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EFFECT OF WASTEWATER ON THE GROWTH OF PEA PLANT

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ABSTRACT

We investigated the effects of wastewater on the growth of pea (*Pisum sativum* L.). We isolated several fungal phytopathogens from wastewater samples by serial dilution techniques. There were six different treatments to assess the impact of wastewater as well as fungal phytopathogens on pea plants. After 21 days treatment, growth parameters and disease symptoms on pea plants were recorded. By physiochemical analysis, composition and nature of freshwater and wastewater samples was compared with Institute of Food and Agricultural Sciences irrigation water standards. We found that wastewater and fungal pathogens are toxic and lethal to pea plants at a considerable level, affecting growth. We suggest that wastewater contaminants not only affect the metabolism of plants but also it affects the soil properties negatively. Our analysis of waste water samples suggest that the quality of waste water nearly corresponded to given IFAS water standards used for irrigation excluding alkalinity.

Key-words: Waste water, pea plant, phytopathogenic fungi, physio-chemical analysis.

INTRODUCTION

Fresh water is a non-renewable resource consumed widely by human activities. Variant forms of water i.e. 70 % in agriculture, 20 % in industrialization and 10 % in domestic utilities have evoked fresh water scarcity (FAO, 2003).

In many developing countries including Pakistan, waste water is considered as an alternate resource to cover fresh water deficiency (Cisse, 1997; Parveen *et al*., 2012; Rehman *et al*., 2013). It is widely used for irrigation (Rattan *et al*., 2001). Waste water is domestic or industrial, but its quality relies on its discharge and form involving liberation of salts, trace elements organic and inorganic noxious chemicals, nitrate concretes, and microbial pathogens (Okoh *et al*., 2007). Effluents mixed in waste water contain NaCl salts which deposit in high amount causes salinity and soil logging when irrigated with waste water (Sheikh and Irshad, 1980).

Domestic waste water comprise of wastes from washing purpose, food preparation, laundry, body sanitary, plant food nutrients and other products. These waste water reservoirs are also become filled with unwanted additives of tanneries, textile plants, paints, mechanical workshops, electroplating units, pulp, pharmaceutical industries, cattle hair shaving, glass, chemical, paper boards, ceramics, petroleum, oxygen demanding and food industrial wastes(Beg, 1994; Abedi and Najafi, 2001). Domestic sewage water has trivial effects, as compared with enormously common pollutants discharged into Karachi rivers through industrial sewage water. In and around Karachi waste water is used to irrigate vegetables crops, however, it is not known if this water is contaminated with fungal pathogens and do they cause damage to the vegetable crop.

Garden Pea belongs to family Fabaceae is a vegetable considered as important legume next to soybean, ground nuts and beans (Hules, 1994). It is cultivated mainly as cool (Rabi) season crop in Pakistan and throughout the world. Pea crop occupies third position among the major grain legumes in Pakistan (Aslam *et al*., 2000; Kazmi *et al.*, 2002). Production and yield of peas are very low in Pakistan as compared to most of the countries of the world (Achakzai, 2012). We set out to isolate phytopathogenic fungi from wastewater. We also investigated the effect of these phytopathogenic fungi on the growth and development of pea plants.

MATERIALS AND METHODS

To investigate the effect of waste water on the growth and development of pea plants, we set up experiments in screenhouse at the Department of Botany, Federal Urdu University, Gulshan-e-Iqbal Karachi. Pea seeds were sown in the month of December to February, planted in 5.5 cm diameter clay pots containing soil at 400 g/pot mixed with manure. Thirty pots were used. Pea plants were grown upto flowering stage.

Waste water samples were collected from two different sites: one from Malir River near Korangi crossing and the second sample from Lyari River at Lyari express highway near Gulshan-e-Iqbal. Freshwater was used as control. Water serial dilution technique was performed in which 2 different concentrations of waste water samples were prepared (1/1000 & 1/10,000) to isolate fungal specimen from waste water samples and transferred onto petri plates containing Sabroud Dextrose Agar medium. Isolated fungi were identified using standard references (Ellis 1971; Barnett and Hunter, 1972).

