

STUDY OF HOMEOPATHIC DRUGS ON SEED GERMINATION AND FUNGAL GROWTH

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Abstract

In vitro, seeds of mung bean, sunflower, okra and mash bean were treated with homeopathic drugs namely *Arnica montana* and *Thuja occidentalis* (30C) were evaluated against root rot fungi. Different concentrations like 100, 75 and 50% v/v were tested to investigate seeds germination and inhibition of root rot fungi such as *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Results indicated that treated seeds of mung bean, sunflower, okra and mash bean with pure homeopathic drugs (100% v/v) by *A. montana* and *T. occidentalis* (30C) showed complete germination (100%), greater root length and excellent inhibition of root infecting pathogens. However, tested seeds treated with 75 and 50% v/v concentrations (prepared from 30C) by homeopathic drugs, respectively recorded significant increase in germination, root length and maximum zone of inhibition.

Key words: Homeopathic drugs, Seed treatment, Concentrations, Germination, Inhibition of root rot fungi.

Introduction

Plant pathogens particularly fungi poses the most visible threats to crop fields (Fravel, 2005). The losses of world crop production due to fungal diseases estimated as 12% or more in developing countries (Horbach *et al.*, 2010). Root rot pathogens like *M. phaseolina*, *R. solani* and *Fusarium* spp. are reported to infect an extensive range of crop plants in Pakistan (Ghaffar, 1988). *F. oxysporum* considered as pathogenic soil inhabitant that has capability to degrade lignin (Sutherland *et al.*, 1983) plus complex carbohydrates (Snyder & Hansen, 1940). *F. solani* and *R. solani* are responsible for causing root rot and damping off diseases on a numerous variety of crop plants (Abu-Taleb *et al.*, 2011). *M. phaseolina* found throughout the globe (Hoes, 1985) and considered an essential plant pathogens in the tropical and subtropical regions (Reuveni *et al.*, 1983) producing the symptoms mainly damping off, charcoal rot, leaf and stem blight, wilt and dry rot (Cowan, 1999). Association of pathogenic fungi enhances the disease intensity and severity on many crop plants (Piecsarka & Abawi, 1978). The aim of controlling plant disease is to increase the growth quality of crop plants (Stephan *et al.*, 1988) by using fungicide application (Perez *et al.*, 2004). However, chemical applications produces environmental and human health risks (Mancini *et al.*, 2008) and pest resistance also becomes a problem (Bhanti & Taneja, 2007). Therefore, substitute methods of disease control in plants are essential (El-Mougy *et al.*, 2004).

Homeopathic drugs used as an alternative control have potential to resist diseases without destruction of an environment (Bonato & Silva, 2003). Application of homeopathic drugs showed positive effect in human beings and animals but in case of using drugs particularly in plants and soil have been developed more recently (Bonato *et al.*, 2006). Presently, homeopathic drugs are being used in the control of plant diseases (Kumar, 1980; Hanif & Dawar, 2015a), plant detoxification of metals for example aluminum (AL), (Rocha *et al.*, 2002; Moretti *et al.*, 2002) and copper (Cu), (Almeida, 2002). Using homeopathic

drugs against plant pathogenic fungi has been reported earlier (Khanna & Chandra, 1980, 1981; Goswami & Das, 1980; Khurana & Gupta, 1981). By using the drugs such as *Filixmas* and *Blatta orientalis* showed maximum inhibition of *Fusarium oxysporum* in the seed mycoflora of wheat (Rake *et al.*, 1989). Khanna & Chandra (1983) found significant results in the inhibition of root rot fungi on tomato caused by *Fusarium roseum*, by the homeopathic preparation of *Kali iodatum* in 149 CH and *Thuja occidentalis* in 87 CH for both pre and post harvest conditions. Homeopathic drugs minimize plant diseases through the activation of induced resistance (Betti *et al.*, 2009). Homeopathic drugs such as, *Thuja occidentalis* (Cupressaceae) has been use to treat amenorrhea, enuresis, psoriasis, cystitis, uterine, carcinomas and rheumatism (Chang *et al.*, 2000). Plant extracts of *Thuja occidentalis* contain anti-diarrheal and anti-viral activity (Deb *et al.*, 2007). *Arnica montana* (Asteraceae) mainly grows on the Central Europe and East, its active constituents identified in its flower, leaves and roots contains chlorogenic acids, lactones, flavonoids, sesquiterpene, carotenoids, tannins, alcohols, essential oil and phenolic acids compounds (Gawlik-Dziki *et al.*, 2011). These compounds are responsible for anti-inflammatory properties (Siedle *et al.*, 2004). *Arnica montana* exhibited anti-bacterial, anti-fungal, anti-septic, anti-oxidant and anti-sclerotic activities (Sugier & Gawlik-Dziki *et al.*, 2009).

