

E-ISSN No: 2789-3545
PRINT-ISSN No: 2789-3537



GU J. Phytosci.



GU JOURNAL OF PHYTOSCIENCES

A Quarterly Peer-Reviewed Journal

Volume: 1 | Issue : 2 | October 2021

Department of Botany
Ghazi University, City Campus
Dera Ghazi Khan- 32200
Punjab, Pakistan

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Published by the Ghazi University and Ghazi University Botanical Society, Dera Ghazi Khan.

Printed at Ghazi University Press, Dera Ghazi Khan-32200, Pakistan. Phone: +92 64 9260135

Copies available from:

Editor-in-Chief,
GU Journal of Phytoscience,
Ghazi University Botanical Society,
Ghazi University Press, Dera Ghazi Khan-32200, Pakistan
Phone: +92 344 2743495

Price: Each issue Rs. 500/ US\$30; Vol. Rs. 3000/US\$ 200 or in exchange

Annual Subscription Fees: Rs. 2500

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Proximate Analysis of Nutritional Composition of Antifungal and Antinematicidal Activities of Different Seaweeds from the Coast of Karachi-Pakistan

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Abstract

Seaweeds inhabit almost everywhere and known as the omnipresent organisms. They are renewable living resources which are also used as feed, wastewater treatment or for the industrial production of phycocolloids, thickening, and gelling agents in food and pharmaceutical industries. This research article is based on chemical composition and nutritional values from the coast of Karachi, Pakistan using sixteen seaweeds belonging to three different Phylum including Chlorophyta, phaeophyta and Rhodophyta. The oil was extracted with n-hexane then it was subjected for physical, chemical and biochemical composition of different marine algae by means of some of the known tests, like tests for lipids, carbohydrates, protein etc. After that the residue of these seaweeds were examine *in vitro* condition for antifungal activities using food poisoning technique against three pathogenic fungi e.g., *F. oxysporum*, *R. solani* and *M. phaseolina* and for nematocidal activities using mortality and hatching test against *M. javanica* species and conclusion, got highly significant results. This study has revealed an interesting array to create a nutritional data to the alternation of an efficient food for Pakistan food industry and envisage pesticides invention for agricultural department.

Keywords: Antifungal; Nematicidal; Nutritional Values; Seaweeds

1. Introduction:

Nutritionally valuable seaweeds have been widely used for human consumption in all over the world in fresh and dried food or as ingredients in wide variety of prepared foods (Ambreen *et al.*, 2012). Availability of approximately 841 species of marine algae was recorded by (Oza & Zaidi, 2000). From nutritional point of view these vast varieties of seaweeds have low calorie food, with high concentration of minerals (Mg, Ca, P, K and I), to

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possess useful untapped biochemical compounds which are great source of indigestible carbohydrates, carotenoid, enzymes, vitamins, proteins, low concentration of lipids, polysaccharides, sterols, antibiotics and many other fine chemicals (Chapman *et al.*, 1980; Fenical, 1982; Colwell, 1983; Stein & Borden, 1984; Qasim, 1991; Jiménez-Escrig & Cambrodon, 1999; Prabha *et al.* 2013). The majority of these compounds are terpenes and polyphenols (Blunt *et al.*, 2006), but chemical composition of these seaweeds varies, which is dependent on the type of species, habitat, time of collection, external conditions such as temperature, light and nutrient concentration in water (Mabeau & Fleurence, 1993; Marinhos *et al.*, 2006). Macro algae can be classified as brown algae (Phaeophycota), green algae (Chlorophycota) or red algae (Rhodophycota). Seaweed mineral content is higher than that of land plants and animals (Ito and Hori, 1989; Ortega-Calvo *et al.*, 1993). Due to the presence of rich beneficial nutrients red and brown seaweeds are mainly used as human food sources in many countries such as China, Japan as raw materials in the manufacture of many seaweed food products, such as jam, cheese, wine, tea, soup and noodles and pharmaceutical uses in the western countries, and Korea even in Asia they have been consumed since ancient time, furthermore they have been commonly utilized in human alimentation. At present, the demand of food supplements from seaweeds has increased in Europe, North America, and South America (Manivannan *et al.*, 2009). Thus, they have been recognized as being beneficial for human and animal health (Fleurence, 1999).

These aquatic habitats have been prolific producers of biologically active compounds (Chanthini *et al.*, 2012; Peres *et al.*, 2012) thus; produce chemically active metabolites in their surroundings. The discovery of metabolites with biological activity from algae increased substantially in the last three decades (Smit, 2004). These active metabolites also known as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, and terpenoids are produced by several species; resultant seaweeds evolved chemical defense mechanisms against their predators and enhanced their survival (Carte, 1996).

1.1. Objectives:

- To explore the nutritional and chemical values of seaweeds from the coast of Karachi.
- To determine the antifungal and antinematicidal activities of collected seaweeds.
- To examine the antifungal activities *in vitro* conditions using food poisoning and for antinematicidal using mortality and hatching test.

2. Materials and Methods:

2.1. Collection of Seaweeds:

Sixteen seaweeds were collected from diverse coastal areas of Pakistan including Buleji (Latitude: 24°50'20" and Longitude: 66°49'31"), Hawksbay (Latitude: 24°51'16.8" Longitude: 66°51'36.17") Beach and Manora (Latitude: 24.80579, Longitude: 66.95721). Seaweed species exposed on sand, rocks and along the waves in floating, were collected in sterilized plastic bags containing water to prevent evaporation, put into ice box and transferred to laboratory immediately. Fresh seaweeds were rinsed with tap water and polished to remove extraneous (any associated epiphytes, salt, sand, microorganisms) and other suspended materials. Samples were washed thoroughly with seawater as well as surface sterilized with 1% sodium hypochlorite (NaOCl). The samples were shade dried for ten days than oven dried at 40±2°C. These dried samples were ground in an electric mixture into powder, stored in polythene bag at room temperature until use.

2.2. Chemical and Biochemical Analysis

2.2.1. Extraction of Oil Content:

The sample of seaweed (10 gm) was soaked in organic solvent *n*-hexane (100 ml) in an air tied container for 3 days at room temperature. After 3 days distilled water (100 ml) was added, in seaweed *n*-hexane extract, shaken the mixture vigorously and left for few minutes until all residue settled down. Firstly, separate *n*-hexane layer and then water layer in a separated beaker. It is observed that *n*-hexane extract was green in color and water extract was brown. *n*-hexane extract was placed on water bath for evaporation and when it was concentrated collect in a vial and used for the determination of different tests.

2.2.2. Saponification Value:

This test is specially used for the determination of oily contents present in seaweed extract. Acid base titration method was used to find out the oily content of SWOS sample. SWOS sample (0.1 gm) was taken in flask 'A' (100 ml) and KOH (20 %, 20 ml) while in flask 'B' add only KOH (20 %, 20 ml) was added it is used as reference. In addition, shake the contents of both flasks, attached air condenser on each flask and placed on water (30 minutes). The reaction mixture had cooled them at room temperature then add phenolphthalein indicator (3 drops) to both the flasks and titrated against oxalic acid until the reaction mixture was decolorized. Saponification value was calculated by this formula

$$\text{Saponification No.} = \frac{[(B - A)N \times 56.1]}{\text{Wt. of sample (1 g)}}$$

where: A= HCl required for titration of the sample in ml

B= HCl required for titration of the blank in ml

N= Normality of the HCl

2.2.3. Acid Strength:

The acid strength of distilled water and SWOS sample was recorded by taking 10 ml of sample in conical flask and NaOH (0.2N) in burette. The normality of distilled water and SWOS sample was calculated by acid-base titration method by using the formula

$$N_1V_1 = N_2V_2$$

2.2.4. Acid Value:

This test is specially used for the determination of amount of free acids contents present in seaweed extract. SWOS sample (0.1 gm) was taken in a conical flask 'A' (250 ml), then added n-hexane solution (25 ml). The reaction mixture was shake gently until all oily contents dissolved in it. Furthermore, add phenolphthalein (2 drops) and titrated against standardized NaOH. The pink color was observed at the end point. Measure the volume of sodium hydroxide titrant used and calculate the acid value according to the following equation

$$\text{Acid Value} = (\text{ml sample} - \text{ml blank}) \times N \text{ NaOH} \times 56.1 \text{ g sample}$$

2.2.5. Cumber Assay:

Dipped the comber strip in seaweeds water extract (SWS, 10 ml) and matched the comber colour changing strips with stander chart. The Comber Test of water samples of seaweeds (SWS) was performed for the determination of carbohydrates, proteins and specific pH.

2.2.6. Molisch's Test:

Carbohydrate present in Seaweed Water Solution (SWS) was determined by Molisch's Test. In a test tube, added test solution (2 ml) and α -naphthol (2 drops) solution then carefully incline the tube and gently added dropwise conc. H_2SO_4 , using a dropper, along the sides of the tube noticed the violet coloration at the junction of the two layers for the presence of carbohydrates.

2.2.7. Biuret Assay:

To put to the presence of protein performed that test in this method, added 10 ml 5% NaOH, few drops of $CuSO_4$ in a test tube then shake vigorously, the solution turns from blue to violet (purple), separated into two equal parts and marked A & B. Test tube A: Add 5 ml seaweeds water solution (SWS) and in Test tube B: Add 5 ml (distilled water), noticeable blue coloration turns from to pink.

2.3. Isolation of Soil-borne fungi:

Fusarium oxysporum, *Macrophomina phaseolina* and *Rhizoctonia solani* were isolated from the roots of brinjal plants obtained from Department of Botany, Federal Urdu University of Arts, Science & Technology, Karachi. The infected roots were surface sterilized with 1% bleach for 2 minutes and inoculated on Potato Dextrose Agar (PDA) media containing Petri plates. The fungi were incubated at 28 ± 2 °C for five days.

2.4. Isolation of Soil-borne fungi:

The dried sample (16g) was extracted in each solvents 100 ml methanol and aqueous at 28°C. After 24 hours, extract was filtered by Whatman filter paper no 1. The methanol and aqueous extract of seaweeds were tested against soil-borne fungi by poison food technique against different fungi including in lab conditions, where seaweeds extract at 2.2ml of each methanol and water stock solution (16% concentration) was added in PDA pour sterilized Petri plates. For antifungal activity, 5 mm disc of actively growing culture of test fungi *F. oxysporum*, *M. phaseolina* and *R. solani* were placed at the center of Petri plates. Each treatment was replicated three times and Petri plates were incubated at 28 ± 2 °C. After five days of inoculation, radial growth of mycelium was measured and compared with the results of control (Hussain et al., 2015). The following formula of percent inhibition was applied for each fungus in treatment.

$$\text{Percent Inhibition} = \frac{Y - Z}{Y} \times 100$$

Where Y = Mycelial growth of pathogen alone (control), Z = Mycelial growth of pathogen along with antagonist.

2.5. Extraction of water and methanolic bioactives:

For organic extract, seaweed biomass was homogenized with water and methanolic solvents at 28°C. After 24 hours the mixture was separated for stock solution by filtration using Whatman filter paper.

2.6. Hatching Test:

A suspension of eggs containing 30-35 eggs /ml was prepared from fresh roots of brinjal plant. For water extract, 1 ml of test extract and 1 ml eggs suspension were prepared. However, for methanolic extract, 2 ml methanolic extract (about 1ml for evaporation in vacuum chamber) were prepared in lab. After complete process of evaporation, 1 ml egg suspension was transferred in glass cavity block, diam., 2.5 cm and kept at room temperature. Each treatment was replicated 3 times and the glass cavity block containing 1 ml egg suspension for water extract and 2 ml methanol with egg suspension was served as positive control and without vehicle is negative control (Manilal et al., 2009). The number of hatched eggs was counted under a low power stereoscopic microscope (x6) after 24, 48, 72-hour exposure time.

2.7. Mortality Test:

Eggs of *M. javanica* were placed in distilled water and incubate at room temperature (25 ± 2 °C) for 24 hours. A suspension of freshly hatched juveniles in distilled water containing (30-35 juveniles/ ml) was prepared. For water extract: 1 ml of test extract and 1 ml juveniles suspension while for methanolic extract: 2 ml methanolic extract (about 1ml for evaporation in vacuum chamber) after complete evaporation 1ml juveniles suspension was transferred in glass cavity block, diam., 2.5 cm and kept at room temperature. Each treatment was replicated 3 times and the glass cavity block containing 1 ml juvenile's suspension for water extract and 2 ml methanol (after evaporation) with juvenile's suspension or one egg mass was served as positive control and without vehicle is negative control. After 24-, 48- and 72-hour exposure, the number of killed juveniles was counted under a low power stereoscopic microscope (x6) (Cayrol et al., 1989).