We analyzed the water samples for the determination of pH, electric conductivity, total dissolved solids (TDS) and total suspended solids (TSS) and alkalinity. The pH of the three samples was determined by using Jenway 3510 pH Meter. Electric conductivity of the samples was determined by using Jenway 4510 Conductivity Meter. Three marked empty beakers along with filter papers were weighed using a balance. Each of the three samples were filtered through funnel and filtrates were collected in 3 separate beakers. Residues on filter papers were left to be air dryand the beaker containing waste water and fresh water samples were placed on hot plate for water evaporation. Then residues along with filter papers and beakers with evaporated samples were weighed. Total Dissolved Solids was calculated by using the following formula:

$$\textbf{TDS (in gm)} = \frac{\textbf{Dry weight of filtrate and beaker(A) – Dry weight of beaker(B)}}{\textbf{Volume of sample}} \times \mathbf{1000}$$

Total Suspended Solids was calculated by with the help of following formula:

$$\textbf{TSS (in gm)} = \frac{\textbf{Dry weight of residue with filter paper (A)– Dry weight of filter paper(B)}}{\textbf{Volume of sample}} \times \mathbf{1000}$$

Waste water and fresh water samples were titrated against 0.05 M of standard HCl using methyl orange as indicator. Color change from yellow to pink was considered as end point and concordant readings were used to evaluate alkalinity with the use of following formula:

$$\textbf{Alkalinity (mg/L as } CaCO_3\textbf{)} = \frac{(V_A - V_B) \times M \times 50000}{V_S}$$

Table 1.Physio-chemical parameters of water and wastewater.

Samples	Parameters	Unit	Results
Fresh Water	pH	-	7.5
Malir Wastewater			6.98
Lyari Wastewater			7.6
Fresh Water	Electric Conductivity	dS/m	0.769
Malir Wastewater			0.892
Lyari Wastewater			1.061
Fresh Water	TDS	mg/L (ppm)	200
Malir Wastewater			160
Lyari Wastewater			680
Fresh Water	TSS	mg/L (ppm)	280
Malir Wastewater			11128
Lyari Wastewater			1040
Fresh Water	Alkalinity	mg/L (ppm)	450
Malir Wastewater			2500
Lyari Wastewater			475

Table 2. Infection % of different water and waste water samples with and without inoculation of fungi.

Name of fungi	**A**	**B**	**C**	**D**	**E**	**F**
Aspergillus flavus	-	-	37.5	-	18.07	8
Aspergillus fumigatus	-	20	-	-	-	-
Aspergillus niger	-	40	41.6	-	-	18.07
Fusarium sp.	-	-	-	14	10	17.97
Macrophomina phaseolina	-	-	-	13.33	40	18.07
Rhizoctoni asolani	-	-	-	15	20	12.5

A= Control, **B**= Malir Wastewater, **C**=Lyari Wastewater, **D**=Fresh water with fungi inoculation, **E**=Malir Wastewater with fungi inoculation, **F**=Lyari Wastewater with fungi inoculation.

- = no colonies.

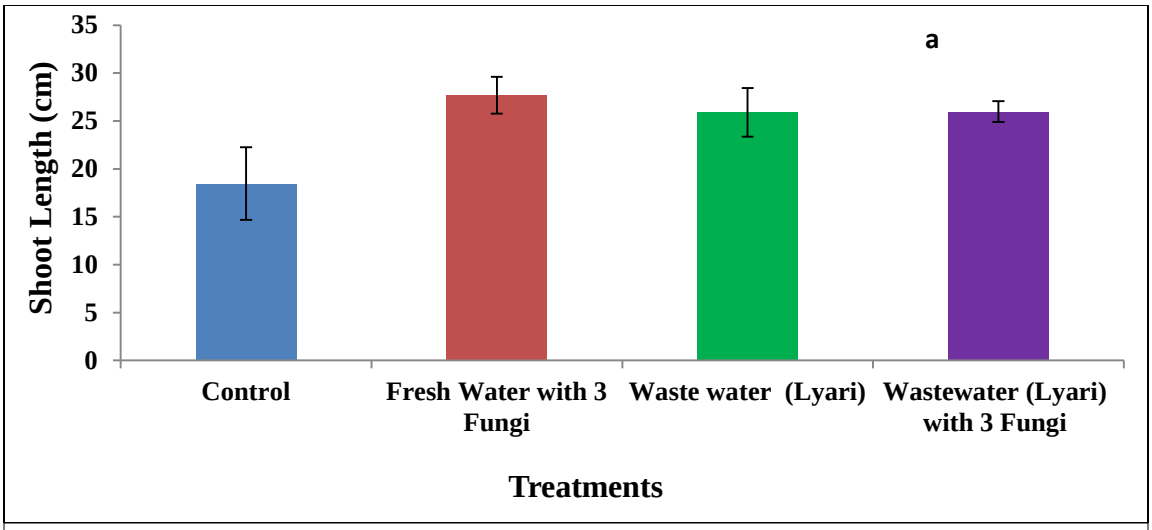
Pure cultures of pathogenic fungi, *Fusarium* sp., *M. phaseolina* and *R. solani* were inoculated near roots in pre-grown pea plants for treatment purpose. Inoculation suspension dose of each fungi was 10 ml. The objective of fungal inoculation was to observe the combine effect of artificial inoculated plant pathogenic fungi with fungal phytopathogens in waste water. There were six distinctive treatments and each treatment consisted of five replicates. Treatments were conducted for 21 days in which 150 ml fresh and wastewaters were irrigated to each replicate. During treatment, various growth parameters including shoot length, number of leaves, symptoms induced by waste water and symptoms induced by inoculated pathogenic fungi were recorded. Plants were harvested after 21st day treatment and final data were collected on root length, shoot and root dry weight, number of pea pods and dry weight of pea pods, infection % of phytopathogenic fungi were determined. Data collected during and after treatment was further assessed for statistical analysis of variance (ANOVA). Analysis comprised of factorial analysis (F-test), Least Significant Difference (LSD) and Duncan Multiple Range Test (DMRT) to equate significant difference among means of treatments (Gomez and Gomez, 1984).