Developing countries shows interest in testing seeds for quality and seed research in pathology (Neergaard, 1977). Seeds attack by fungi caused seed shrunken, abortion, reduced size, necrosis, sclerotisation, seed rot and discoloration, reduced or totally germ failure, mechanical and physiological changes (Shetty & Prakash, 1994). Seeds are considered as the carriers of plant pathogens and thus responsible for producing severe diseases leading to huge loss of crop yield (Janardhanan & Husain, 1980). Seeds carrying infection resulting in immense destruction due to seedling blights (Janardhanan, 1994). Pathogenic fungi produce toxic metabolites which kill embryo (Vidyasekaran *et al.*, 1970).

Present research has done to explore antifungal activity of homeopathic drugs in the inhibition of root rot fungi namely; *Rhizoctonia solani*, *Fusarium oxysporum* and *Macrophomina phaseolina* by treated crop seeds with homeopathic drugs.

Materials and Methods

Homeopathic drugs like *Arnica montana* and *Thuja occidentalis* (30C) were purchased from the medicinal market of Karachi. 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 treated with Dr. Willmar Schwabe homeopathic drugs like *A. montana* and *T. occidentalis* (30C) with the concentrations of 100, 75 and 50% v/v, respectively whereas, seeds treated with sterilized water and absolute alcohol (MERCK) for about 10-15 mins. served as control and dried aseptically. Four seeds of mung bean, sunflower, okra and mash bean were placed in each Petri plates containing PDA (Potato Dextrose Agar) medium supplemented with antibiotics (penicillin @ 100,000 unit/L and streptomycin @ 200 mg/L) to inhibit the growth of bacteria and in the centre test fungus was inoculated. Root infecting pathogens like *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina* were replicated thrice and plates were incubated for one week at room temperature (26-31°C). Observed the germination percentage (Swati *et al.*, 2013), root length and fungal growth inhibition percentage (Pinto *et al.*, 1998; Loksha & Benagi, 2007). Data were analyzed by using two way analysis (ANOVA) followed by the least significant difference (LSD) test at P = 0.05 as given by Gomez & Gomez (1984).

Results and Discussion

Mung bean and mash bean seeds when treated with *A. montana* and *T. occidentalis* @ 100, 75 and 50% v/v concentrations showed complete germination ($p < 0.05$) but in case of sunflower and okra seeds when treated with both homeopathic drugs @ 100% v/v showed 100% germination followed by 75 and 50% concentrations. Tested seeds when treated with sterilized water and absolute alcohol served as control showed significant reduction ($p < 0.05$) in germination and produced severe infection which was visible on seed surface as well as roots easily infected by root rot fungi but also failed to produce zone of inhibition. Highest zone of inhibition and significant root length ($p < 0.05$) was observed on mung bean, sunflower, okra and mash bean seeds treated with *A. montana* and *T. occidentalis* @ 100% concentrations (Fig. 1A). Maximum zone of inhibition and root length were recorded when seeds were treated with 75 and 50% v/v concentrations respectively by both homeopathic drugs (Fig. 1B). Highest inhibition was recorded by *A. montana* with different concentrations and was found to be the best

