Int. J. Biol. Res., 3(1): 13-18, 2015.

CEREALS PROTECTION FROM SOIL BORNE ROOT INFECTING FUNGI BY THE USE OF HOMEOPATHIC DRUGS

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ABSTRACT

Homeopathic drugs like *Thuja occidentalis* and *Natrum muriaticum* with different concentrations (0.05% and 0.1% v/v) were tested for antifungal activity by using paper disc diffusion and agar well diffusion methods. Both drugs showed zone of inhibition against *Macrophomina phaseolina* and *Fusarium oxysporum* at 0.1% while *Rhizoctonia solani* was not inhibited by any of the homeopathic drug. Seed treatment with *T. occidentalis* at 0.1% significantly improves growth parameters in millet plant while root infecting fungi were reduced by using both drugs at 0.1%. Chlorophyll content was increased by using both homeopathic drugs. In case of wheat plant, both drugs enhanced growth parameters, chlorophyll contents and reduced root infecting fungi.

KEYWORDS: Millet and wheat plant; Paper disc and agar well diffusion methods; Root infecting fungi; *Natrum muriaticum*; *Thuja occidentalis*.

INTRODUCTION

Homeopathic drugs, used as an alternative method for controlling diseases, have ability to induce production of secondary metabolites that are harmless to the environment. These drugs isolated from plants, animals and rocks, having biological activity, have been focused *In vitro* and *In vivo* studies. According to Bonato and Silva, (2003), homeopathic drugs are prepared from mother tincture which consists in serial aqueous dilutions coupled with dynamization phases. Homeopathy is eco-friendly, leaving no residue in the environment. *Thuja occidentalis* (Cupressacae), commonly called as white cedar, is a well known medicinal plant. It is native to eastern North America and Europe as an ornamental tree (Chang *et al*., 2000). It is used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, rheumatism, amenorrhea and other skin problems (Shimada, 1956). Homeopathic drugs used in different purposes like in the control of pathogens and pests, increase of secondary metabolites in plants, detoxification of plants which are contaminated by metals like aluminum and copper and promotes plant physiology. Drugs like *Filixmas* and *Blatta orientalis* completely control *Fusarium oxysporum* in the seeds of wheat (Rake *et al*., 1989). Kolisko, (1923) observed the action of homeopathic drugs on plant growth. Seed borne fungi and germination of *Abelmoschus esculentus* was control by using homeopathic drugs (Saxena *et al*., 1988). Khanna and Chandra, (1983) in India found significant results in the control of tomato rot, caused by *Fusarium roseum*, by the homeopathic preparation of *Kali iodatum* in 149 CH and *Thuja occidentalis* in 87 CH for pre and post harvest conditions. Sinha and Singh, (1983) found that growth of aflatoxins producing fungi, *Aspergillus parasiticus*, was controlled by using Sulphur (200 CH). *Lachesis* and *Chimaphila* (200 CH) reduce 50% of virus content of tobacco mosaic virus in tobacco leaf (Verma *et al*., 1989). Rolim *et al*. (2005) observed that powdery mildew of tomato was reduced by the usage of 100 CH *Kali iodatum.*

T. occidentalis is also know to have antibacterial, anti cancer, antibody production, anti-HIV activity, antispasmodic, antioxidant, radioprotective, antiatheroscelorosis, neuropharmacological and insecticidal activity (Brijesh *et al*., 2012). *Natrum muriaticum* is actually common edible salt which is used for hypotension as well as hypertension in higher and lower potencies. It is particularly useful in treating backache, constipation, skin problems like warts, pimples, boils, psoriasis and excessive oiliness (Sarao, 2013).

This paper presents usage and understanding of homeopathic drugs and their impact on plant growth, and physiology of cereals (millet [*Panicum miliaceum* L.] and wheat [*Triticum aestivum* L.]).