2.8. Analysis of Data:

Data were also analyzed and subjected to analysis of variance (ANOVA) depending upon the experimental design according to Gomez & Gomez (1984). The follow up of FANOVA included Least Significant Difference (LSD).

Duncan’s multiple range tests were also used to compare the treatment means. Analysis of variance (ANOVA) or factorial analysis of Variance (FANOVA) was followed by standard error of the difference between means (SED). Cluster analysis and principal component analysis were carried out using the software program PC-ORD Version 5.10 (McCune & Mefford, 2005).

3. Results:

In present results, acid value of different seaweeds were detected and observed. Out of all species of seaweeds, *S. polypodiodes* and *U. rigida* showed 0.4 and 0.3 mg/g respectively highest acid values as compared to all other species. However, when iodine value of different seaweeds were observed. The maximum results were found in *V. sp.*, *D. dicotoma* and *M. afaqhusinii* with 39.5, 37.2 and 37 values in mg/g as compare to others seaweeds species, respectively. The pH of different seaweeds were tested. Maximum pH value 9 was recorded in *C.sp.*, *J. laminarioides*, *J. adhaerens*, *G. corticata* and *H. musiformis* as compared to all other marine seaweeds. In saponification test, almost all species of seaweeds showed different saponification strength. Maximum value were recorded in *I. stellata*= 34.5, *C. sinuosa*=33.7, *P. tetrastromatica*=32.3 and *C. racemosa*= 31.6 respectively as compared to control and other all seaweeds (Fig. 1).

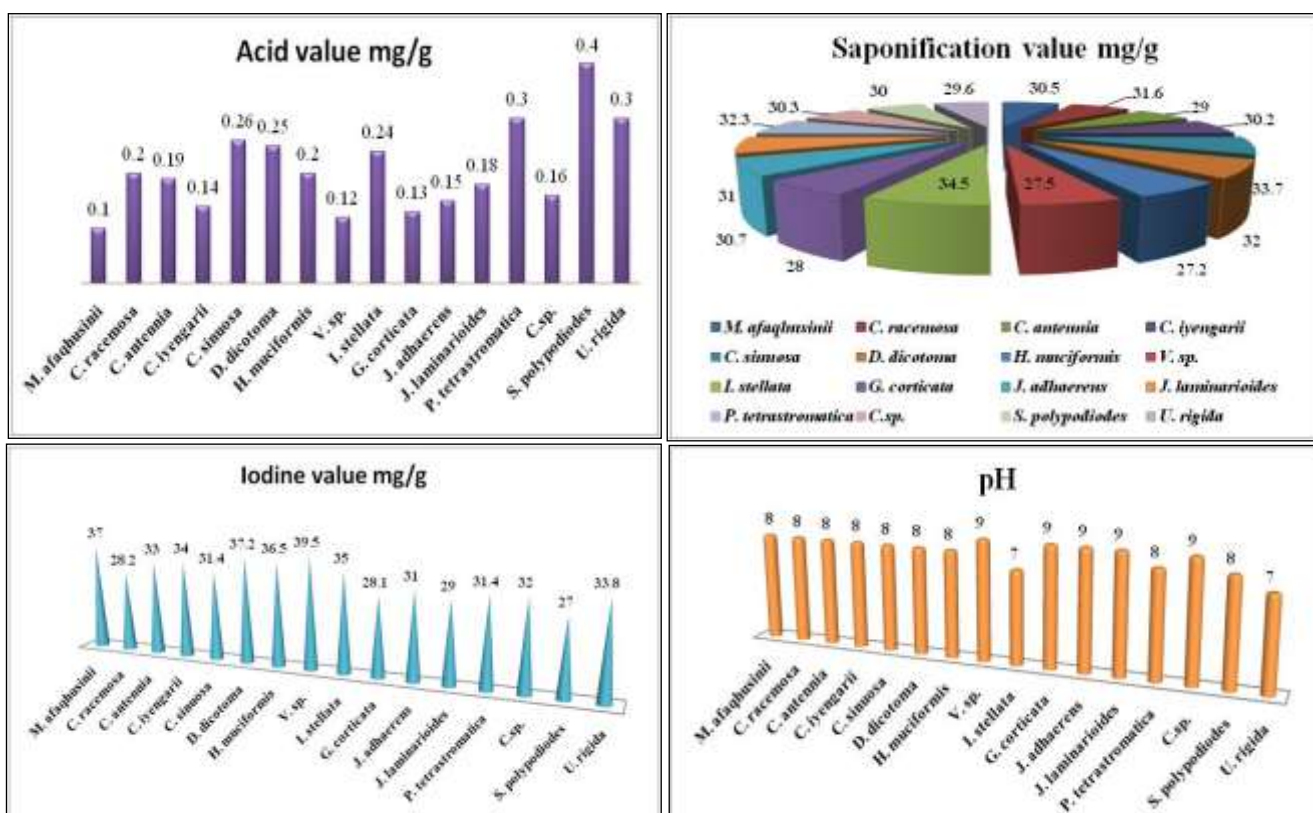


Fig.1: showing acid, Iodine, Saponification (mg/g) and pH values of different seaweeds

3.1. Mortality Test

Approximately all extracts of seaweeds showed nematocidal activity against root-knot nematodes. However, the extract of *M. afaqhusinii*, *D. dicotoma*, *I. stellata* and *J. laminariode* exhibited maximum nematocidal activity (mortality %) as compared to other plant extracts during 72 hours of exposure (Fig. 2).

3.2. Hatching Test

In hatching test, a decrease was recorded in egg hatching with increase in the concentrations of different plant extracts. The eggs were treated to each seaweed extract for 72 hours. *D. dicotoma* extracts showed highly mortality rates with mean of 91 after 72 hours of exposure (P<0.05). However, Methanol extract of seaweeds

showed aggressive results. Overall results indicated that extract of *D. dicotoma* and *M. afaqhusinii* with mean 94 and extract of these seaweeds were more toxic as compared to other plant extracts (Fig. 2). Inhibition of egg hatching, and larval mortality were reduced with increase in dilution of all plants extract. Egg hatching increased corresponding to an increased-to-increased time of exposure.

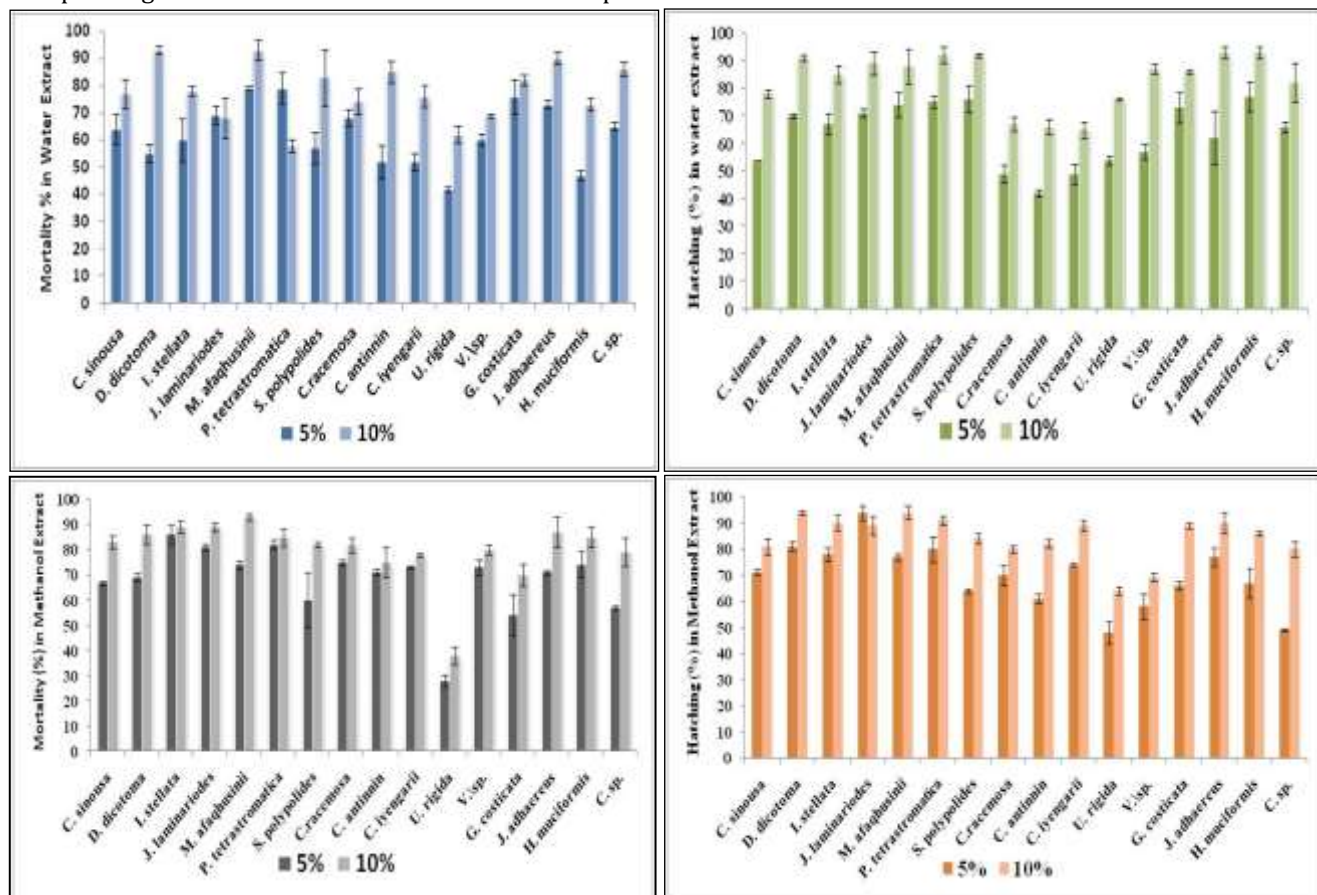


Fig. 2. showing mortality and hatching (%) of different seaweeds in water and methanol extract.

3.3. Antifungal Activity

Three fungi *R. solani*, *M. phaseolina* and *F. oxysporum* were tested against the water extracts of sixteen selected seaweeds which have antifungal activities (Table 1). Maximum inhibitory activities were recorded in *F. oxysporum* against all extracts of selected seaweeds.

Table1: Mean and Standard Error of different seaweeds against soil-borne fungi in water and methanol extract.

| Name of Seaweeds | Soil-borne fungi | | |
|----------------------------------|----------------------|----------------------|------------------|
| | <i>F. oxysporum</i> | <i>M. phaseolina</i> | <i>R. solani</i> |
| | Water extract | | |
| <i>Caulerpa racemosa</i> | 2.8±0.11 | 3.3±0.05 | - |
| <i>Centroceras sp.</i> | 4±0 | 5±0 | - |
| <i>Chaetomorpha antinnin</i> | 1.9±0.11 | 2.9±0.05 | 4.9±0.05 |
| <i>Codium iyengarii</i> | 1.5±0 | 5±0.57 | 4.8±0.11 |
| <i>Colpomenia sinoua</i> | - | - | - |
| <i>Dictyota dicotoma</i> | 4.1±0.05 | 4.1±0.11 | - |
| <i>Gracilaria costicata</i> | 1.3±0.17 | 2.9±0.25 | 6±0.57 |
| <i>Hypnea muciformis</i> | 2.33±0.17 | 3.6±0.11 | 5.1±0.05 |
| <i>Iyengaria stellata</i> | - | - | - |
| <i>Jania adhaereus</i> | 3.33±0.72 | - | - |
| <i>Jolya laminariodes</i> | 4.3±0.17 | 4.53±0.17 | - |
| <i>Melanothamnus afaqhusinii</i> | 3.4±0.23 | 5.2±0.11 | - |

| | | | |
|-----------------------------------|----------|-----------|-----------|
| <i>Padina tetrastromatica</i> | 1.3±0.17 | 4±0.11 | 4±0.28 |
| <i>Steochospermum polypolides</i> | 3.9±0 | 3.3±0.17 | 3.3±0.23 |
| <i>Ulva rigida</i> | 2.3±0.17 | 4.53±0.17 | 6.13±0.17 |
| <i>Valaniopsis sp.</i> | 4±1.15 | 3.2±0.11 | 3.5±0 |
| Methanol extract | | | |
| <i>Caulerpa racemosa</i> | 1±0.11 | 3±0.57 | - |
| <i>Centroceras sp.</i> | 4.3±0.17 | 5±0.28 | 5±0.57 |
| <i>Chaetomorpha antinnin</i> | 1.3±0.11 | 5.2±0.20 | 4±0.23 |
| <i>Codium iyengaraii</i> | 1±0.11 | 3.2±0.11 | 4±0.57 |
| <i>Colpomenia sinouosa</i> | - | - | - |
| <i>Dictyota dicotoma</i> | 3.1±0.05 | 4±1.15 | - |
| <i>Gracilaria costicata</i> | 1.4±0.23 | 1±0.11 | 4±0.57 |
| <i>Hypnea muciformis</i> | 4.6±0.34 | 3±0.28 | 4.8±0.26 |
| <i>Iyengaria stellata</i> | - | - | - |
| <i>Jania adhaereus</i> | 3.9±0.17 | 3.33±0.33 | 4±0.57 |
| <i>Jolyna laminariodes</i> | 4±0 | 3.9±0.11 | - |
| <i>Melanothamnus afaqhusinii</i> | 2.9±0.05 | 5±0.57 | 5±1.15 |
| <i>Padina tetrastromatica</i> | 0.3±0.11 | 4.2±0.23 | 4±0.57 |
| <i>Steochospermum polypolides</i> | 3.6±0.11 | 3±1.15 | 3.3±0.17 |
| <i>Ulva rigida</i> | 2.5±0.28 | 4±0.57 | 6±0.57 |
| <i>Valaniopsis sp.</i> | 4±1.15 | 2.3±0.17 | 3±0.28 |