drug in controlling the *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina* and also showed highest root length (Table 1). Emphasis on methods has been made which are simple, economic, reproducible and effective but some methods such as blotter and deep freezing suppressed seed germination (Limonard, 1968). Non synthetic media plays an important role in germination of seeds because of the characteristic requirements of moisture and oxygen available for germination (Swati, 2006). Seeds treated with different concentrations of homeopathic drugs plated on PDA medium showed better results in germination, root length and showed maximum zone of inhibition against root rot fungi which was also reported by Farrag & Moharam (2012) when cucumber seeds treated with 1, 2 and 3% peppermint extract exhibited highest seed germination and reduced infection of pathogenic fungi on PDA medium. *T. occidentalis* in 30 M and 200 M showed significant result against *Aspergillus flavus* whereas, by using 50 M against *A. niger* (Gupta & Srivastava, 2002). Singh *et al.* (1980 and 1981) proved antiviral effectiveness of homeopathic drugs against animal and plant viruses. *In vitro*, *T. occidentalis* (30C and 200C) showed maximum inhibition against *Candida albicans* (Gupta *et al.*, 2015). Saxena *et al.* (1988) inhibited twenty two genera of fungi on okra seeds when treated with *T. occidentalis*. Hanif & Dawar (2015b) reported that by using the concentrations of 100, 75 and 50% v/v of *T. occidentalis* and *A. montana* (30C) used as a seed treatment and soil drenching methods inhibit root rot fungi and enhanced the growth of crop plants. *T. occidentalis* and *A. montana* (30C) pellets showed positive results when used *In vitro* and *In vivo* experiments against root rot fungi (Hanif *et al.*, 2015). Use of Ceresan for seed treatment avoid the seeds rot in storage and obtain better sprouting of seedlings of *Oroxylum indicum* (Mehrotra, 1990). Seed treatment is an excellent method to protect seed from seed borne and soil borne pathogens which permit the seed to germinate and become established as a healthy seedling (Chang & Kommedahl, 1968). Treatment of crop seeds with fungal and bacterial antagonists showed effectiveness in the inhibition of root rot fungi (Dawar *et al.*, 2008, Ikram & Dawar, 2015). *B. thuringiensis* when applied as seed dressing showed significant increase in germination, shoot length, shoot weight, root length and root weight (Sheikh *et al.*, 2006). Similarly, seed dressing with *Bacillus subtilis* control variety of crop diseases including *Rhizoctonia solani* (Merriman *et al.*, 1974). Seed dressing increase yield and minimize economic losses by reducing pathogenic fungi (Martha *et al.*, 2003). There is a need to enhance the yield and improve seed quality of the crops therefore; treatments of seed should be done as a routine practice due to cheap insurance against possible adversity at a later stage (Bilgrami & Dube, 1976).

Present studies showed that seed treatment with homeopathic drugs *In vitro* was found to be better and cheap method for the inhibition of root rot fungi which protect seeds and roots. For that reason, experiment would be carried out in the field to improve the yield and increase the productivity of crop plants.

