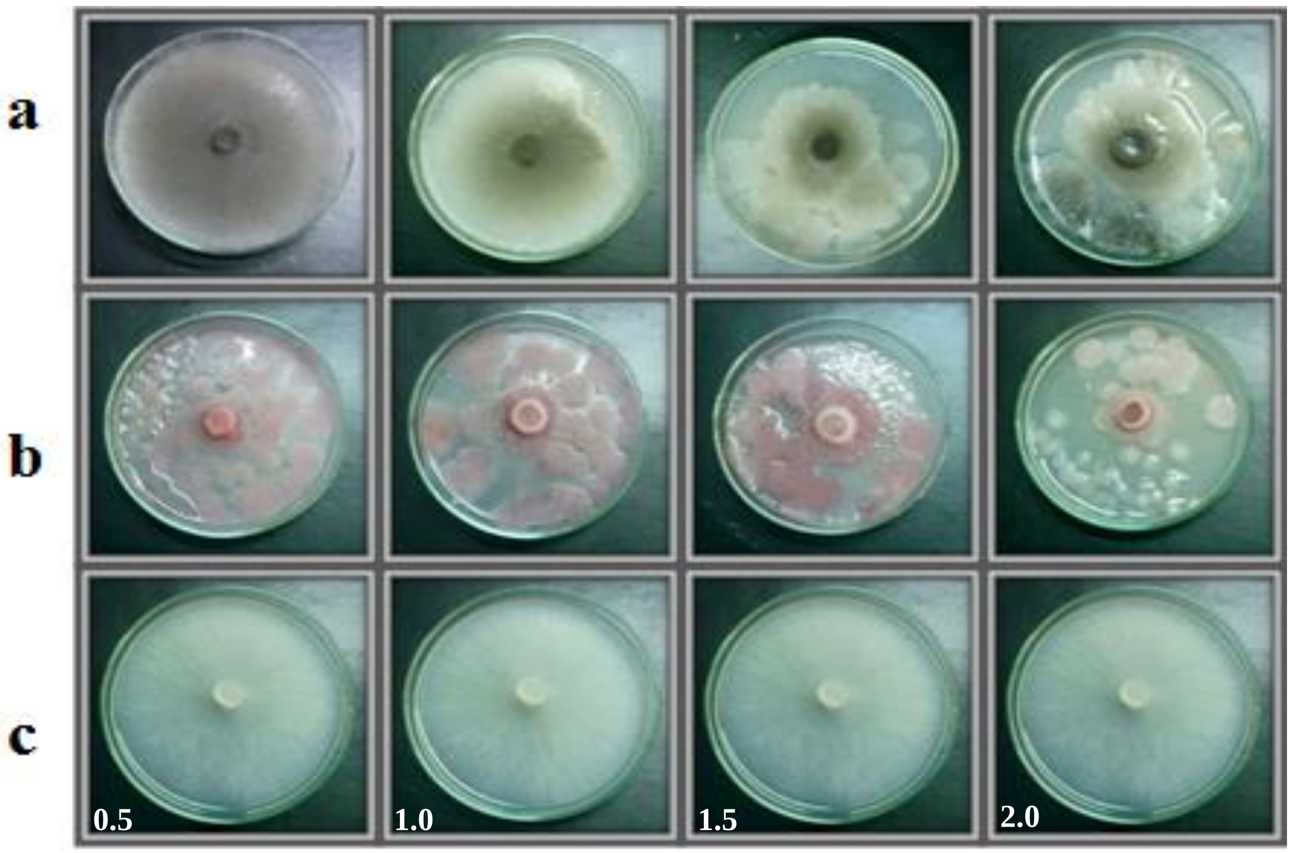
(mL)