MATERIALS AND METHODS

Collection of drugs: Two homeopathic drugs *Natrum muriaticum* (200) and *Thuja occidentalis* (30) were purchased from medicinal market of Karachi, Pakistan and concentrations like 0.1 and 0.05% v/v were prepared from these drugs by using sterilized distilled water.

Laboratory work: Two different methods were employed to observe antifungal activity against root infecting fungi. In paper disc diffusion method, Whatmann's number 1 paper disc (6 mm) were soaked in 0.05 and 0.1% potencies of each drugs separately and were placed on potato dextrose agar (PDA) poured Petri plates supplemented with antibiotics (streptomycin, penicillin). Discs soaked in sterilized distilled water were placed served as control. Root infecting fungi like *R. solani, F. oxysporum* and *M. phaseolina* were placed in the middle of the plate. Each disc was pressed down for complete contact with agar surface. Treatments were replicated thrice and the plates were incubated at room temperature (25-30°C) for 5 days and the zone of inhibition was measured in cm (Tariq, 2012).

Same procedure was applied in Agar well diffusion method and instead of paper disc, wells were made around the three corners of Petri plate and in the middle, root infecting fungi were placed. Wells were supplemented with respective concentrations of homeopathic drugs and for the comparison, control (well supplemented with sterilized distilled water) was used (Tariq, 2012).

Soil properties: Soil used in the experiment was obtained from the experimental field of the Botany Department, Karachi University, sieved to discard large pebbles and was filled in the pots (8 cm diameter) containing 300g. The soil examined was sandy loam with sand (70%), silt (19%) and clay (11%), pH (7.6-8.0), moisture holding capacity (24.01%) (Keen and Raczkowski, 1922). Natural infestation of *M. phaseolina* (1-3 sclerotia/g), *R. solani* (5-10%) and *Fusarium* species (3000 cfu/g soil) was also recorded (Sheikh and Ghaffar, 1975; Wilhelm, 1955; Nash and Synder, 1962).

Seed treatment and soil drenching: Millet (*Panicum miliaceum* [L.]) and wheat (*Triticum aestivum* [L.]) seeds were sterilized in 2% sodium hypochlorite solution for 5 minutes, washed twice in sterilized distilled water to remove all dust particles from seeds. Subsequently washed seeds were treated with suspension of homeopathic drugs with different concentrations for 10 minutes and then air dried for 3-4 hours in laminar flow chamber and then sown in the plastic pots. Similarly, 20 mL solution of each drug with different concentrations was transferred in the pots, keeping overnight and then seeds of millet and wheat were sown in each pot separately. Replicate each treatment thrice and the pots without homeopathic drugs served as a control. The pots were randomly placed in the screen house of Department of Botany, University of Karachi.

Estimation of growth parameters and colonization by root rot fungi: After 30 days of germination, plants were uprooted and examined for growth in terms of shoot length, shoot weight, root length, root weight, chlorophyll a and chlorophyll b. Roots were washed under running tap water, surface sterilized by 1% calcium hypochlorite, $Ca(OCl)_2$ (2-3 minutes) and then five pieces were cut and placed on the PDA poured plates. The antibiotics, penicillin @ 100,000/L and streptomycin @ 20 mg/L were added to the PDA. The Petri plates were then incubated for 5 days at room temperature for fungal growth on the root pieces (Short *et al*., 1980).

Chlorophyll content: Fresh leaf material (1 g) was crushed in 80% acetone and centrifuged at 1600 rpm 3 times for 5 minutes. The absorbance of the solution was measured at 645 and 663 nm (Arnon, 1949). The chlorophyll contents were determined using the extinction coefficient of Mackinney (1941).

Statistical analysis: The data were analyzed for two-way analysis (ANOVA) as per experimental design. Means were separated using Duncan's multiple range test (DMRT) at $p<0.05$ (Sokal and Rohlf, 1995).