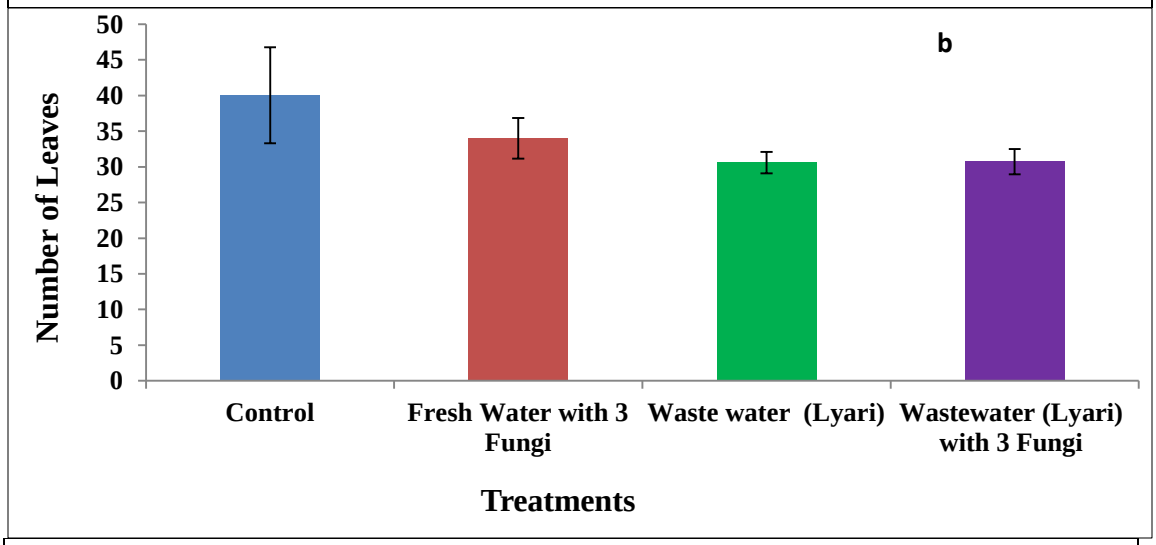
RESULTS AND DISCUSSION

We isolated fungal phytopathogens viz., *Penicillium* sp., *Aspergillus flavus, Aspergillus niger and A. fumigatus* from waste water samples. Certain physio-chemical tests were performed to evaluate either the parameters of water and wastewater samples coincided or elevated from Institute of Food and Agricultural Sciences (IFAS) irrigation water standards as shown in Table 1.These standards were used for irrigation water because no specific standard values exist for waste water usage for irrigation purpose in Pakistan (WHO, 2006).The pH of all three samples were 7.5 (basic), 6.98 (acidic) and 7.6 (basic). The given range of IFAS standard level was 6.5- 8.0 showing fresh values of pH of fresh water, Malir waste water and Lyari waste water did not seem to cause any damaging effect on plants. Duncan *et al*. (2009) reported that pH levels between 6.5- 8.5 of any water samples are suitable for watering purpose of crops. Electric conductivity denoted in dS/m, vary according to Institute of Food and Agricultural Sciences (IFAS standards). Fresh water which exhibited very low level of conductivity (0.769 dS/m) produced few detrimental results. Low values were demonstrated by Malir (0.892 dS/m) and Lyari waste water (1.061 dS/m) samples reported by Lauchli and Luttge (2002). These waste water samples showed low salinity which indicated slight brackish properties (Toor and Lusk, 2011). If salinity in water and waste water are not controlled, it may result in low productivity or yield (Alobaidy *et al*., 2010). We found total dissolved solids (TDS) of fresh water weight as 200 mg/L (ppm) and for Malir waste water, it was 160 mg/L (ppm) which produced very low amount of salts and small harmful effects. However, Lyari waste water contained 680 mg/L (ppm), taken as low amount of salts that is suitable for irrigation according to IFAS irrigation water standards. Low amount of total solids may cause water stress in pea plants which leads to plant injury and also creates toxic accumulation in soil (Lauchli and Luttge, 2002).We also noted 280 mg/L (ppm) total suspended solids (TSS) in fresh water, 11128 mg/L (ppm) in Malir waste water, and 1040 mg/L (ppm) in Lyari waste water. No data of TSS is available in Institute of Food and Agricultural Sciences (IFAS) standards. In present study, alkalinity was measured to be 450 mg/L (ppm) in fresh water, 2500 mg/L (ppm) in Malir waste water and 475 mg/L (ppm) in Lyari waste water. We found much higher alkaline levels in all samples than reported in Institute of Food and Agricultural Sciences (IFAS) standards. Alkalinity represents concentration dependency; more alkalinity in irrigation water indicates more acid consumption to mitigate the pH to required limit producing opposition in soil pH levels.