In water extract, maximum inhibitory activities were recorded in *F. oxysporum* against all extracts of selected seaweeds but three seaweeds *G. costicata* and *P. tetrastromatica* showed 85.56% and *C. iyengaraii* showed 83.33% promising inhibition results. However, some seaweeds extracts did not prove inhibitory activity against two pathogenic fungi *R. solani* and *M. phaseolina* but *C. racemosa*, *G. costicata* and *S. polypolides* showed good results against *M. phaseolina*, while *S. polypolides* and *Valaniopsis sp.* showed more than 60% results against *R. solani* (Fig. 3). In methanolic extract, seaweeds were significantly reduced the mycelial growth of *F. oxysporum*, *M. phaseolina* and *R. solani* as compared to control. Maximum 96.67% inhibition % was recorded against *F.oxysporum* by *P. tetrastromatica*, while *G. costicata* showed 88.89% of inhibition against *M. phaseolina*. However, some seaweed including *M. afaqhusinii*, *S. polypolides* and *Valaniopsis sp.* showed more than 60% activity against *R. solani* (Fig. 3).

The results of ANOVA for effect of aqueous and methanol extracts of different seaweeds were observed on these three soil-borne fungi. All treatments showed highly significant differences (F=82.41, P<0.001) and inhibited the growth of selected soil-borne fungi including *F. oxysporum*, *M. phaseolina* and *R. solani* (F=78.49, P<0.001). However, in methanolic extract, all most all treatments observed highly significant differences (F=30.6, P<0.001) and helped out to suppress the growth of common soil-borne fungi (*F. oxysporum*, *M. phaseolina* and *R. solani*) with (F=12.94, P<0.001).

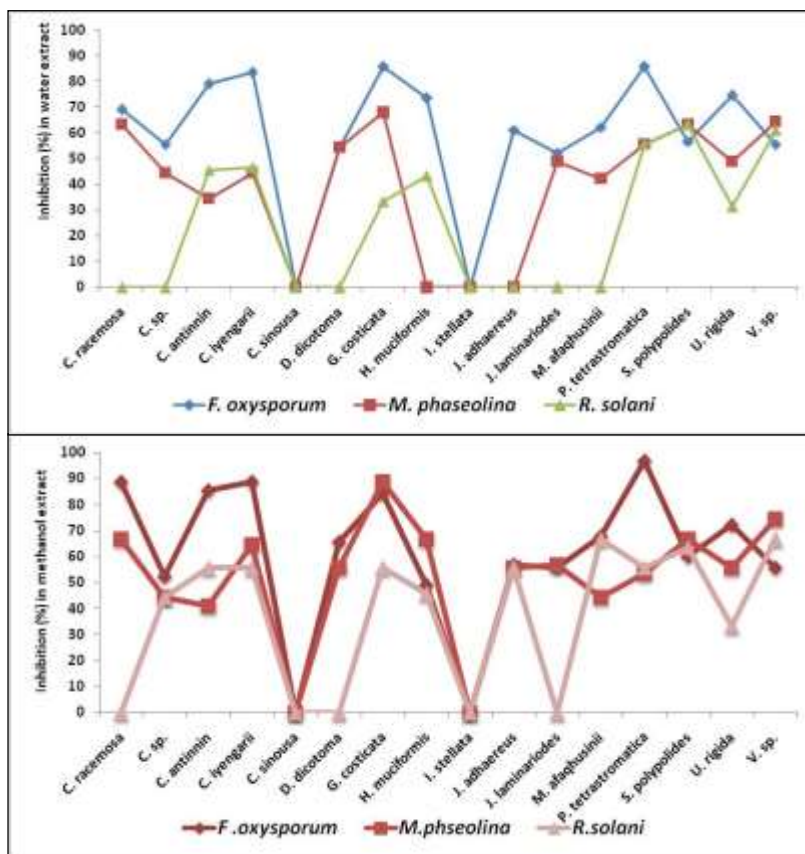


Fig. 3: Inhibition % of different Seaweeds against soil-borne plant pathogenic fungi in water and methanolic extract

4. Discussion:

Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. The functions of these secondary metabolites are defense mechanism against herbivores, fouling organisms and pathogens chemical defense mechanisms against herbivore; for example, grazer-induced mechanical damage triggers the production of chemicals that acts as feeding deterrents or toxins in seaweeds (Watson & Cruz-Rivera, 2003). The sea offers a reservoir of useful seaweeds with biodynamic activities. There is little attention has been given to their potential as antimicrobial agents, but it is expensive and risky because of expenses and production of environment hazards (Zareen & Khan, 1984; Akhter, 1991; Abid et al., 1997; Abid et al., 2005). Special attention has been reported for antiviral, antibacterial and/or antifungal activities against human pathogens as showing antibacterial (Shyamali et al., 1982; Uzair et al., 2006; Ahmed et al., 2008;), antifungal (Usmanghani & Shameel, 1986; Shaikh et al., 1990; Ara, 2001), anti nematocidal (Naqvi et al., 1992), antiviral (Grag, 1994), anti-inflamantary, analgesic (Bruckner, 2002) and cytotoxic (Moore et al., 1993; Khan, 2000; Newman & Cragg, 2004) and biostimulant properties of seaweeds are explored for use in agriculture. Several studies of marine plant extracts confirm the present antifungal and antimicrobial study (Shameel & Tanaka, 1992; Alam et al., 1994; Shameel et al., 1996; 2000; Aliya and Shameel, 1999; Hameed et al., 2000; 2001; Rizvi & Shameel, 2001; 2003; 2005).

The nematocidal activities of seaweeds would be a great help to completely contrast or at least reduced the nematode disease in economically important plants, which caused heavy losses to crop plants and adversely affect economy of our country (Rizvi & Shameel, 2006), used as a fertilizer for many years and widely used biostimulant in agriculture (Hattori, 1999). Different seaweeds exhibited very significant nematocidal activities (Whapham et al., 1994; Ara et al., 1996; Sultana et al., 2000; Noreen et al., 2002; Zaki et al., 2005). Seaweeds contained elaborate secondary metabolites that play a significant role in the defense of the host against predators and parasites which offer a potential novel approach to control population of plant parasitic nematodes (Paracer et al., 1987; Jacobs et al., 2003). Seaweed extracts have been reported to increase plant resistance to pests and diseases, plant growth, quantity and yield (Jolivet et al., 1991; Verkleji, 1992; Pardee et al., 2004). Application of seaweed to plants resulted in decreased levels of nematode attack (Ara et al., 1997; Wu et al., 1997; 1998). Sultana et al. (2011) reported that seaweeds like *Spatoglossum variabile*, *Halimeda tuna* and *Melanothamnus afaqhusainii* showed more or less similar suppressive effect on root rotting fungi and root-knot nematode to chemical fungicides (Topsin-M) and nematocide (carbofuran). In a large number of marine algae, antimicrobial activities are attributed to the presence of acrylic acid. Seaweeds contain 1- aminocyclopropane-1-carboxylic acid (ACC), which has antimicrobial activity (Nelson & Van-Standen, 1985). Our present study supported by the results of Paracer et al. (1987); Ara et al. (1996; 1997); Zaki et al. (2005); Abid et al. (2005) and Rizvi & Shameel (2006).

5. Conclusion:

This research article is based on chemical composition and nutritional values from the coast of Karachi, Pakistan using sixteen seaweeds belonging to three different Phylum including Chlorophytocota, pheophycota and Rhodophycota. The residue of the seaweeds was examined *in vitro* condition for antifungal activities using food poisoning technique against three pathogenic fungi e.g., *F. oxysporum*, *R. solani* and *M. phaseolina* and for nematocidal activities using mortality and hatching test against *M. javanica* species and conclusion, got highly significant results. This study has revealed an interesting array to create a nutritional data to the alternation of an efficient food for Pakistan food industry and envisage pesticides invention for agricultural department. It is concluded that seaweeds are renewable living resources which are also used as feed, wastewater treatment or for the industrial production of phycocolloids, thickening, and gelling agents in food and pharmaceutical industries.

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Allelopathic Effects of Sunflower (*Helianthus annus* L.) against *Luffa cylindrica* (L.) Roem

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Abstract

The Sunflower (*Helianthus annus* L.) has been considered for allelopathic plant and it has potential properties against different weeds and crop plants. The sunflowers usually release the allelochemicals to influence the growth and germination of different plants and crops. In present study, screenhouse and lab-based experiments were carried out to explore the allelopathic effect of *H. annus* against test crop *Luffa cylindrica* (L.) Roem. The petri-plate method was applied in lab by the application of whole plant extract of Sunflower with different concentrations (4, 8 and 10%). The seedling germination of test crop *L. cylindrica* was evaluated in petri-plate experiment and the control seedling was treated with distilled water in lab-based conditions. However, clay pot method was applied in greenhouse conditions. The soil was amended by whole plant powder extract with concentration of 4, 8, and 12g was inoculated in soil of clay pot and control plant were filled without soil treatment inoculation. The growth parameters like percent germination, germination index, radicle and plumule elongation, seedlings growth, fresh and dry weights and chlorophyll contents of test crop *L. cylindrica* were studied. During this research study, the plumule lengths of *L. cylindrica* were much more affected by the extract concentration as compared to control. Powder also contained phytotoxic compounds that caused remarkable reduction in plant heights and chlorophyll contents of test species. In addition, it can be concluded from this research observed the allelopathic effects of sunflower extract and powder showed inhibitory effects on different growth parameters of *L. cylindrical*.

Keywords: Allelopathic Effect; Sunflower; Petri-Plate Experiment; Pot Experiment

1. Introduction:

The Sunflower cultivation is done throughout the world as well as in Pakistan particularly concerned in Sindh, Punjab and Khyber Pakhtoonkhawa. as it is a main source of vegetable oil used for different purposes (Anjum & Bajwa, 2007). It is recognized as an important crop in several areas of Pakistan due to suitability of the crop to local agroclimatic conditions, its importance as source of edible oil and protein, resistance to drought and its short duration (Kamal & Bano, 2009). However, yields of some crops following sunflower are lower than normal, possibly because of inadequate nutrition and chemical inhibition (Kamal & Bano, 2008). More than 200 natural allelopathic

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© 2021 (Accepted for publication in August 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

compounds have been isolated from different cultivars of sunflower (Kamal & Bano, 2009). Sunflower leaf extracts caused reduction in radical and hypocotyl length of mustard seedling (Wardle *et al.*, 1991; Bogatek *et al.*, 2006).

The allelopathical chemical compounds are also known as allelochemicals that are usually produced by plants (Chon & Kim, 2002). The allelopathic phenolic compounds have inhibitory activities against the growth of neighboring crops (Jalili *et al.*, 2007). The allelopathic activity of Sunflower has been reported on subsequent crops as well as on weeds (Macais *et al.*, 2002). Several phytotoxic allelochemicals that have been identified in Sunflower residues are chlorogenic acid, isochlorogenic acid, scopolin and alpha-naphthol derivatives described by Kupidlowaska *et al.* (2006).