RESULTS

Lab experiment: *N. muriaticum* and *T. occidentalis* drugs were used to test antifungal activity against root infecting fungi. Paper disc method showed effective results in producing zone of inhibition. Maximum zone of inhibition was recorded against *M. phaseolina* (1.1 cm) at 0.1% concentration of both drugs. However, 0.05% concentration of both drugs was also effective against *M. phaseolina* and *F. oxysporum* as compared to control. No zone of inhibition was observed against *R. solani* at any concentration of both drugs (Table 1).

In case of well method, relatively large zone of inhibition was recorded against *M. phaseolina* in both drugs (1.35 and 1.30 cm, respectively) used at 0.1% concentration. However, *F. oxysporum* also showed zone of inhibition at 0.05 and 0.1% concentrations but most effective result was achieved when 0.1% concentration (0.9 cm) of *T. occidentalis* was used. Both drugs did not show effective results against *R. solani* growth (Table 1).

Pot experiment: Shoot length (35.00 cm), shoot weight (1.10 g), root length (19.75 cm), root weight (0.29 g) were highest when seeds were treated with *T. occidentalis* used at 0.1% concentration. Both drugs at 0.05% also showed better result in improving plant growth (Table 2). Both homeopathic drugs significantly ($p<0.01$) increased chlorophyll content of millet leaves when compared with control. Chlorophyll "a" was highest when seed treated with *N. muriaticum* at 0.1% (0.32 mg/mL) while maximum chlorophyll "b" was recorded when seeds were treated with *T. occidentalis* (0.15 mg/mL) at 0.1% (Table 2). Colonization of millet roots by root infecting fungi was observed to be reduced in all the treatments except for control ($p<0.05$). However, *M. phaseolina* was reduced when seeds were treated by *T. occidentalis* at 0.1% while *R. solani* and *F. oxysporum* was reduced when seed treated with *N. muricatum* at 0.1% (Fig. 1).

In case of wheat, soil treatment with *T. occidentalis* at 0.1% concentration showed maximum shoot length (20.3 cm) and shoot weight (0.33 g). Root length (19.40 cm) was enhanced by the use of seed treatment with *N. muriaticum* while increased root weight (0.36 g) was recorded when seeds were treated with *T. occidentalis* at 0.1% concentration (Table 3). Chlorophyll "a" (0.22 mg/mL) and chlorophyll "b" (0.11 mg/mL) were enhanced due to seed treatment with 0.1% concentration of *T. occidentalis* followed by soil treatment with 0.1% concentration of *T. occidentalis* and *N. muriaticum* ($p<0.01$). Both drugs were effective in reduction of root infecting fungi in wheat plant. *M. phaseolina* and *R. solani* was reduced to when seeds were treated with *T. occidentalis* at 0.1% concentration. However, soil treatment with *N. muriaticum* at 0.1% was effective in reduction of *Fusarium* species followed by soil treatment with *N. muriaticum* at 0.05% concentration (Fig. 1).

Table 1. Effect of homeopathic drugs in the inhibition of root infecting fungi.

Drugs	Concentrations (ppm)	Root infecting fungi		
		Fusarium oxysporum	***Macrophomina phaseolina***	***Rhizoctonia solani***
	Well method (cm)			
Control	-	FG	0.6	FG
T. occidentalis	500	0.7	1.2	FG
T. occidentalis	1000	0.9	1.35	FG
N. muriaticum	500	0.60	0.8	FG
N. muriaticum	1000	0.65	1.30	FG
	Paper Disc method (cm)			
Control	-	0.2	FG	FG
T. occidentalis	500	0.3	0.9	FG
T. occidentalis	1000	0.55	1.1	FG
N. muriaticum	500	0.20	0.4	FG
N. muriaticum	1000	0.25	1.5	FG