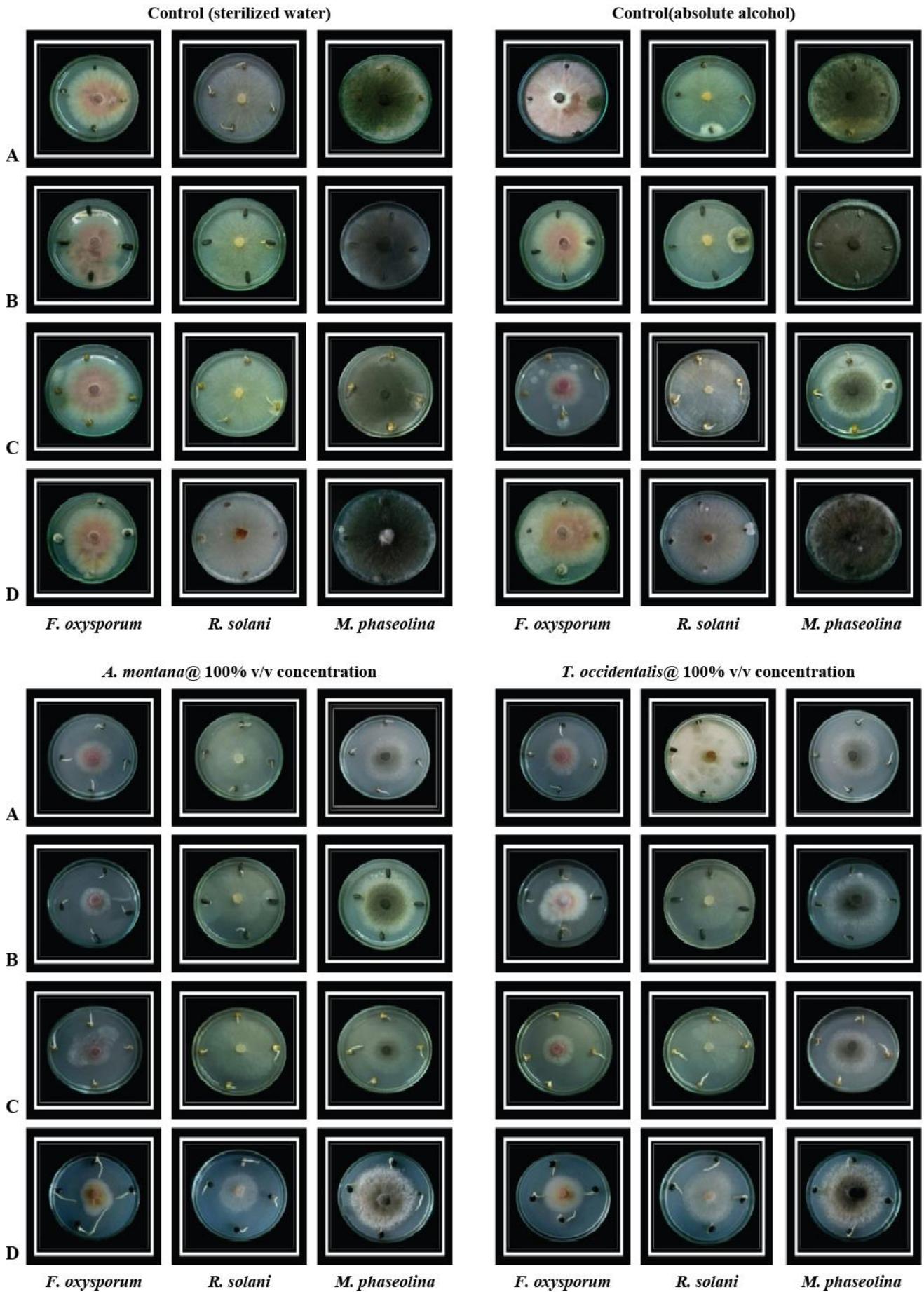


Fig. 1A.(Cont'd.).