Table 3. ANOVA of different growth parameters and its symptoms during and after treatment.

Growth Parameters And Symptoms Noted During Treatment															
Days	Length of Shoot			Number of Leaves			Chloroxis			Necrosis			Wilting		
	F-value	P-value	LSD 0.05	F-value	P-value	LSD 0.05	F-value	P-value	LSD 0.05	F-value	P-value	LSD 0.05	F-value	P-value	LSD 0.05
Before Inoculation															
1st Day	6.01	0.0010***	6.91	5.33	0.0020**	6.62	-	-	-	-	-	-	-	-	-
After inoculation															
3rd Day	9.61	0.0000***	6.94	11.94	0.0000***	7.17	28.12	0.0000***	3.84	23.88	0.0000***	4.1	20.87	0.0000***	4.33
6th Day	3.49	0.0403*	7.22	3.37	0.0446*	9.03	3.43	0.0423*	2.2	4.29	0.0211*	2.17	8.44	0.0014**	3.73
9th Day	3.24	0.0499*	6.89	5.23	0.0105*	8.3	5.47	0.0088**	2.11	3.52	0.0392*	2.41	8.28	0.0015**	5.62
12th Day	3.78	0.0315*	6.79	0.38	0.7668ns	9.5	7.06	0.0031**	2.59	7.52	0.0023**	2.32	14.5	0.0001***	5.33
15th Day	3.64	0.0355*	6.73	0.27	0.8461ns	12.17	0.89	0.4672ns	1.48	6.69	0.0039**	2.2	8.72	0.0012**	7.86
18th Day	3.78	0.0317*	6.73	0.13	0.9393ns	13.24	3.65	0.0353*	1.72	5.48	0.0087**	6.36	32.91	0.0000***	5.73
21th Day	0.92	0.4514ns	11.1	1.19	0.3440ns	15.11	4.73	0.0150*	1.48	4.37	0.0198*	5.4	14.44	0.0001***	7.49
Growth Parameters Noted After Treatment															
Growth parameters	Root Length			Root Dry Weight			Shoot Dry Weight			Number of Pea Pods			Dry Weight of Pea Pods		
	F-value	P-value	LSD 0.05	F-value	P-value	LSD 0.05	F-value	P-value	LSD 0.05	F-value	P-value	LSD 0.05	F-value	P-value	LSD 0.05
	2.14	0.0948ns	2.59	8.37	0.0001***	0.04	16.3	0.0000***	0.04	3.2	0.0517ns	0.47	2.12	0.1367ns	0.1


a
Shoot Length (cm)
35
30
25
20
15
10
5
0
Control
Fresh Water with 3 Fungi
Waste water (Lyari)
Wastewater (Lyari) with 3 Fungi
Treatments


b
Number of Leaves
50
45
40
35
30
25
20
15
10
5
0
Control
Fresh Water with 3 Fungi
Waste water (Lyari)
Wastewater (Lyari) with 3 Fungi
Treatments