FG = Full growth of root infecting fungi

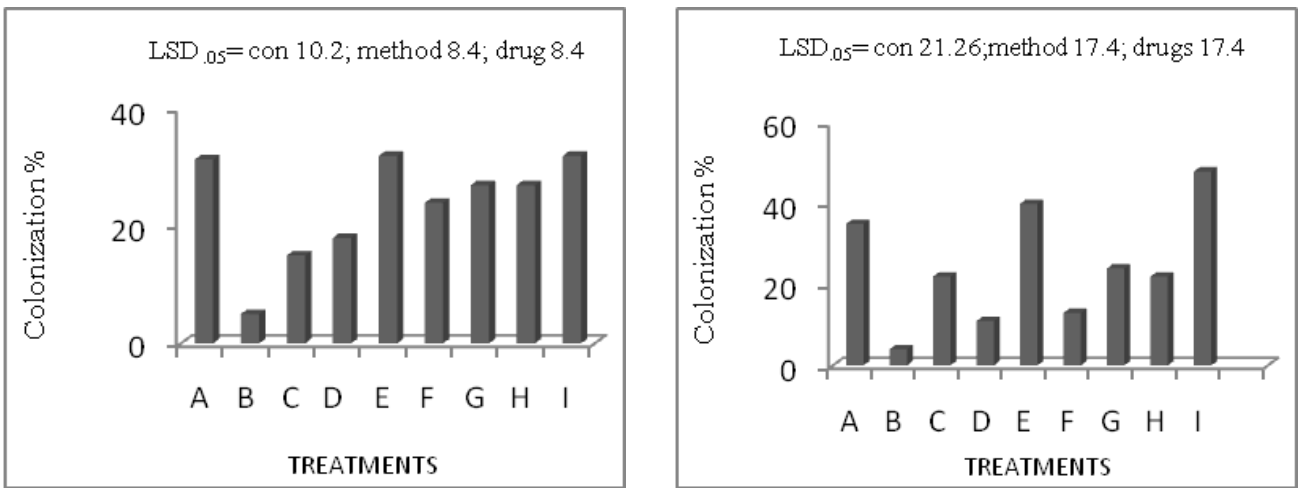
Table 2. Effect of homeopathic drugs on the growth of millet plant.

Drugs	Concentration (ppm)	Method	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Chlorophyll a (mg/mL)	Chlorophyll b (mg/mL)
Control	-	-	24.56	0.65	13.50	0.28	0.26	0.12
T. occidentalis	500	Seed treatment	30.11	0.50	16.50	0.22	0.31	0.12
T. occidentalis	500	Soil drenching	25.05	0.42	15.00	0.20	0.16	0.03
T. occidentalis	1000	Seed treatment	35.00	1.10	19.75	0.29	0.30	0.15
T. occidentalis	1000	Soil drenching	25.00	0.45	17.21	0.14	0.24	0.10
N. muriaticum	500	Seed treatment	25.26	0.44	15.00	0.20	0.31	0.10
N. muriaticum	500	Soil drenching	31.35	0.36	13.33	0.14	0.15	0.08
N. muriaticum	1000	Seed treatment	31.50	0.70	14.22	0.08	0.32	0.13
N. muriaticum	1000	Soil drenching	24.56	0.36	13.46	0.13	0.15	0.08
$LSD_{0.05}$	Concentrations		3.37	0.27	2.60	0.04	0.25	0.12
	Methods		3.07	0.22	1.67	0.03	0.06	0.10
	Drugs		3.07	0.22	1.67	0.03	0.06	0.10