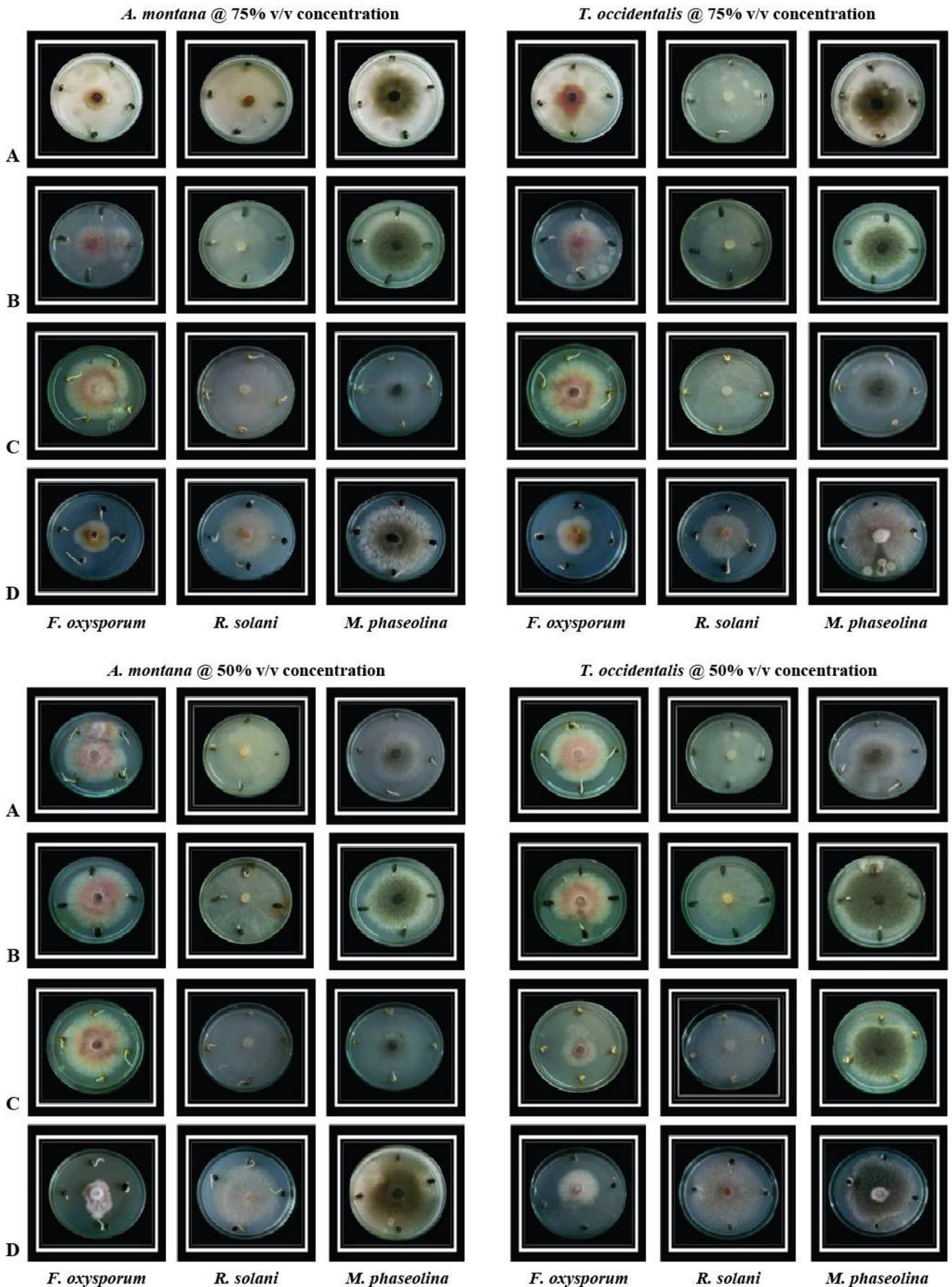


Fig. 1A. = *In vitro*, inhibition of root rot fungi by homeopathic drugs treated crops seeds on PDA (Potato Dextrose Agar) medium Where; **A**= Mash bean (*Vigna mungo* (L.) Hepper), **B**= Sunflower (*Helianthus annuus* L.), **C**= Mung bean (*Vigna radiata* (L.) R. Wilczek.), **D**= Okra (*Abelmoschus esculentus* (L.) Moench) seeds.