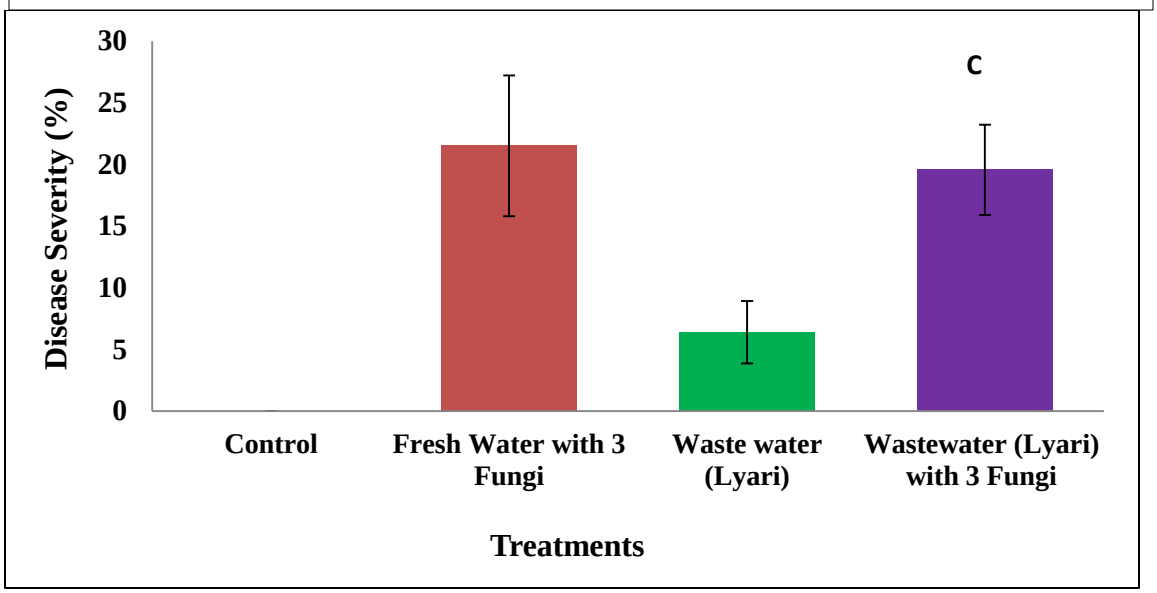
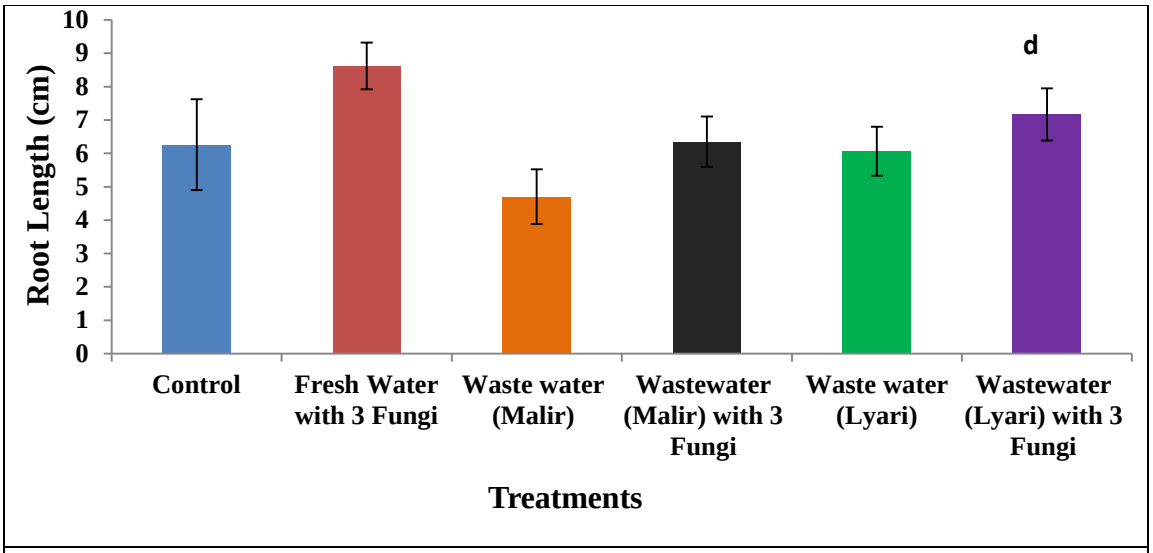
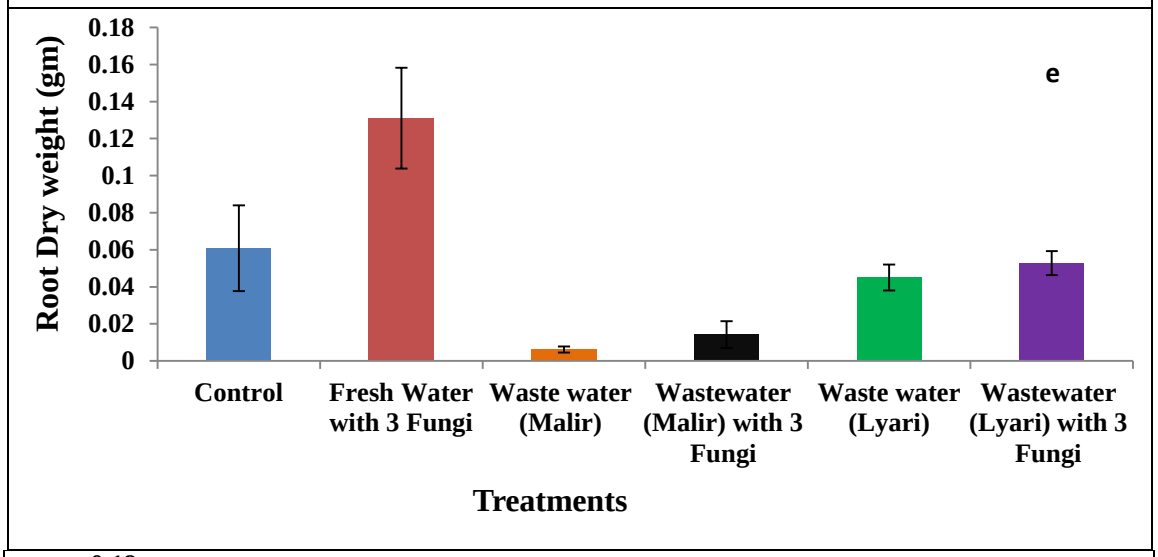