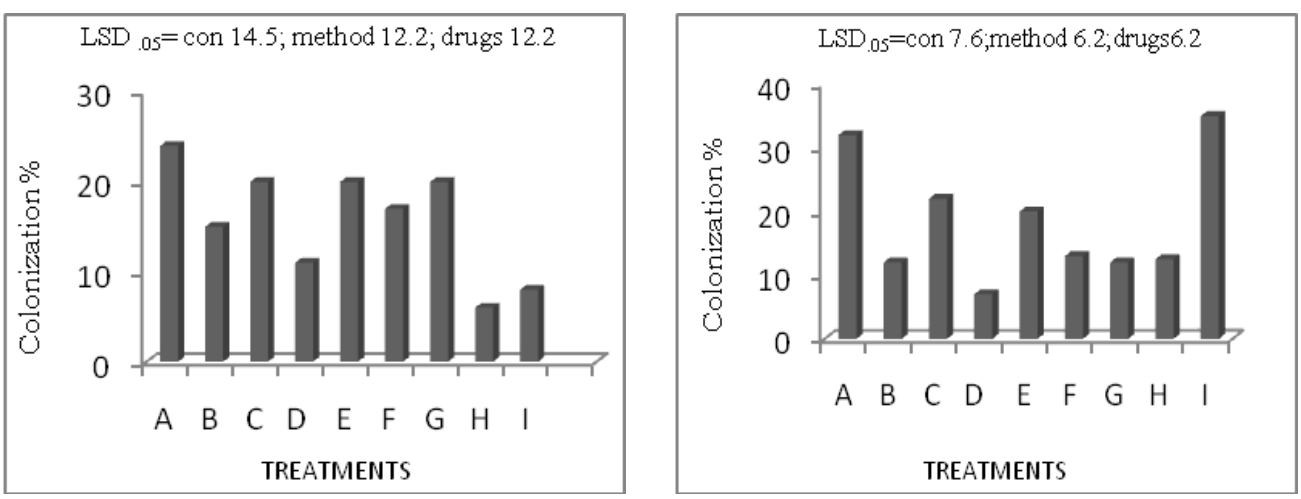
Table 3. Effect of homeopathic drugs on the growth of wheat plant.

Drugs	Concentration (ppm)	Method	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Chlorophyll a (mg/mL)	Chlorophyll b (mg/mL)
Control	-	-	15.55	0.24	19.80	0.22	0.092	0.08
T. occidentalis	500	Seed treatment	17.78	0.31	18.75	0.22	0.10	0.08
T. occidentalis	500	Soil drenching	17.00	0.26	18.56	0.32	0.17	0.08
T. occidentalis	1000	Seed treatment	18.32	0.30	16.85	0.36	0.22	0.11
T. occidentalis	1000	Soil drenching	20.30	0.33	15.50	0.27	0.12	0.10
N. muriaticum	500	Seed treatment	19.70	0.24	17.26	0.24	0.10	0.02
N. muriaticum	500	Soil drenching	16.62	0.31	16.77	0.26	0.17	0.07
N. muriaticum	1000	Seed treatment	15.97	0.33	19.40	0.25	0.18	0.06
N. muriaticum	1000	Soil drenching	16.96	0.24	18.83	0.31	0.10	0.10
$LSD_{0.05}$	Concentrations		2.8	0.05	3.06	0.06	0.03	0.07
	Methods		2.2	0.04	2.49	0.05	0.02	0.02
	Drugs		2.2	0.04	2.49	0.05	0.02	0.02