The test crop *Luffa cylindrica* (L) M. Roem. (Sponge gourd) belongs to the family Cucurbitaceae and is grown mainly for the tough fibrous netting that remains after the pulpy flesh is removed from the mature fruit. It is a climbing, hairy, smooth vine, reaching a length of 12m or more. *Luffa* (*Luffa cylindrica* and *Luffa acutangula*) reportedly originated in subcontinent has vigorous climbing vine and is a dicot genus of about six species occurring in the tropical regions of the world (Nyananyo, 2006). It is a tropical and subtropical plant and has been widely cultivated in the Middle East and India, China, Japan, and Malaysia (Porterfield, 1955). It is widely cultivated for its fruits which are used as vegetable. In Pakistan, it is mainly cultivated in Sindh, Punjab, Baluchistan, and Khyber Pakhtoon Khuwan (Ali, 1977). Sunflower is often water-soluble substances that are released into the environment through root exudation, leaching and decomposition of plant residues. Several Asteraceae species have been reported as allelopathic effects on other plant species, reducing seed germination and emergence of subsequent small-grain crops when grown in rotation (Muehlchen *et al.*, 1990). Several putative allelochemicals have been isolated from Asteraceae and their allelopathic potential demonstrated in bioassays. For example, allyl-isothiocyanate (ITC) isolated from sunflower residues inhibited the germination and growth of various grass species (Vaughn & Boydston, 1997).

1.1. Objectives:

- To explore the allelopathic effects of sunflower against Sponge Gourd.
- To determine the different plant parameters to test crop and allelopathic effect during plant growth.

2. Materials and Methods:

2.1. Collection of Material:

The experimental work was carried out in research laboratory and screen house of Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus Karachi, Pakistan. The seeds of Sunflower and Sponge Gourd (*L. cylindrica*) were collected from a local Seed market. The Sunflower plants were cultivated in the field of Department of Botany, as the plants became young before flowering, the plants were collected.

2.2. Preparation of Plant Extract:

The collected plants were air dried in screen house, after drying plants were ground by Wiley mill (Thomas Wiley Lab Mill, Model 4). For the preparation of aqueous extracts, add 4, 8 and 12 g whole plant powder in marked beakers and 50 ml of distilled water was liquescing in each beaker for left the suspension for the time of 24 hours. After 24 hours, these suspensions were centrifuged by centrifuge machine and filtered through Whattman No.1 filter paper and add distilled water to make up the volume up to 100 ml of each concentration and kept the solution into marked conical flasks. All the glass wares were sterilized in autoclave at 121°C and 15 lb pressure for 30 minutes to avoid microbial contamination.

2.3. Petri-Plate Experiments:

The seeds of *L. cylindrica* were surface sterilized with 0.1 % mercuric chloride solution for 1-2 min and washed with distilled water. All the Petri plates were marked as control, 4, 8 and 12 %, along with the 5 replicates, respectively. Total 10 sterilized seeds of test crop *L. cylindrica* were kept in petri-plates along with uniform distance. 2 ml plant powder extracts were poured in the replicates of each concentration while distilled water was poured as a control. The extracts were poured at alternate days. The petri plates of test crop *L. cylindrica* were placed at room

temperature in laboratory. The germination of seeds was recorded regularly on daily basis, while the radicle and plumule lengths were taken in alternate days.

2.4. Pot Experiment:

Pot experiments were conducted in screen house and in an open field of Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus Karachi, Pakistan. The sandy loam soil and natural humus fertilizer was used in the ratio of 8:2. The soil was free from fungal population, insects, and other pathogenic organisms. All the pots were marked as control, 4, 8 and 12 g. Total 500 g soil was weighed and incorporated with plant extract powder, while the control pots were filled only with 500 g soil. Pots were kept in screen house and irrigated for 2-3 days, and pots were prepared for sowing after 3 days.

2.5. Germination and Plants growth:

The seeds of *V. radiata* was surface sterilized as described previously. Total 10 sterilized seeds were sown in each pot. The germination record was noted on daily basis. After 8 days of germination, the plants were thinned and only four plants were left in each pot. After thinning, the plants height was measured weekly. All the pots were kept in open field due to required critical day light.

2.5.1. Germination Percentage

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

2.5.2. Germination Index

The Germination Index was calculated by given formula of Khandakar and Bradbeer, (1983).

$$\text{Germination index (S)} = \left[\frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} \dots \dots \right] \times 100$$

Where S is the germination index, N1/1, N2/2.... are the ratio of number of the seeds germinated per day

2.5.3. Seedling Vigour Index:

The Seedling Vigour Index (S.V.I.) was calculated according to the following formula of Abdul-baki and Anderson (1973).

$$\text{S.V.I} = (\text{Root length} + \text{Shoot length}) \times \text{Germination (\%)}$$

2.5.4. Inhibition Percentage (%)

Inhibition percentage was calculated by following formula of Surendra and Pota, (1978).

$$\text{Inhibition (\%)} = 100 - \left(\frac{E_2}{E_1} \right) \times 100$$

Where, I= % Inhibition, E1= Response of Control plant, E2=Response of Treatment plant

2.6. Germination and Plant Growth

The pot experiment was terminated after one month and all plants of each pot of experiments were uprooted carefully for taken fresh weight, dry weight, detection of chlorophylls content and carotene.

2.7. Detection of Chlorophyll Contents

2.7.1. Preparation of Samples

For preparation of samples to detection of chlorophyll content, 2 g fresh leaves of each concentration (control, 4%, 8% and 12%) were carried out. These leaves were crushed with 2 ml of 80% acetone in pestle and mortar, and then filtrate the extract with the help of muslin cloth. The extract and 2 ml of 80% acetone was centrifuged for 2-3 minutes at 1500 rpm. The supernatant was poured in a separate test tube and debris were washed with 2 ml of 80% acetone. The solution was centrifuged, and the supernatant was transferred into the same test tube. The procedure was repeated until the debris become colorless. The pestle and mortar were rinsed with 80% acetone and collect the residuals of washing in the same test tube. Make up the volume up to 10 ml with 80% acetone.

2.7.2. Determination of Chlorophyll Content

The chlorophyll content (chlorophyll a, chlorophyll b and carotenoids) was determined by using Spectrophotometer and 80% acetone were poured in the cuvette (at least two-third) it was considered as blank. The Spectrophotometer was kept on constant at 0 absorbance for each wavelength (663 nm, 645 nm, and 510 nm). The blank was used to minimize the amount of 80% acetone in each concentration (control, 4%, 8% and 12%). The O.D of each concentration was carried out and calculated the amount of chlorophyll a, chlorophyll b and total chlorophyll by using the formula given by Arnon, (1949) and content of carotenoids was determined by the formula suggested by Harborne, (1973).

$$\text{Chlorophyll "a"} \left(\frac{\text{mg}}{\text{g}} \right) = 12.7 (A_{663}) - 2.69 (A_{645}) \times (V|1000) \times W$$

$$\text{Chlorophyll "b"} \left(\frac{\text{mg}}{\text{g}} \right) = 22.9 (A_{645}) - 4.68 (A_{663}) \times (V|1000) \times W$$

$$\text{Total Chlorophyll} \left(\frac{\text{mg}}{\text{g}} \right) = 20.2 (A_{645}) + 8.02 (A_{663}) \times (V|1000) \times W$$

Where, A= Absorbance at specific wavelength, V= Final volume of Chlorophyll extract in 80% acetone, W= Fresh weight of tissue extracted

2.7.3. Determination of Carotenoids

The carotenes content can be calculated by following formula presented by Harborne, (1973).

$$\text{Amount of Carotenes in mg} = \frac{4 \times \text{O. D.} \times \text{Total volume of sample}}{\text{Weight of plant tissues}}$$

3. Results:

3.1. Petri-Plate Experiment:

The Petri-Plate experiment was conducted in lab conditions of Department of Botany. The data of germination percentage is given in Table. 1 that describe the minimum 84% germination was recorded in the treatment of 12% extract as compared to control 4 and 8%. The bias was obtained as 100% < 100% < 92% < 84% (Fig. 1). The result of inhibition % revealed that the highest 16 percent inhibition was observed in the treatment of 12% extract while lowest percent 2% inhibition was obtained in 4% extracts as compared to control. The trend of percent inhibition shown as 0% < 2% < 8% < 16%. However, the germination index was affected as the concentrations of extract were increased; the germination index was gradually inhibited as 98 % < 90% < 88% < 75% (Table 1).



Fig. 1: Effects of *H. annus* on germination % of *L. cylindrica*

The data of seedling Vigour Index (S.V.I.) determined that the highest value 793.42 was observed in control and lowest value 255.13 was noted in the treatment of 12% extract. The S.V.I. value was decreased as the strength of extracts were increased. This trend was obtained as 793.42 < 696.478 < 282.85 < 255.13. The compilations of radicle length presented that the extracts of *H. annus* reduced the radical lengths of treated plants as compared to control, as the strength of extracts was increased. The inclination was obtained in 18.42 < 12.478 < 10.53 < 9.85 cm manner. The percent inhibition in radicle length substantiated that the concentration of extracts in highest amount inhibited the radicle lengths as compared to control. The maximum 46.52% inhibition in radicle lengths was reported in 12% treatment while minimum 0% recorded in control. These results showed as 0 % < 32.3 % < 42.83 % < 46.52%. The details of plumule length annotated that the length of plumule was significantly decreased as the concentrations of extract were increased. The maximum 4.15cm reduction in plumule length was observed in 12% extract treatment as compared to control, 4% and 8%. The reduction trend showed as 8.60 < 6.75 < 6.09 < 4.15cm. The data of inhibition percent in plumule length annotated that the maximum 62.32% inhibition was obtained in plumule length at concentration of 12% extract treatment as compared to control while minimum 11.74% inhibition was recognized in 4% extract treatment.

Table 1: Different plant growth parameters of *L. cylindrica* affected by *H. annuus* in Petri-Plate experiment

| Treatments | Different Plant Growth Parameters in Petri-Plate Experiment | | | | | | | |
|------------|---|-----|----|--------|-----------|-------|------------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Control | 100% | 98% | 0 | 793.42 | 7.75±0.33 | 0 | 18.4±0.64s | 0 |
| 4% | 100% | 90% | 0 | 696.47 | 6.84±0.32 | 11.74 | 12.47±0.76 | 32.30 |
| 8% | 92% | 88% | 8 | 282.85 | 2.96±0.64 | 61.80 | 10.53±1.13 | 42.83 |
| 12% | 84% | 75% | 16 | 255.13 | 2.92±0.35 | 62.32 | 9.85±0.04 | 46.52 |

1= Germination (%), 2= Germination Index, 3= Inhibition (%) in germination (%), 4= S.V.I, 5= Plumule length, 6= Inhibition (%) in plumule length, 7= Radicle length, and 8= Inhibition (%) in radicle length

3.2. Pot Experiment:

The pot experiment was conducted in screen house of Department of Botany. The data of germination was observed that maximum 96% germination percentage was found in control while in treatments, as the amount of powder was increased, the germination percentage was decreased as in 4, 8, and slightly reduction in 12g powder extract treatment. The decreasing trend shown as 96 % < 90 % < 88 % < 86%. The highest 10.4% inhibition in germinated seeds was found in 12 g powder extract as compared to control, 4, and 8 g powder extract treatment. The trend of inhibition shown as 4% < 6.25% < 8.33 % < 10.4%. The data of germination index demonstrated that the germination index was affected as the increase of amount of the powder extract treatment. The seeds germinated with 49.3 greatest rate index in control while in 12g powder extract, seeds germinated with 44 lowest rate index.

The declined bias shown as $49.3 < 45.6 < 45.3 < 44$ (Table 2).