C
Disease Severity (%)
30
25
20
15
10
5
0
Control
Fresh Water with 3 Fungi
Waste water (Lyari)
Wastewater (Lyari) with 3 Fungi
Treatments

Fig.1. Con'd…


Root Length (cm)
10
9
8
7
6
5
4
3
2
1
0
d
Control
Fresh Water with 3 Fungi
Waste water (Malir)
Wastewater (Malir) with 3 Fungi
Waste water (Lyari)
Wastewater (Lyari) with 3 Fungi
Treatments


Root Dry weight (gm)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
e
Control
Fresh Water with 3 Fungi
Waste water (Malir)
Wastewater (Malir) with 3 Fungi
Waste water (Lyari)
Wastewater (Lyari) with 3 Fungi
Treatments

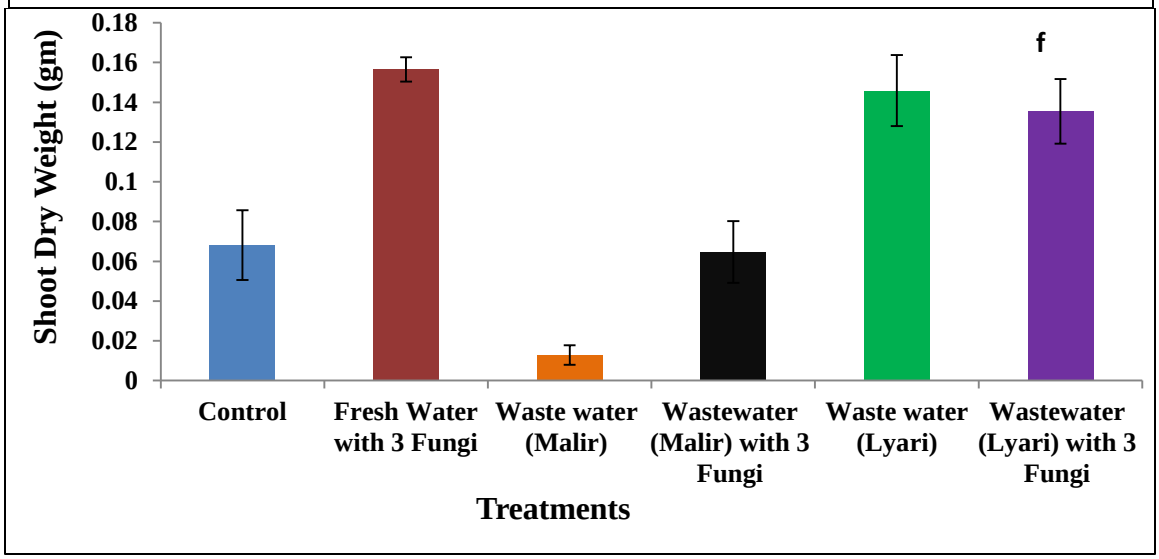

Shoot Dry Weight (gm)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
f
Control
Fresh Water with 3 Fungi
Waste water (Malir)
Wastewater (Malir) with 3 Fungi
Waste water (Lyari)
Wastewater (Lyari) with 3 Fungi
Treatments