***T. occidentalis* @ 75% v/v conc.** **Fig. 1. Cont'd.**



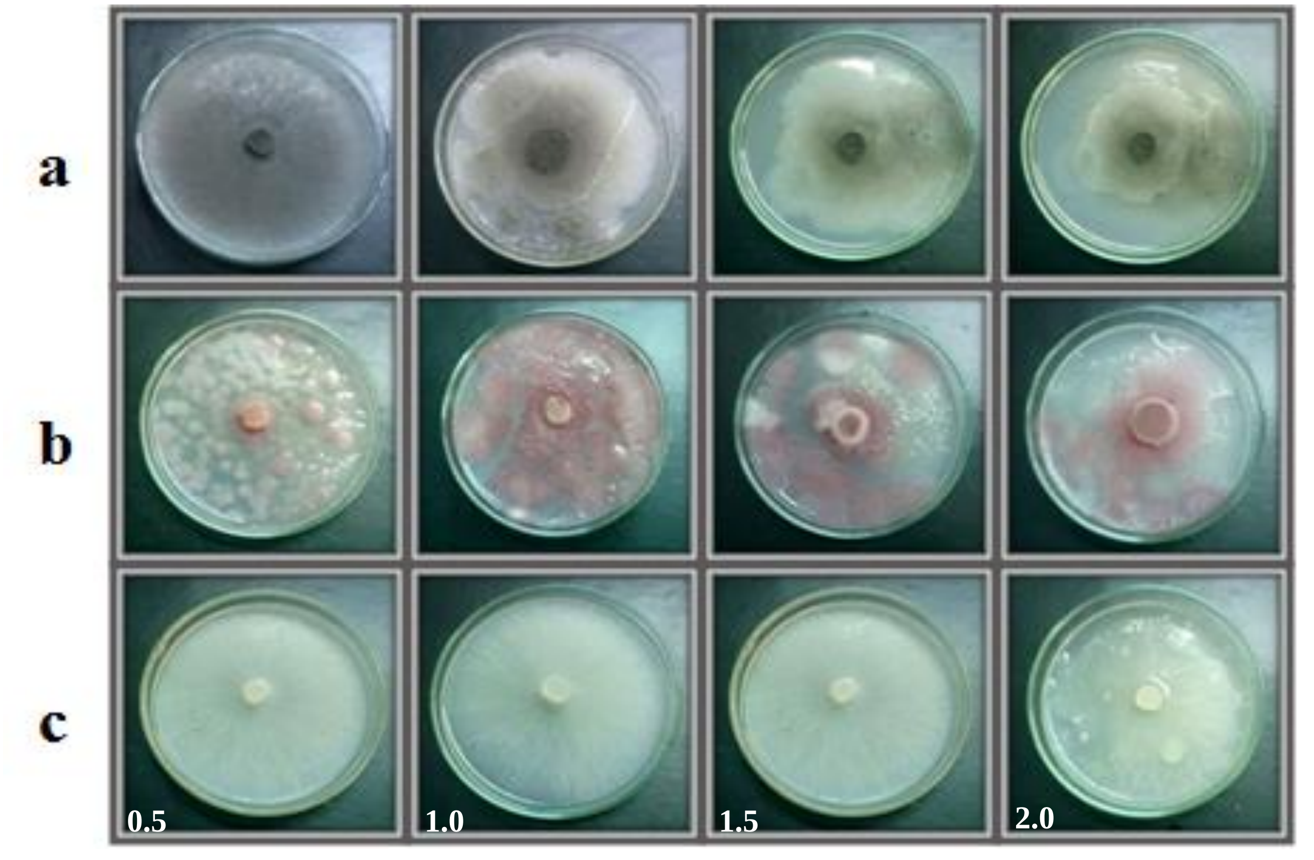
(mL)

***A. montana* @ 50% v/v conc.**



(mL)

***T. occidentalis* @ 50% v/v conc.** **Fig. 1. Cont'd.**



(mL)

Fig. 1. Inhibition of root infecting fungi on PDA incorporated with homeopathic drugs concentrations.
Where; Conc. = Concentration, a = *M. phaseolina*, b= *F. oxysporum* and c= *R. solani*.

DISCUSSION

A. montana and *T. occidentalis* @100% v/v concentration was found to be excellent in the inhibition of root rot fungi and most effective dose was observed at the dose of 2.0 and 1.5 mL which completely suppressed the pathogen *In vitro* followed by 75% v/v concentration. Similar results were obtained *In vitro* when *A. montana* and *T. occidentalis* pellets (30C) showed highest zone of inhibition used at 100% v/w concentration followed by 75 and 50% v/w which showed maximum inhibition of *F. oxysporum, M. phaseolina* and *R. solani* (Hanif *et al*., 2015). *Thuja* (30 and 200C) drugs found to be effective against *Aspergillus flavus* causing cutaneous aspergillosis and *Thuja* (50M) against *Aspergillus niger* causing otomycosis in human (Gupta, 2002). All potencies of *Thuja* (Q, 30C, 200C, 1M, 10M, 50M) drugs showed high degree of inhibition against *Bipolaris* spp., followed by *Curvularia* spp., *Exserohilum* spp. and *Aspergillus flavus* (Asha *et al*., 2014). Homeopathic drugs such as *Thuja* and *Natrum muriaticum* exhibited significant inhibition of *Fusarium* spp on sunflower seeds (Hussain *et al*., 2000). Drugs such as *Filixmas* and *Blatta orientalis* control *Fusarium oxysporum* in the seeds of wheat (Rake *et al*., 1989). Sinha and Singh (1983) demonstrated that aflatoxins produced by fungi transmitted through stored product contaminations can be control by application of *Sulphur* in 200 CH which showed 100% inhibition of *Aspergillus parasiticus*. Verma *et al.* (1989) found that homeopathic drugs like *Lachesis* and *Chimaphila* in 200 CH can be used before and after inoculation reduces 50% of tobacco mosaic virus content in tobacco leaf disc. *In vitro, Thuja occidentalis* showed significant result against *Aspergillus flavus* in 30M and 200M, whereas in 50M found promising result against *Aspergillus niger* (Gupta and Srivastava, 2002). Homeopathic drugs proved antiviral effectiveness against animal and plant viruses (Singh *et al*., 1981; Singh and Gupta, 1985). Saxena *et al.* (1988) inhibited seed borne fungi and germination on okra seeds when treated with homeopathic drugs. *Thuja occidentalis* extracts were tested *In vitro* showed antibacterial against *Escherichia coli, Citrobacter, Shigella flexenari, Yersinia aldovae, Staphylococcus aureus* and *Pseudomonas aeruginosa* while antifungal activity against *Saccharomyces cereviciae, A. parasiticus, Macrophomina, Trichophyton rubrum, Fusarium solani* and *Candida albicans* (Jahan *et al*., 2010). Present research showed that when *T. occidentalis* and *A. montana* (100% v/v conc.) used at 2.0 and 1.5 mL showed the promising results in the inhibition of root rot fungi. Therefore, it represents an environmental friendly strategy and can be applied in field for the management of root rot fungi.

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