Table 2: Different Plant growth parameters of *L. cylindrica* affected by *H. annuus* in pot experiment

| Treatments | Different Parameters in Pot Experiment | | | | | | |
|------------|--|------|-------|------|------|------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | 96% | 49.3 | 4% | 12.9 | 0 | 3.16 | 1.5 |
| 4g | 90% | 45.6 | 6.25% | 11.2 | 13.1 | 3.57 | 1.605 |
| 8g | 88% | 45.3 | 8.33% | 10.2 | 21 | 3.08 | 1.2 |
| 12g | 86% | 44 | 10.4% | 9.33 | 28 | 3.4 | 1.31 |

1= Germination (%), 2= Germination Index, 3= Inhibition (%) in germination (%), 4= Plant height, 5= Inhibition (%) in plant height, 6= Fresh weight (g), 7= Dry Weight (g)

The facts substantiated that there was significant decrement 9.33 scrutinized in heights of 12g treated pot plants, while in the other pots as the amount of powder extract increased the plant height slightly decreased 11.2 to 10.2 in 8 and 4g treated pot plants as compared to control. The decrement course was obtained as $12.9 < 11.2 < 10.2 < 9.33$ (Fig. 2). The compilations regarded that there is a remarkable inhibition found in the treated plants of 12g powder extract treatment due to greater amount of powder as compared to control plants. The trend shown as $0 < 13.1 < 21 < 28$. The fresh weight was found maximum 3.57g in 4g treatment whereas lowest 3.08g fresh weight was obtained in 8g powder extract treatment. Minimum 1.2g dry weight was recognized in 8g powder extract treatment as compared to control 1.5g. The facts and figures demonstrated that there is declined bias archived in fresh weight and dry weight of the treated plants (Table 2).



Fig. 2: Effects of *H. annuus* on plant heights of *L. cylindrica*.

3.1.1. Chlorophyll content:

The data of chlorophyll contents and carotenoids is presented in Table 3. It showed that powder extract treatment in high 12g concentration treatment caused reduction of chlorophylls and carotenoids as compared to control.

Table 3: Chlorophyll contents of *Luffa cylindrica* affected by *Helianthus annuus*

| Treatments | Chlorophyll (a) mg/g | Chlorophyll (b) mg/g | Total Chlorophyll mg/g | Carotene |
|------------|-------------------------|-------------------------|---------------------------|----------|
| Control | 0.052 | 0.048 | 0.100 | 6.7 |
| 4g | 0.044 | 0.042 | 0.087 | 6.1 |
| 8g | 0.033 | 0.040 | 0.070 | 4.82 |
| 12g | 0.024 | 0.040 | 0.068 | 4.68 |

4. Discussion:

Germination of seedling is the first crucial stage for growth and development of the plant. At this stage, different metabolic changes take place which is necessary for the growth of the plant. It is indicated from our results that the different strengths of extract caused inhibition in the germination of seedlings of *L. cylindrica*. These results are in accordance with the findings of Kamal & Bano, (2008) described that germination of seedlings of wheat was reduced due to aqueous extract of Sunflower. Our results supported with the findings of Ahmed *et al.* (1995) who stated that the aqueous extract of *H. annuus* have significant inhibitory effect on germination and seedling growth of Cotton. Similarly, the germination of seedlings of *L. cylindrica* was highly affected as the strengths of extracts were increased; different soluble phenolic compounds are incorporated in water extract of allelopathic plants that have inhibitory effects on germination and growth of other weeds and crop plants (Kruidohf *et al.*, 2010). Kupdisdowska *et al.* (2006) and Oracz *et al.* (2007) strongly supported our results who investigated that seed germination of *Sinapis alba* (mustard) was completely inhibited by sunflower extract. Our results supported with the results of Mubeen *et al.* (2012) who elucidate that sunflower extract has inhibitory effects on germination index of *Dactyloctenium aegyptium*. In our results, rate of germination index on the seeds germination of *L. cylindrica* was highest. This showed that extract applied to the germinating seeds have negative effects on germination index of the seeds. Radicle and plumule elongation are an important aspect in the life cycle of plant. Under stress conditions if these aspects are affected then the growth and development of the plant also affected. Our results justified by the findings of Anderson *et al.* (1978) who documented that radicle and plumule elongation was inhibited due to the whole plant extract of sunflower. Our estimations were not interacting with the determination of Chung & Miller, (1995) and Turk & Tawaha, (2002) who annotated that radicle growth was much more affected than shoot length due to the extracts of allelopathic plants.

In *L. cylindrica*, there was significant reduction depicted in radicle and plumule elongation over the control plants. The highest 12% concentration of extract was significantly inhibiting the roots and shoots length of seedlings. Extract has inhibitory effects on roots and shoot lengths of each treatment. Extracts of allelopathic plants usually contain phytotoxic allelochemicals that may direct influence the growth of the subsequent crops. These results are similar with the findings of Chaturvedi & Jha, (1992) reported that inhibitory effects of extracts in seedling growth is just because of the presence of phytotoxic allelochemicals. Our estimations are co-related with the interpretation of Xuan *et al.* (2005) who enumerated that allelochemicals of phytotoxic plants when exposed to the susceptible plants affects the growth and development of plants. Similarly, in our research studies the plant heights of *L. cylindrica* was completely inhibited when amount of powder in highest amount incorporated with soil. The plant heights of treatments significantly lower 9.3 cm at 12g powder as compared to control plants. These findings are interacted with the adjudicates of Batish *et al.* (2002) enlightened that germination and growth of millet, sorghum, corn and cluster bean was decreased due to sunflower residues incorporated in soil. Our results also supported with the findings of Shaikat & Siddiqui, (2001) stated that there was remarkable reduction caused in plant growth due to inhibitory phytotoxic compounds accumulated in soil released by allelopathic plants.

The decrement in fresh weights and dry weights of treated plants of *L. cylindrica* was recorded as strength of powder was increased. Our testaments are in accordance with the findings of Sahoo *et al.* (2010) who manifested that dry weights of chilli, soya bean, maize and lady finger was inhibited due to higher concentrations of extract *Mangifera indica*. Another most important parameter of plant growth is chlorophyll content. Chlorophyll contents are the essential pigments in growth of plants, as they provide basic framework in photosynthesis. The amount of chlorophyll contents destroyed under stress conditions which tremendously affected the metabolic processes of plants. In our studies, the allelochemicals contained by sunflower powder showed inhibitory effects on the chlorophyll contents of *L. cylindrica*. Our interpretations are strongly supported by Lorenzo *et al.* (2011) who elucidate that allelopathic compounds have inhibitory effects that interfere the physiological processes like photosynthesis. Farhoudi & Lee, (2012) documented that chlorophyll content of *Lolium* spp. as well as *Avena*

ludoviciana seedlings reduced due to the application of safflower extract. These results supported our estimations in which the chlorophyll contents were inhibited due to the residues of sunflower. Our results are also in agreement of consistence of Stupnicka-Rodzynkiewicz *et al.* (2006) who elucidate that chlorophyll contents and photosynthetic rate were affected by allelochemicals released by plants residues.

5. Conclusion:

During present research study, we observed the allelopathic effects of sunflower extract and powder applied to test crop *L. cylindrica*. Both extract and powder showed inhibitory effects on different plant growth parameters which all are essential for plant growth. In addition, it can be concluded from this research observed the allelopathic effects of sunflower extract and powder showed inhibitory effects on different growth parameters of *L. cylindrica*.

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Ethnoflora of Tehsil Kharian, District Gujrat-Punjab, Pakistan

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Abstract

Peoples of all civilizations always depend upon plants for their primary needs (foodstuff, protection, warmth and medication). In the present study an ethno-medicinal analysis was conceded out in Kharian, District Gujrat, Punjab-Pakistan. A series of surveys were conducted in support citations of essential ethno flora along with information beginning from local community about the medicinal uses of plants in their surroundings. As many as 49 plants were identified and characterized regarding ethnobotanical uses. Various plant parts are used for medical use. The plants were belonging to different families. The plants were selected, and herbarium sheets were submitted in herbarium established at University of Gujrat, Gujrat. These surveys were carried out from February 2018 to April 2019 in close vicinity of University of Gujrat (UOG), Pakistan.

Keywords: Ethnobotany; Gujrat; Kharian; Medicinal plants; Pakistan

1. Introduction:

Pakistan is fairly a huge state gifted with a diversity of climates, ecological zones and topographical regions (Hussain *et al.*, 2008) as well as occupies a unique position among developing countries. It has all the four weathers for that having well potential within the variety of medicinal plants due to its diverse atmospheric and edaphic dynamic which reflects multiplicity and valuable therapeutic plant legacy. Gujrat is an ancient and famous district of Punjab (Pakistan) situated between two famed rivers, the Jhelum River and the Chenab River. Because of its closeness with the rivers, the land is good for cultivation of rice and sugar cane as chief crops. District Gujrat spreads over an area of 3,192 square kilometers and cover three tehsils, Gujrat, Kharian and Sarai Alamgir. Climate is moderate and the average rainfall at Kharian 75 cm and Gujrat 67 cm.

Kharian is the major city of Tehsil Kharian, which governs various adjacent rural and urban areas (villages and town). It is situated approximately midway between the capital cities of Pakistan, Islamabad (125 km) also the provincial capital of Punjab, Lahore (145 km). It was used during British Rule to transport goods across South Asia. The main railway line also passes through Kharian, thus providing good transportation to the northern and southern parts of Pakistan. Kharian Cantonment is one of the largest cantonments (Army Base) of Pakistan. It was constructed

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© 2021 (Accepted for publication in September 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

in late 1950s with the help of USA. The cantonment has a bunch of amenities, amongst which are a generous services mess/club to offer a site for social actions, an elegant mosque and a gorgeous park. There is also a demanding shopping spot, a vigorous equipped military hospital (CMH Kharian) and two-degree colleges, both for girls and boys. Kharian Cantt. is also a center for education, both national and international-level schools and colleges are available, within the premises of Garrison. Besides all these Kharian is also blessed with an immense ethano flora most of the surrounding area is rural so for that people mostly depends upon herbal medicine. According to botanical definition that herb is a plant which cannot form a woody stem and they are annuals and perennial. Medicinal herb is distinct from botanical term 'herb' these are the plants which are referring to use in medicine and so for that it is called herbal medicine. Besides herbs, shrubs and woody trees, angiosperms, gymnosperms and bryophytes are also used as medicinal plants.

The history of innovation and utilization of diverse therapeutic plants is as elderly since the history of invention and exploitation of plants for foodstuff (Ibrar, 2002). Medicinal plants occupy an input position in conventional health concern organization for both human and animals' health. Many allopathic drugs also include extort full of curative plants (Rashid & Arshad, 2002). Since last 100 years, science of ethno botany has developed and tendency is changing from sheer certification process to a much practical one, which accentuate on conservation and sustainable use of plant resources. Almost 80% people of the world depend on the traditional system of health care (Ahmad, 2005). Herbal medicines have fewer elevation effects and herbs are easily available from nature. Unani method is dominant inside Pakistan although the ethno medicinal plants utilization is also seen in distant region. (Ahmad *et al.* 2003). In Pakistan, ethnobotany is newly established. The modern conventional healthcare is hindered with vast dilemma of dangerous medicine, chronic diseases, challenging infections, auto immune disorder and degenerative disorder, allergic infections have shifted peoples from allopathic medicine toward herbal medicine, Pakistan has about 6,000 species of wild plants of which about 400 to 600 are considered to be medicinally important (Hamayun *et al.*, 2003). A figure of remedial plants is recognized concerning their properties and appropriate use at the community level (Khan, 2002). A chain of documents on medicinal and indigenous employment of medicinal plants in Pakistan has been published throughout the country (Bhatti *et al.*, 2001; Qureshi & Bhatti, 2008; Qureshi *et al.*, 2002; 2009; 2010; Ahmad *et al.*, 2010).

Most of our rural population relies upon traditional methods to cure the disease they believe on herbal medicine using different parts of plants like root, stem, leaves, bark. A variety of plant extracts are used to reduce the pathogenic effects of the disease. Herbal method is also very important due to its cheapness; plants are less expensive and easily available globally.

1.1. Objectives:

- To cure the diseases by herbal medicines using different parts of plants like root, stem, leaves and bark.
- A variety of plant extracts are used to reduce the pathogenic effects of the disease.

2. Materials and Methods:

2.1. Study Area:

Kharian is located on the Grand Trunk Road (colloquially known as the GT road). This is the road which connects Kharian, all the way from Bangladesh, through India across Pakistan and to Afghanistan. Many field trips of Tehsil Kharian were conducted regarding plant collection from Feb 2014 to May 2014. It is situated between two famous cities of Pakistan Lalamusa and Jehlum.

2.2. Survey:

Several field surveys were accomplished in Kharian to obtain information regarding use of herbal medicine and common medicinal plants in the area during 2018-19. An attempt was too made to verify the ethno medicinal utilization from native farmers, herbal doctors, healers (Hakeems) and herbal dealers (Pansars) in Kharian Bazaar.

2.3. Interviews and Questionnaire:

Ethnobotanical information was acquired during oral interviews and planned questionnaire beginning from locality and the elderly people. Females and male both were interviewed. In this way more than 50 people who

aware with the ethno flora and mostly used on the local resources for survival, were interviewed in the form of oral discussion and documented questionnaires.