Fig.1. Con'd…

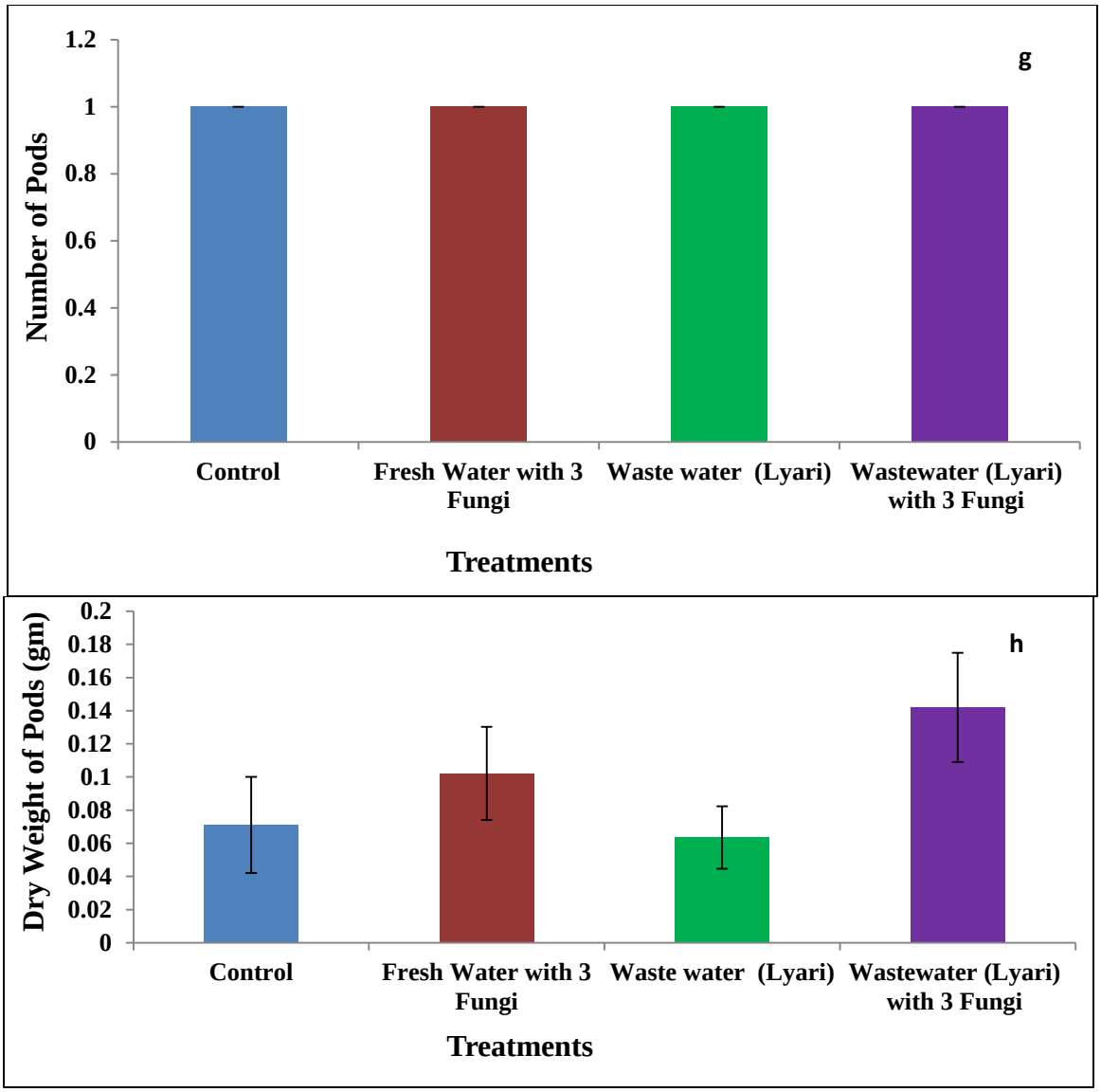


Fig.1. a to h. shows effect of different treatments on growth parameters on 21st day after inoculation. Bars show mean ± SE.

Infection % after treatments on pea plant samples are shown in table 2. In fresh water samples, fungal phytopathogens were absent, however, Fresh water irrigated pots inoculated with fungi showed various levels of fungal infection on pea plants. *Rhizoctonia solani* showed maximum infection (15%), whereas *Macrophomina phaseolina* showed minimum (13.33%) in fresh water and fungi inoculated samples. In Malir wastewater samples, *Aspergillus niger* and *Macrophomina phaseolina*, both represented maximum 40% infection rate, whereas *Aspergillus fumigates* showed minimum (20%). Conversely in Malir wastewater irrigated pots inoculated with fungi were analyzed, *Macrophomina phaseolina* showed maximum (40 %) infection. Minimum (10%) infection was caused by *Fusarium* sp. In Lyari wastewater samples *Aspergillus niger* showed maximum (41.6 %) infection and *Aspergillus flavus* showed minimum (37.5 %) infection. In contrast, Lyari wastewater sample with fungal inoculation demonstrated *Aspergillus niger* and *Macrophomina phaseolina* maximum (18.07 %) infection and *Aspergillus flavus* showed minimum (8%) infection.