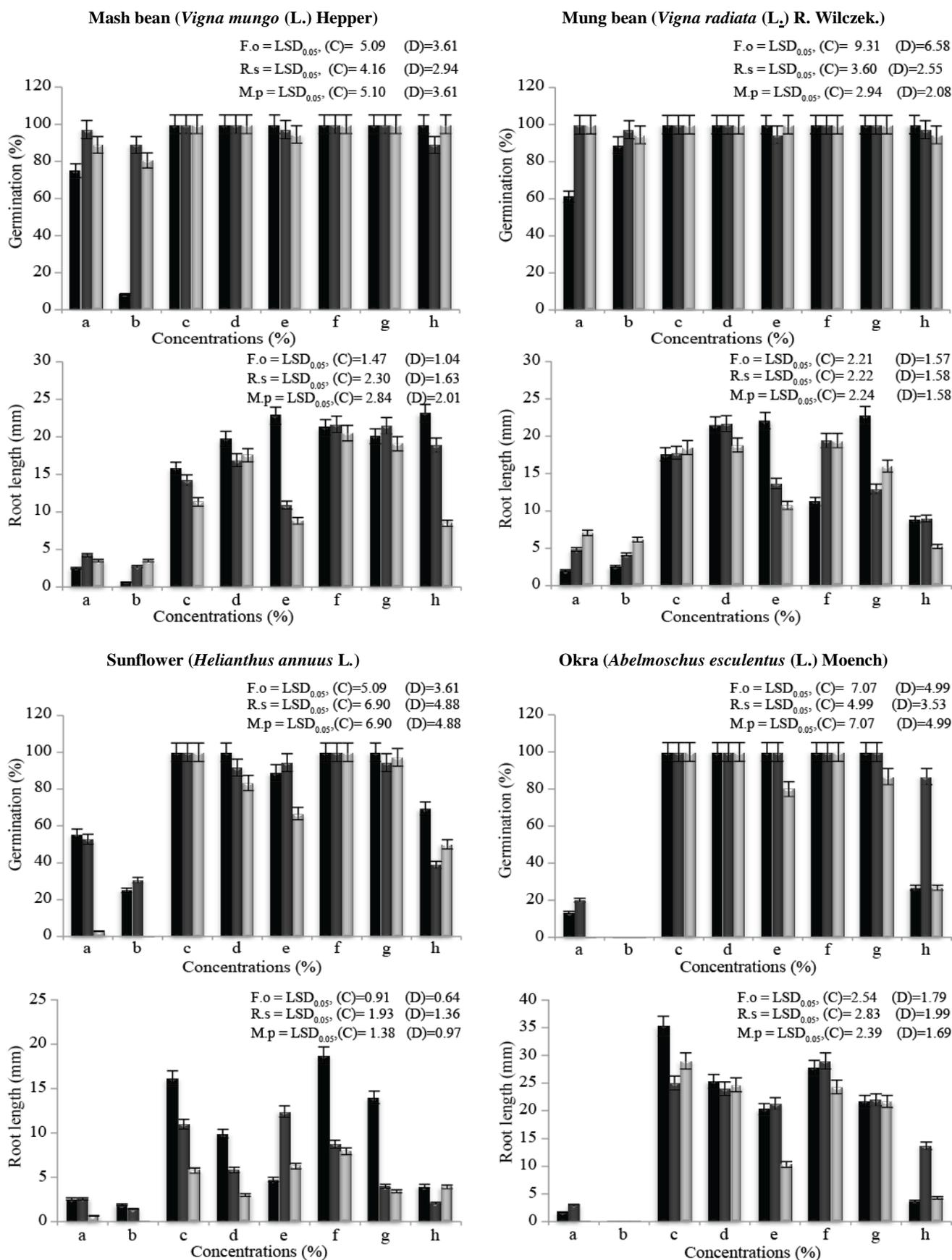


Fig. 1B. Effect of seed treatment with *Arnica montana* and *Thuja occidentalis* (30C) in the control of root rot fungi on crop seeds.

KEY: F.o = *Fusarium oxysporum* R.s = *Rhizoctonia solani* M.p = *Macrophomina phaseolina* a = Control (Sterilized water), b = Control (Absolute alcohol) c = A@100%, d = A@75%, e = A@50%, f = T@100%, g = T@75%, h = T@50% v/v concentrations, (C) =Concentrations, (D) =Drugs

Table 1. *In vitro*, inhibition of root rot fungi by *A. montana* and *T. occidentalis* (30C) on treated crop seeds.

