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% w/w 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% w/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 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-glycosylacturonic), sesquiterpene lactones (armonidol, 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-sclerotie, 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 & Kuhnt, 2011; Pjelvjakušić 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 flavonoids, polysaccharides (Naser et al., 2005) essential oils (Svajdlenka et al., 1999) diterpenes, monoterpenes (α-thujone, β-thujone, fenchone), and sesquiterpene (Chang et al., 2000) polysaccharides as well as flavonoids (kercefin, camphilerol), 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. Wiekzck. 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 & Rakowski, 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 mung bean, mash bean, sunflower and okra with different concentrations of A. montana and T. occidentalis at 75 and 50% v/v 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): 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 mung 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 3OC) 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. montana* and *T. occidentalis* @ 50% v/v concentration (prepared from 3OC) 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, anti diarrheal 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 (Bonifim 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 phaseollina* (Hanif et al., 2015). Asha et al., 2014 reported that by using all potencies of *Thuja* (Q, 3OC, 20OC, 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 (Sumila et al., 2011) also against diarrhea (Gaskin, 2005). *T. occidentalis* methanolic extracts at 10% v/v concentrations showed significant inhibition of *Aspergillus* sp., *Microsporum* 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, G1 production by *Aspergillus parasiticus* (strain # = MTCC-411) in grundnuts 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 (Srivastava & 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 (Benzé & 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


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