Macrophomina phaseolina (Tassi) Goid. causes root rot, stem rot and pod rot on 500 hosts of the fungus containing several cereal plants and legumes was reported by Dhingra and Chagas (1981) and Sinclair (1982). Symptoms of rot in pea pods infected with *M. phaseolina* has been observed. *M. phaseolina* are known as high temperature pathogen because the disease caused by these fungi spread more severely and rapidly under dry

conditions and high temperature (Grover and Sakhuja, 1981). We have also noted a similar pattern of infection in present study.

Aspergillus species are found in compost, decaying plant matter, stored grains and in soils of warmer climates. For instance, *Aspergillus niger* is widely distributed in soil, its spores are often associated with organic materials and soil. *Aspergillus niger* is also causative agent for many rot diseases in plants (Sharma, 2012). It has been observed in a broad range of habitats because itcan colonize a wide variety of substrates (Perfect *et al*., 2009; Perrone *et al.*, 2007).We found *A.niger* in waste water samples causing rot disease in Pea. We also isolated other fungal phytopathogens which were *A. fumigatus, A. flavus*, *Rhizoctonia solani* and *Fusarium* sp. from waste water and fresh water inoculated and uninoculated samples.

Rhizoctonia solani Kühn is a soil-borne pathogenic fungus with vast host range; causes rot root diseases and damping off seedling in a variety of crops, ornamentals and trees (Naz *et al.,* 2008). We also found rotting in roots of pea plants infected with these fungi isolated from fresh water inoculated and waste water (Malir and Lyari) inoculated samples.

The genus *Fusarium* includes many species that cause plant diseases, such as vascular wilts, root, stalk and cob rots, collar rot of seedlings, and rots of tubers, bulbs and corns. For example, *Fusarium oxysporum* (formaespeciales) is an important plant pathogen, which causes vascular wilt diseases and some root rots. This effect of *Fusarium* was also examined in this experiment, which results in death of pea plants in inoculated fresh and waste water samples.

Table 3 shows ANOVA values of various growth parameters and symptoms caused by fungal pathogens during and after treatment. Length of shoot growth from 1^{st} to 18^{th} day treatments showing highly significant to significant P values followed by sudden death of pea plants on 21^{st} day of treatment induced by fungal pathogens and waste water detrimental effects denoting non-significant P values.Number of leaves were increased from 1^{st} day to 9^{th} day of treatments presented highly significant to significant P values. Decrease in number of leaves were noted on 12^{th} to 21^{st} day treatments due to sudden death of plants by waste water exposure and fungal infection indicating non-significant P values. Symptoms such as chlorosis and necrosis caused by fungal plant pathogens affected pea plants resulting in highly significant to significant P values due to disease infection severity. Wilting showed highly significant P values from treatments (1^{st} day to 18^{th} day) affecting pea plants which lead to death of pea plant samples on 21^{st}day. Root Length represented non-significant effect after treatment due to root rot caused by inoculated and waste water isolated fungal pathogens. Root and shoot dry weights were severely infected by waste water and inoculated fungal plant pathogens demonstrating highly significant effect after treatment.Number and dry weight of pea pods were unaffected after treatment presenting non-significant P value. Waste water samples produced positive effect of growth at start of treatments but caused negative influence in combination with fungal phytopathogens due to high rate of infection resulting in death of pea plants.It is evident that Malir waste water sample severely affected pea plants whereas Lyari waste water sample responded to growth up to a certain limit as compared with fresh water. Physio-chemical parameters signifies that waste water samples nearly correspond with Institute of Food and Agricultural Sciences (IFAS) irrigation water standards because no specific standard limits are promulgated by World Health Organization (WHO) for waste water reuse in irrigation which can be followed in Pakistan. Treatments affect pea plants in both significant and non-significant manner. If these waste water samples will be further used for irrigation in future, drastic changes in soil properties will occur leading to reduced yield and accumulation of unwanted additives which results in decomposition of soil nutrients for further cultivation. More research studies on waste water adequate treatment, standards formation of these waste water samples must be required for their use as marginal quality water.

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