2.4. Preservation of the plants:

Plants were collected during surveys and field trips. Collected plants were photographed and dried by standard herbarium method. Standard methods were followed with regard for collection of plant materials, drying, mounting, preparation and preservation of plant specimens described by (Nasir & Ali, 2001). Dried were preserved in plastic envelopes and arranged alphabetically with their botanical name, local name and family and also parts of plant used as herbal medicine (root, stem, leaf, flower, bark).

3. Results:

During the present study, a total of 49 species belonging to 30 families of angiosperms were documented from Tehsil Kharian, District Gujrat, Punjab-Pakistan (Table 1.). The data for habits and life forms of plants including herb, shrub, climber and trees were recorded. The highest number of species were recorded from the family Mimosaceae (5 spp.), followed by Myrtaceae and Rutaceae (4 spp.), respectively.

Table 1: Brief account of ethno-flora of Tehsil Kharian, District Gujrat-Punjab and their traditional uses

| Family Name | Plant Name | Common Name | Parts Used | Ethnomedicinal Uses |
|---------------|-------------------------------------|-------------------------|------------------------|--|
| Amaranthaceae | <i>Amaranthus graecizans</i> subsp. | Phulari | Leaves | Inflammations, Piles, Gonorrhoea |
| | <i>Achyranthes aspera</i> L. | Puth Kanda, Chaff Plant | Whole Plant | Kidney problems and cough. |
| Anacardiaceae | <i>Mangifera indica</i> L. | Aam, Mango | Leaf and Seed | Earache, Vomiting. |
| Annonaceae | <i>Polyalthia longifolia</i> | Ulta ashok | Leaves, Root, stem | Fever, diabetes, hypertension, skin diseases & helminthiasis |
| Apiaceae | <i>Anethum graveolens</i> L. | Soy | Leaves, flowers, fruit | Seed are given to females to increase of milk secretion acting as lactagogue. Powder obtained from the seeds in combining <i>Terminalia chebula</i> and black salt commonly known as <i>Phakki</i> and is used for flatulence. The seeds are also used in making pickles acting as an appetizer. |
| Apocynaceae | <i>Catharanthus roseus</i> | Sada Bahar | Leaves | Diabetes mellitus |
| Areaceae | <i>Phoenix dactylifera</i> L. | Khajur, Date | Fruit | General body weakness |
| Asphodelaceae | <i>Aloe vera</i> (L.) Burm. f. | Kwargandal, Aloe | Leaf | Rheumatism, body weakness and in the treatment of pimples or acne. |
| Asteraceae | <i>Carthamus oxycantha</i> M. Bieb. | Poli, Carthamus | Seed | Grind seed flour is used to treat ulcer problems. |
| Brassicaceae | <i>Brasica compestris</i> L. | Sarsoon | Leaves, oil | Leaves are used as potherb to expel abdominal worm and to treat constipation. The same is supposed as good appetizer and mild laxative. The oil obtained from the seed is applied to the body as antimicrobial and anti- |

| | | | | |
|----------------|---|--------------------------|--------------------------------|---|
| | | | | lice agent. The <i>Brassica</i> oil mixed with <i>Lawsonia alba</i> (<i>Mehndi</i>) is applied to athlete foot to relieve. |
| Chenopodiaceae | <i>Chenopodium album</i> L. | Bathu, Goose Foot | Whole Plant | Jaundice |
| Convolvulaceae | <i>Convolvulus arvensis</i> L. | Vahri, Bind Weed | Whole Plant | Constipation, control dandruff |
| Cucurbitaceae | <i>Cucumis melo</i> var. <i>agrestis</i> Naudin | Chibbar, Wild Watermelon | Fruit and seed | Dried powdered plant used to treat skin infections, stomach problems. Fruit (<i>Karaila</i>) is prescribed as a potherb to sugar patients. Stomachic, gas trouble and constipation, blood purifier to treat boils and pimples, improve the liver functioning and kill the abdominal worms. |
| | <i>Momordica balsamina</i> L. | Jangli Kareela | Fruit and seeds | |
| Cuscutaceae | <i>Cuscuta reflexa</i> Roxb. | Akash Bail, Dodder | Stem | Paralysis, Hair treatment |
| Euphorbiaceae | <i>Chrozophora tinctoria</i> (L.) Juss. | Leaves | Neeli Booti | The leaves are boiled in water and the obtained juice is given orally to relieve chest burning acting as stomachic. |
| | <i>Ricinus communis</i> L. | Hernoli, Castor oil | Seed | Constipation, Stomach and bowels problems |
| Lathyraceae | <i>Lawsonia alba</i> Lam. | Mehndi | Leave, fruit | Leaves ground and made into powder applied for hair dying. The powder of leaves mixed with milk is orally given to cattle after mating and is supposed to act as cooling agent. Leaves are mixed with the <i>Brassica</i> oil and made into paste which is externally applied to athlete foot to relieve. |
| Malvaceae | <i>Bombax ceiba</i> L. | Simbal | Flowers, Roots, bark and seeds | Dysentery, Stimulant, blood purification, constipation, snake bite and gonorrhoea. |
| | <i>Hibiscus rosa sinensis</i> L. | Gurhal, Shoe Flower | Flower | Apply paste to reduce burning sensation |
| | <i>Abutilon indicum</i> L. | Peeli buti | Leaves and flowers | As a resolvent, analgesic, inflammations, diarrhea, bleeding piles and toothache. |
| Meliaceae | <i>Azadirachta indica</i> (L.) A. Juss. | Nim | Leave, fruit, stem | Leaf extract is used for blood purification and as cooling agent. Fruit extract and pulp are supposed to a tonic for liver and stomach. The paste of leaves is externally applied to remove skin allergy and itching. The same is applied on heads acting as antilice agent |
| Mimosaceae | <i>Acacia nilotica</i> (L.) Deliled. | Desi Kikar | Fruit and seeds | The juice of roots/bark mixed with sugar is given to cure jaundice. Leaves, bark of young ranches, |

| | | | | |
|------------|--|------------------------|--------------------------|--|
| | | | | flowers, gum and unripe pods are taken in equal quantity and made into powder and is given to treat spermatorrhoea and premature ejaculation. The branches are used as tooth stick to strengthen the gums. |
| | <i>Cassia fistula</i> L. | Amaltas, Golden Shower | Seed | Gastric problems |
| | <i>Acacia nilotica</i> (L.) Delile. | Kekar, Gum Arabic | Pod | Gonorrhoea |
| | <i>Dalbergia sissoo</i> Roxb.ex DC. | Tali, Rosewood | Bark | Nosebleed |
| | <i>Dalbergia sissoo</i> Roxb.ex DC. | Tali, Rosewood | Bark | Nosebleed |
| Moraceae | <i>Morus nigra</i> L. | Kala Toot, Mulberry | Root, leaf and fruit | Bad thorax, stomach worms. |
| | <i>Ficus religiosa</i> L. | Pipal | Bark, Fruit, Seed | Asthma, urinary problems, constipation and vomiting |
| Myrtaceae | <i>Eucalyptus camaldulensis</i> Dehnh. | Sofeda, Eucalyptus | Leaf | Common cold, nose infections, common cold |
| | <i>Psidium guajava</i> L. | Amrood, Guava | Fruit | Improvement of appetite, and stomach problems. |
| | <i>Syzygium cumini</i> (L.) Skeels | Jaman, Jambolan | Seed | Diabetes. |
| | <i>Eucalyptus globules</i> | Sufaيدا | Leaves, Seeds | Cold, cough, throat lozenges, malaria and toothache. |
| Pinaceae | <i>Pinus roxburghii</i> Sarg. | Chir | Bark, Resin | Burns and scalds, boils, cough and gastric troubles. |
| Poaceae | <i>Saccharum spontaneum</i> L. | Sarrout | Whole plant | Improvement of appetite and in the treatment of abdominal pain |
| | <i>Zea mays</i> L. | Makai | Stigma of female flowers | Urinary disorders, Bladder cleaning and kidney disorders. |
| | <i>Avena sativa</i> Linn. | Jao, Oat | Seeds | Tension and skin allergies. |
| Punicaceae | <i>Punica granatum</i> L. | Anar, Pomegranate | Exocarp of fruit | Dysentery and menstrual irregularities. |
| Rhamnaceae | <i>Ziziphus jujuba</i> Mill. | Baer, Jujube | Leaf and fruit | Skin infections where pus is present and iron deficiency |
| Rosaceae | <i>Rosa indica</i> L. | Gulab, Rose | Flower and seed | Eye disorders and heart disease. |
| Rutaceae | <i>Citrus limon</i> (L.) Burm. f. | Nimboo, Lemon | Fruit | Toothpowder for teeth diseases and in infections Fruit is used as a tonic, blood purifier and appetizer and given to the constipating patients. The fruit pericarp is mixed in sweet dishes due to their fragrance. |
| | <i>Citrus grandis</i> (L.) Osbeck | Chakotra | Fruit | The smell of pericarp is supposed to prevent vomiting during road journey. |
| | <i>Murraya exotica</i> | Marva | Leaves & roots | Anthelmintic, blood disorders, skin diseases, carminative, tonic, purgative, Stomachic, leprosy, |

| | | | | |
|---------------|---------------------------------------|---------------------------|---------------------|---|
| | <i>Citrus sinensis</i> (L.) Osbeck | Kinno/Malta | Fruit | diarrhoea and dysentery. Fruit is used as a tonic and appetizer and given to the constipating patients. The fruit pericarp is cooked for the preparation of sweet dishes (<i>Mutanjan/Zarda</i>), due to their fragrance. The pericarp of fruit is rubbed and snuffed to stop vomiting; a common complaint happened during road journey. |
| Solanaceae | <i>Withania coagulens</i> L. | Chota ak | Fruit & Seed | Digestive disorders, gastritis, diabetes and blood purification. |
| | <i>Solanum nigrum</i> L. | Kainch Mainch, Nightshade | Leaf | Abnormal and painful secretions from ears. Leaves are smoked to relieve asthma. The paste of leaves is applied to hairs to expel lice (anti-lice). Slightly warm leaves are applied externally for removing swellings. The plant is boiled in water and is applied in home to expel insects (insecticide) and the juice is used in insect bite |
| | <i>Datura alba</i> Nees | Dhatura | Whole plant, leaves | |
| Typhaceae | <i>Typha latifolia</i> | Typha | Leaves and Pollens | Astringent, diuretic, sedative and anticoagulant. It is used in the treatment of kidney stones and painful menstruation |
| Vitaceae | <i>Vitis vinifera</i> L. | Angoor | Fruit | Fruit is used as a general tonic and laxative. The fresh fruit is given to patients suffering from typhoid fever. |
| Zingiberaceae | <i>Elettaria cardamomum</i> Maton. | Ilaichi | Seeds and fruit | Fruit is boiled in water and given to treat nausea and vomiting. |

4. Discussion:

Herbal medicines are used since ancient times but now a day's new trends make (medicinal plants) more popular. Plants show, Medicinal effect because they have specialized chemicals secondary metabolites like phenolics, alkaloids, terpenes, and alcoholic compounds. In the present investigation, many herbal plants were investigated because of their medicinal effects. The 80 percent of people relies on the herbs plants for the manufacturing of medicines that are used for the treatment of various diseases (Joudi *et al.*, 2010). Due to the lack of modern communications, as well as poverty, ignorance and unavailability of modern health facilities, most people especially rural people are still forced to practice traditional medicines for their common day ailments (Azaizeh *et al.* 2003). Most of these people form the poorest link in the trade of medicinal plants (Khan, 2002).

5. Conclusion:

Rapid human population growth is a principal cause of diminishing the medicinal plant vegetation. Certain species are disappearing rapidly. The documentation and survey indicate that Tehsil Kharian, District Gujrat-Punjab has very high potential flora for medicinal purpose. Therefore, it is an urgent need for our local communities and educated peoples that they should be directly involved in creating the awareness about medicinal plant vegetation and their significance.

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Air Contamination in Metropolitan Environs and Wellbeing Related Snags: A Case Study

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Abstract

This study explores the effects of ambient air pollutants on urban environment and human health using both qualitative and quantifiable assessment methods. The investigation of (one-hour average) particulate matter (PM₁₀μm, PM_{2.5}μm) and toxic gases (CO, SO₂, and NO₂) revealed that the study area (Quetta city) was obviously contaminated by air pollutants. Seasonally air quality indexes (AQI) of all the investigated air pollutants were pragmatic highest during autumn and lowest during winter season. Individually, CO remained moderate to harmful for the people whose belong to sensitive group, SO₂ was found good to moderate, while NO₂ was testified moderate to harmful for the people of sensitive group. SPM₁₀μm and SPM_{2.5}μm fall in the categories of very harmful for all the peoples of the urban areas during all the four seasons. Data regarding impact of air pollutants on human health exhibited that the percentage of peoples suffering from, Headache, Eye irritation, Nose/ Throat and Chest/Lungs/Asthma diseases were found highest as compared to Blood pressure and heart diseases in the urban area.