Homeopathic drugs	Concentrations/ Growth inhibition (MIC)											
	<i>Fusarium oxysporum</i> (%)				<i>Rhizoctonia solani</i> (%)				<i>Macrophomina phaseolina</i> (%)			
	C±SD	100±SD	75±SD	50±SD	C±SD	100±SD	75±SD	50±SD	C±SD	100±SD	75±SD	50±SD
Mash bean (<i>Vigna mungo</i> (L.) Hepper)												
<i>A. montana</i>	0.0 ± 0.0	74.82 ± 2.08	72.60 ± 6.03	40.73 ± 1.53	0.0 ± 0.0	65.18 ± 1.53	65.18 ± 3.05	38.51 ± 2.08	0.0 ± 0.0	75.56 ± 2.00	63.71 ± 1.53	48.16 ± 1.53
<i>T. occidentalis</i>	0.0 ± 0.0	82.20 ± 4.58	68.20 ± 1.53	38.4 ± 4.51	0.0 ± 0.0	87.30 ± 2.52	55.60 ± 3.61	32.70 ± 5.86	0.0 ± 0.0	72.70 ± 1.53	46.70 ± 3.00	42.20 ± 2.65
LSD _{0.05} (Conc.) =	4.04				3.61				2.25			
(Drugs) =	2.85				2.55				1.59			
Sunflower (<i>Helianthus annuus</i> L.)												
<i>A. montana</i>	0.0 ± 0.0	89.62 ± 2.52	61.50 ± 4.16	17.10 ± 2.08	0.0 ± 0.0	68.16 ± 2.08	64.40 ± 2.65	11.80 ± 1.53	0.0 ± 0.0	56.2 ± 3.79	57.80 ± 4.00	10.40 ± 1.53
<i>T. occidentalis</i>	0.0 ± 0.0	65.20 ± 2.08	46.70 ± 1.00	27.60 ± 5.03	0.0 ± 0.0	73.30 ± 1.00	43.80 ± 3.06	17.80 ± 2.00	0.0 ± 0.0	68.20 ± 3.06	41.60 ± 1.53	18.50 ± 3.21
LSD _{0.05} (Conc.) =	3.31				2.38				3.19			
(Drugs) =	2.34				1.68				2.26			
Mung bean (<i>Vigna radiata</i> (L.) R. Wilczek.)												
<i>A. montana</i>	0.0 ± 0.0	73.30 ± 8.72	48.89 ± 1.00	40.00 ± 2.00	0.0 ± 0.0	65.18 ± 9.29	52.60 ± 1.53	37.10 ± 1.53	0.0 ± 0.0	71.10 ± 6.24	60.73 ± 3.51	33.30 ± 3.61
<i>T. occidentalis</i>	0.0 ± 0.0	88.20 ± 1.53	60.00 ± 5.57	64.40 ± 2.65	0.0 ± 0.0	61.50 ± 6.03	42.96 ± 1.53	43.78 ± 1.53	0.0 ± 0.0	59.30 ± 4.16	65.18 ± 1.53	35.56 ± 3.61
LSD _{0.05} (Conc.) =	4.93				4.97				4.35			
(Drugs) =	3.48				3.52				3.08			
Okra (<i>Abelmoschus esculentus</i> (L.) Moench)												
<i>A. montana</i>	0.0 ± 0.0	65.11 ± 2.08	54.00 ± 1.53	46.60 ± 1.53	0.0 ± 0.0	55.60 ± 1.00	44.44 ± 1.00	36.20 ± 3.06	0.0 ± 0.0	42.90 ± 2.08	31.80 ± 1.53	18.44 ± 1.53
<i>T. occidentalis</i>	0.0 ± 0.0	57.80 ± 2.00	51.11 ± 1.00	42.20 ± 1.00	0.0 ± 0.0	48.90 ± 1.00	42.90 ± 1.53	28.90 ± 2.00	0.0 ± 0.0	44.44 ± 2.00	31.70 ± 3.78	28.90 ± 3.00
LSD _{0.05} (Conc.) =	1.68				1.87				2.61			
(Drugs) =	1.18				1.32				1.84			

Where; C= Control, Conc. = Concentrations and SD = ± Standard deviation, MIC = Minimum inhibitory concentration

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