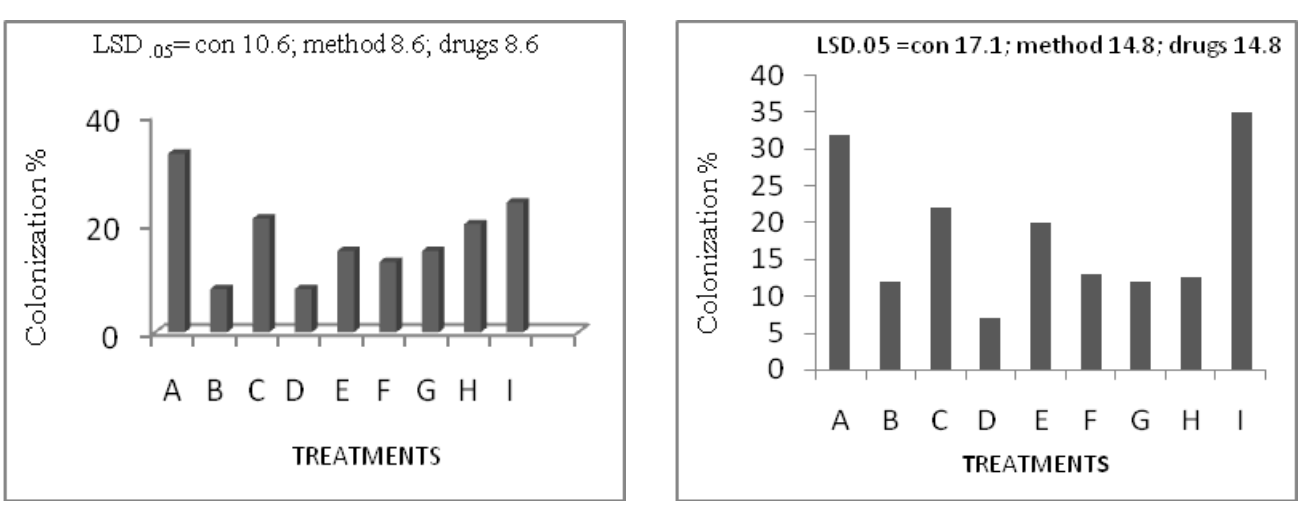
Wheat **Millet**



Macrophomina phaseolina



***Fusarium* species**



Rhizoctonia solani

Fig. 1. Effect of different concentrations of homeopathic drugs on the root infecting fungi of Wheat and Millet.
A= control, B= seed treatment with *T. occidentalis* 1000ppm,C= seed treatment with *T. occidentalis* 500ppm, D= seed treatment with *N. muriaticum* 1000ppm, E= seed treatment with *N. muriaticum* 500ppm, F= soil drenching with *T. occidentalis* 1000ppm, G= soil drenching with *T. occidentalis* 500ppm, H= soil drenching with *N. muriaticum* 1000ppm, I= soil drenching *N. muriaticum* 500ppm.

DISCUSSION

Seed treatment with *T. occidentalis* at 0.1% showed improvement in plant growth parameters like shoot length, shoot weight, root length, root weight of cereal plants. Root infecting fungi was greatly reduced by the use of homeopathic drugs as seed treatment. Both drugs were effective in reduction of *M. phaseolina, F. oxysporum, R. solani* on millet and wheat roots. Panda *et al*. (2013); Baumgartner *et al*. (2004) recorded same result in pea plant. They found that higher the concentration of drugs, stronger is the action. *N. muriaticum* improves plant acclimatization in the locations that are not suitable for growth and development, also helpful for plants that are under stress due to drought and frost. Results obtained by Lensi *et al.* (2010) confirmed that the addition of *N. muriaticum* to *Phaseolus vulgaris* L. population gave positive response and did not show any sign of toxicity in comparision to other treatments. Same result was recorded by Carvalho (2001), in *Tanacetum parthenium* plants considered to be healthy and increased proline content of leaves due to treatment with *N. muriaticum.*

Pathogens like root rotting fungi interfere with the normal absorption of water and nutrient uptakes by the roots, which directly affects the root functioning and subsequently affecting the rate of photosynthesis. Decreased or increased photosynthetic rate will directly affect the biosynthesis of chlorophyll. Some vascular pathogens inhibits root hair production, some alter the permeability of roots, some leads to the blockage of vascular tissues, all these processes results in inefficient water absorption and altered chlorophyll content (Agrios, 2005).

Present results showed that *M. phaseolina* and *F. oxysporum* growth were suppressed by using 0.1% concentration of *T. occidentalis* in agar disc diffusion and agar well methods. According to Gupta *et al.* (2012) methanolic extract of *T. occidentalis* significantly inhibits *Fusarium* sp., *Microsporium* sp., *Aspergillus* sp., and *Penicillium* sp. Our studies confirm the antifungal properties of the in hand homeopathic drugs at 0.1% concentration.

ACKNOWLEDGEMENTS

Authors are grateful to the Dean Faculty of Science, University of Karachi for providing financial support to carried out research work.

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(Received April 2015; Accepted June 2015)