Keywords: AQI; Particulate Matter; Toxic Gases; Human Health; Quetta City

1. Introduction:

Comprehensive information concerning the effects of air pollutants on human being and surroundings is an obligation for the development of efficient policies to decrease the antagonistic crash of ambient air pollution. Metropolitan air contamination has developed a harsh warning to human health, plants and animals, due to which it is getting a great courtesy of the world nowadays. It is one of the supreme severe snags of the biosphere, mostly rising by over inhabitants, growing road traffic and development (Odilara *et al.*, 2006). At present, more than 600 million people mouthful of air in urban sites worldwide are being bare to hazardous limits of traffic made air pollution (Cacciola *et al.*, 2002). Around 30 % of the respiration illnesses are associated to individual introduction to high level ambient air pollutions (WHO, 2000). At universal level, more than 0.5 million demises per year are

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© 2021 (Accepted for publication in September 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

because of disclosure to ambient contents of PM as stated by AQEG, (2005). At established countries PM emanations are primarily accountable for breathing wellbeing difficulties (Yang, 2002; Shendell & Naehar, 2002; Wang *et al.*, 2003). There are several motives, which accountable for the urban air contamination such as amplified urbanization, absence of consciousness, use of fuels with deprived recital, greater liability on remnant fuels, unproductive rules and guidelines for traffic release and growing amount of motorized as stated by Chauhan & Joshi, (2008) and Joshi & Chauhan, (2008). Contamination of air interrupts the ecological circumstances and can be distinct as disparity in any ecological basic from the worth that would have happened without humanoid action (Tripathi & Gautaum, 2007). Anonymous, (2008) described that all kinds of burning are accountable for the emission of air sand particles in to the atmosphere. These may comprise SO, NO₂, CO₂ and suspended particulates matters, by lesser amounts of poisonous metals, carbon-based particle sand dangerous isotopes.

Quetta city is situated in a thickly populated portion of the mountainous area. It is located in a river valley near the Bolan Pass, which has been used as the way of choice from the coast to central Asia, in going through Afghanistan's area. Quetta, the capital of Balochistan, lies between 29°- 52' to 30°- 15' latitude and 66°- 55' to 67°- 48' longitude, the city is about 1692 meters upstairs sea level. The weather of the area is dry with cold winter, warm summer and has been categorized as temperate desert scrubland type with Mediterranean trend (Qadir, 1968). It has three big crags such as; Chiltan, Zarghoon and Koh-e-Murdar that seem to brood upon this lovely city. Their copper red and russet rocks and crests that are pulverized with snowflake in winters enhance enormous attraction to the city.

1.1. Objectives:

- To investigate the actual status of particulates and gaseous pollution in the metropolitan environs (Quetta city).
- To assess the disparity hazards conferring to detailed events for numerous health connected consequences between the overall inhabitants.

2. Materials and Methods:

2.1. Site Selection and Air Sampling:

After initial studies and pitch tours of urban zone (Quetta city) and surrounding areas, different stretches from Hazargangi Chiltan National Park and Wali Tangi Zarghoon area (Control sites) were selected for the sampling. Air sampling was done for the estimation of air born dust (SPM 2.5 & 10 µm) concentration and gaseous contents (CO, SO₂, NO₂). A walk-through review of different localities of Quetta city was carried out. On the basis of valuation of traffic compactness (vehicle count) and the quantity of notice able car exhaust fumes/smoke and roadside dust, eleven different locations were selected, and samples was taken seasonally. These locations were in cluded; Quetta cantt, Hazara town, Shabaz town, Manan chowk, Meezan chowk, Satellite town (Mini market block 3), Jinnah Road (near science college), Zarghoon road (Railway crossing), Mitha chowk (Abdul Satar Road), Golimar chowk (Bolan Medical Complex), Sariab road (Near fly over). For the comparison two control areas such as; hazargangi chiltan national park (located near Quetta at a distance of 18Km on Quetta Mastung road towards N W at 30° 07'N longitude, 66° 58 'E and 1700 m altitude) and wallitangi zarghoon area (situated "approximately 20 km east of Quetta at an elevation of about 8,350 ft) were selected for the sampling and then average was made.

2.2. Analysis of Air Sampling:

Flying PM (SPM 2.5 & 10 µm) was projected for 1 hour at every 30 days interval. For dimension Respire able Dust Sampler (RDS) were used as per methods (Ogunsola *et al.*, 1994; UNEP/WHO, 1994b), which operated at an average flow rate of 1.0 - 1.5m³/min pre-weighted glass fiber filters (GF/A) of Whatman. The device for particulates was kept at a height of 2 meters from the ground (Mueller & Smith, 1991). The content of NO₂, SO₂ and CO was assessed by standard modified method of Jacobs & Hochheiser, (1958) and West & Gaeke, (1956). The concentration of particulates matter and gases data was recorded in µg/m³/hr, ppm and ppb.

2.3. Air Quality Index (AQI):

Air Quality Index of discrete pollutants was intended with the concentration values using the computer based AQI calculator and following the method given by Rao & Rao, (1998).

2.4. Gradation of Air Quality Index:

The spectrum of AQI was divided as five grades or classes [good (G), moderate (M), harmful for sensitive people (Hsp), harmful for all the people (Hap) and very harmful (Vh)] for all the population referring to a previous study (Rao & Rao, 1998).

2.5. Impact Analysis on Human Health:

Effects of dust on hominid were determination by an opinion poll. A questionnaire was prepared and distributed among those workers (Shopkeepers) whose were directly exposed to the air pollutants on designated eleven (11) roads (from where air samples were taken) by using randomize sampling method. Data about different case registered in unlike diseases groups such as Heart patients, Nose/Throat patients, Blood pressure, Chest/Lungs/Asthma patients, Eye irritation patients and headache problems was also collected from different hospitals of the city. Data was composed on monthly basis and than average was made (data of the adult male patients was only taken).

3. Results:

The air parameters recorded from urban area (Quetta city) provided knowledge about how the air converts dirty and suffocative during daytime due to anthropological activities. So, the dust and gases pollutants anticipated a cloudy arrival all over city sites and transparency of the area is significantly lost (Fig 1).

3.1. Carbon Monoxide (CO):

Average AQI of CO during the study period was in between 74 – 108 ppm top to bottom respectively, with seven locations including Quetta cantt, Hazara town, Shabaz town, Manan chowk, Satellite town and Sariab road, indicated moderate (M) index and other remaining four locations such as Jinnah Road, Zarghoon road, Metha chowk and Golimar chowk, showed harmful for sensitive people (Hsp) as illustrated in Table 1. Overall mean values of CO at all the investigated sites during spring, summer and winter season were established 76, 98 and 74 ppm, in that order with standing moderate values, while autumn showed harmful index for sensitive peoples (118ppm). However, control site comprised good (G) (40 ppm) index during spring and winter and moderate with summer and autumn (50 & 56 ppm) (Table 1).

3.2. Sulphur Dioxide (SO₂):

Average value of AQI for SO₂ was noted in the range of 32 – 51 top to bottom respectively, out of 11 locations eight sites such as Quetta Cantt, Hazara Town, Shabaz Town, Sariab road, Manan Chowk, Mezan Chowk, Setalite Town and Jinnah Road showed good (G) index and other three locations (Zarghoon road, Metha chowk and Golimar chowk) indicated moderate (M). Overall mean AQI of SO₂ at all city sites were recorded to be 39, 50 and 36 ppb, indicating spring, summer and winter with good (G) index, respectively and autumn (51 ppb) moderate (M) as illustrated in Table 1. In the present study control sites showed good (G) AQI for SO₂ in all the seasons.

3.3. Nitrogen Dioxide (NO₂):

Average AQI for NO₂ during the study period was found in the ranged 50 – 97 ppb, from top to bottom respectively. Out of eleven locations, ten (Hazara town, Shabaz town, Sariab road, Manan chowk, Mezan chowk, Setalite town, Jinnah Road Zarghoon road, Metha chowk and Golimar chowk) designated moderate (M) and remaining one Quetta cantt showed good (G) AQI. Overall mean AQI of NO₂ at urban sites were reported as 76, 99 and 73 ppb with moderate spring, summer and winter respectively, while autumn performed (102 ppb) harmful for sensitive peoples as demonstrated in Table 1.

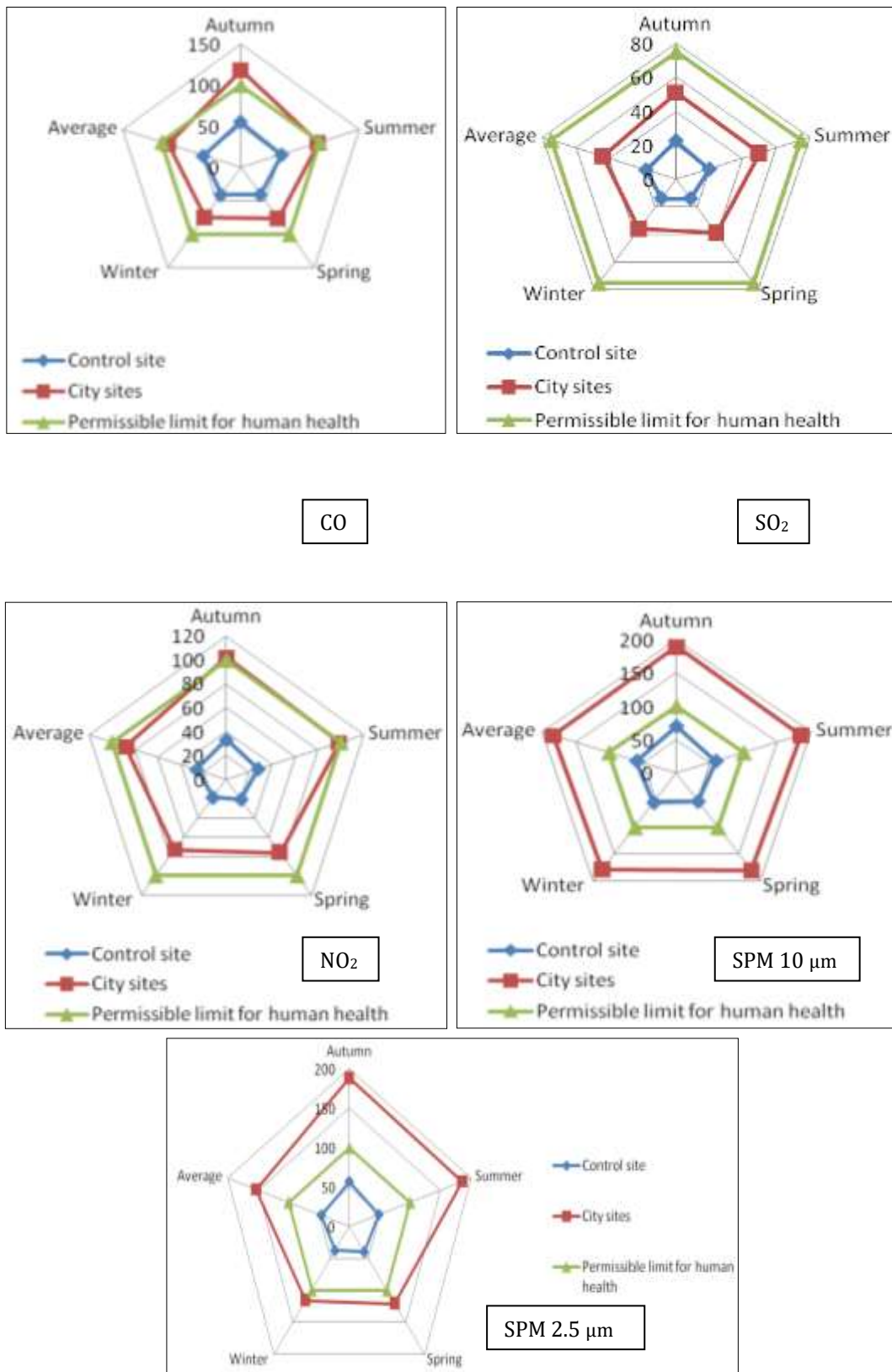


Fig. 1: Comparison of Air Quality Index (AQI) of different air pollutants (CO, SO₂, NO₂, SPM 10 & 2.5 µm) during different seasons between city site, control area and permissible limits

3.4. Suspended Particulate Matters (SPM 10 & 2.5 µm):

Average AQI of SPM10 was exhibited in the ranged 144 – 219 $\mu\text{g}/\text{m}^3$ top to bottom respectively, with three locations such as Quetta cantt, Hazara town and Shabaz town demonstrated harmful for sensitive peoples. Other five locations such as Manan chowk, Mezan chowk, Jinnah Road, Metha chowk and Setalite town, showed harmful for all the people (Hap) and remaining three sites (Zarghoon road, Sariyab road and Golimar chowk) stand for very harmful (Vh). Overall mean values of AQI for SPM10 μm from city sites were accounted 108,185, 190 and 178 $\mu\text{g}/\text{m}^3$ with harmful for all the people that was highly significantly high then control site which showed 52, 59, 70 and 54 $\mu\text{g}/\text{m}^3$ with moderate (M) index values during spring, summer, autumn and winter, correspondingly (Table 1). The concentrations of SPM10 μm from urban area were enormously high when compared to the permitted limits given by WHO, 2006 (Fig 1). Average values of AQI for SPM 2.5 μm was recorded between 94 – 175 top to bottom respectively. Out of 11 locations only one location (Quetta cantt) be evidence for moderate (M) index. Other three such as Hazara town, Shabaz town and Jinnah Road indicated harmful for sensitive peoples and remaining seven locations (Sariyab road, Manan chowk, Setalite town, Metha chowk, Zarghoon road, Mezan chowk and Golimar chowk) represented harmful for all the people (Hap) as illustrated in Table 1. Overall average AQI of SPM 2.5 at all the urban sites was found 121 and 116 $\mu\text{g}/\text{m}^3$ with harmful for sensitive peoples during spring and winter. Whereas during summer and autumn index values was 186 & 189, with harmful for all the people (Hap). However, control site confirmed good (G) AQI values during spring, summer & winter and moderate during autumn (Table 1).

Table 1: Air Quality Index of CO in the atmosphere of Quetta city during different seasons of the year.

| <i>Locations</i> | <i>Spring</i> | <i>Summer</i> | <i>Autumn</i> | <i>Winter</i> | <i>Average</i> |
|--|---------------|---------------|----------------|---------------|----------------|
| <i>Carbon Monoxide (CO)</i> | | | | | |
| Quetta Cantt | 63 M | 83 M | 89 M | 60 M | 74 M |
| Hazara Town | 69 M | 85 M | 92 M | 66 M | 78 M |
| Shabaz Town | 69 M | 89 M | 97 M | 66 M | 80 M |
| Manan Chowk | 73 M | 118 Hsp | 116 Hsp | 71 M | 95 M |
| Sariyab Road | 81 M | 100 M | 123 Hsp | 78 M | 96 M |
| Mezan Chowk | 67 M | 121 Hsp | 125 Hsp | 73 M | 97 M |
| Setalite Town | 78 M | 108 Hsp | 128 Hsp | 76 M | 98 M |
| Jinnah Road | 81 M | 111 Hsp | 131 Hsp | 78 M | 101 Hsp |
| Zarghoon Road | 80 M | 118 Hsp | 138 Hsp | 77 M | 103 Hsp |
| Metha Chowk | 83 M | 104 Hsp | 142 Hsp | 80 M | 102 Hsp |
| Golimar Chowk | 85 M | 118 Hsp | 147 Hsp | 83 M | 108 Hsp |
| Mean | 76 M | 098 M | 118 Hsp | 74 M | 092 M |
| <i>Mean value of Control Site</i> | 40 G | 52 M | 56 M | 40 G | 047 G |
| <i>Sulphur Dioxide (SO₂)</i> | | | | | |
| Quetta Cantt | 24 G | 39 G | 39 G | 24 G | 32 G |
| Hazara Town | 26 G | 41 G | 41 G | 26 G | 34 G |
| Shabaz Town | 27 G | 40 G | 41 G | 26 G | 34 G |
| Sariyab Road | 41 G | 52 M | 54 M | 41 G | 47 G |
| Manan Chowk | 43 G | 54 M | 54 M | 43 G | 49 G |
| Mezan Chowk | 44 G | 54 M | 55 M | 43 G | 49 G |
| Setalite Town | 44 G | 52 M | 55 M | 43 G | 49 G |
| Jinnah Road | 44 G | 54 M | 55 M | 43 G | 49 G |
| Zarghoon Road | 44 G | 54 M | 55 M | 44 G | 50 M |
| Metha Chowk | 46 G | 54 M | 55 M | 44 G | 50 M |
| Golimar Chowk | 46 G | 55 M | 55 M | 46 G | 51 M |
| Mean | 39 G | 50 G | 51 M | 36 G | 44 G |
| <i>Mean value of Control Site</i> | 14 G | 20 G | 23 G | 14 G | 18 G |
| <i>Nitrogen Dioxide (NO₂)</i> | | | | | |
| Quetta Cantt | 36 G | 62 M | 66 M | 33 G | 50 G |
| Hazara Town | 49 G | 64 M | 68 M | 47 G | 57 M |
| Shabaz Town | 50 G | 66 M | 72 M | 48 G | 59 M |
| Setalite Town | 85 M | 103 Hsp | 104 Hsp | 82 M | 94 M |
| Sariyab Road | 85 M | 103 Hsp | 104 Hsp | 82 M | 94 M |

| | | | | | |
|---|----------------|----------------|----------------|----------------|----------------|
| Manan Chowk | 87 M | 103 Hsp | 104 Hsp | 84 M | 95 M |
| Jinnah Road | 87 M | 103 Hsp | 104 Hsp | 83 M | 95 M |
| Mezan Chowk | 89 M | 103 Hsp | 105 Hsp | 86 M | 96 M |
| Metha Chowk | 88 M | 103 Hsp | 105 Hsp | 88 M | 96 M |
| Golimar Chowk | 88 M | 103 Hsp | 105 Hsp | 86 M | 96 M |
| Zarghoon Road | 90 M | 103 Hsp | 105 Hsp | 88 M | 97 M |
| Mean | 76 M | 99 M | 102 Hsp | 73 M | 88 M |
| <i>Mean value of Control Site</i> | 21 G | 28 G | 033 G | 19 G | 26 G |
| <i>Suspended Particulate Matters (SPM) 10 µm</i> | | | | | |
| Quetta Cantt | 140 Hsp | 147 Hsp | 150 Hsp | 139 Hsp | 144 Hsp |
| Hazara Town | 143 Hsp | 149 Hsp | 153 Hap | 141 Hsp | 147 Hsp |
| Shabaz Town | 146 Hsp | 151 Hap | 155 Hap | 144 Hsp | 149 Hsp |
| Manan Chowk | 186 Hap | 192 Hap | 197 Hap | 185 Hap | 190 Hap |
| Mezan Chowk | 189 Hap | 194 Hap | 198 Hap | 187 Hap | 192 Hap |
| Jinnah Road | 191 Hap | 196 Hap | 200 Hap | 188 Hap | 194 Hap |
| Metha Chowk | 193 Hap | 198 Hap | 204 Vh | 192 Hap | 197 Hap |
| Setalite Town | 194 Hap | 198 Hap | 208 Vh | 192 Hap | 198 Hap |
| Zarghoon Road | 197 Hap | 202 Vh | 215 Vh | 194 Hap | 202 Vh |
| Sariab Road | 198 Hap | 207 Vh | 221 Vh | 196 Hap | 206 Vh |
| Golimar Chowk | 211 Vh | 223 Vh | 235 Vh | 204 Vh | 219 Vh |
| Mean | 180 Hap | 185 Hap | 190 Hap | 178 Hap | 183 Hap |
| <i>Mean value of Control Site</i> | 052 M | 059 M | 070 M | 054 M | 059 M |
| <i>Suspended Particulate Matters (SPM) 2.5 µm</i> | | | | | |
| Quetta Cantt | 78 M | 106 Hsp | 113 Hsp | 72 M | 94 M |
| Hazara Town | 90 M | 113 Hsp | 121 Hsp | 82 M | 102 Hsp |
| Shabaz Town | 98 M | 116 Hsp | 122 Hsp | 89 M | 107 Hsp |
| Jinnah Road | 130 Hsp | 206 Vh | 112 Vh | 125 Hsp | 144 Hsp |
| Sariab Road | 127 Hsp | 206 Vh | 209 Vh | 130 Hsp | 168 Hap |
| Manan Chowk | 130 Hsp | 209 Vh | 211 Vh | 126 Hsp | 169 Hap |
| Setalite Town | 131 Hsp | 206 Vh | 211 Vh | 126 Hsp | 169 Hap |
| Metha Chowk | 131 Hsp | 208 Vh | 213 Vh | 126 Hsp | 170 Hap |
| Zarghoon Road | 132 Hsp | 210 Vh | 213 Vh | 128 Hsp | 171 Hap |
| Mezan Chowk | 132 Hsp | 221 Vh | 215 Vh | 128 Hsp | 174 Hap |
| Golimar Chowk | 134 Hsp | 221 Vh | 215 Vh | 130 Hsp | 175 Hap |
| Mean | 121 Hsp | 186 Hap | 189 Hap | 116 Hsp | 153 Hap |
| <i>Mean value of Control Site</i> | 040 G | 049 G | 057 M | 038 G | 046 G |

G = Good, **M**= Moderate, **Hsp**= Harmful for sensitive peoples, **Hap**= Harmful for all the peoples, **Vh**= Very harmful, **Hsg**= Harmful for sensitive group, **Ha**= Harmful for all

3.5. Impact of Air Pollution on Human Health:

Total average data taken from different hospitals of Quetta city during 2013 about the different diseased cases, registered was prominent as 2.5 % Heart cases, 18.1% Nose/Throat cases, 7.6 % Blood pressure cases, 26.3 % Eye irritation cases, 24.0 % Chest/Lungs/Asthma cases and 21.6% were Headache cases (Table 2). Questionnaire's survey indicated that the percentage of people suffering in Heart, Nose/Throat, Blood pressure, Chest/Lungs/Asthma, Eye irritation, and Headache diseases were 1.8, 19.3, 7.0, 22.9, 26.1 and 21.1 %, respectively (Table 2).

Table 2: Percentage of patients registred in different hospitals and Questionnaires report of study area during various seasons of the year and their correlation coefficient.

| Data Reports | Seasons | Patients | | | | | |
|---------------|---------|----------|-------------|----------------|--------------------|----------------|----------|
| | | Heart | Nose/Throat | Blood Pressure | Chest/Lungs/Asthma | Eye irritation | Headache |
| Hospital Data | Spring | 2.5 | 15.4 | 6.7 | 23.5 | 24.5 | 21.4 |

| | | | | | | | |
|-------------------------|-----------------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|
| | Summer | 2.5 | 18.7 | 7.5 | 25.6 | 26.5 | 22.5 |
| | Autumn | 2.9 | 20.9 | 9.6 | 28.2 | 30.4 | 23.6 |
| | Winter | 2.2 | 17.4 | 6.4 | 18.5 | 23.8 | 18.8 |
| | Mean | 2.5 ±0.3 | 18.1±2.3 | 7.6±1.4 | 24.0±4.1 | 26.3±3.0 | 21.6±2.1 |
| Questionnaire Data | Spring | 1.7 | 18.0 | 7.0 | 22.4 | 25.2 | 20.2 |
| | Summer | 2.0 | 20.1 | 7.4 | 23.2 | 26.5 | 22.2 |
| | Autumn | 2.5 | 23.0 | 8.2 | 25.5 | 28.3 | 24.0 |
| | Winter | 1.0 | 16.2 | 5.2 | 20.3 | 24.4 | 20.5 |
| | Mean | 1.8±0.5 | 19.3±2.5 | 7.0±1.0 | 22.9±1.9 | 26.1±1.5 | 21.7±1.5 |
| Statistical Data | Co-coefficient | 0.96*** | 0.82*** | 0.82*** | 0.98*** | 0.99*** | 0.81*** |

*** = highly significant

Correlation coefficient of all the diseases categories showed highly significantly relationship between data collected from different Hospitals of Quetta city and Questionnaire report. Present study also exhibited that all the diseases categories were with highest percentage during autumn and lowest during winter season (Table 2). This might be due to that the Quetta city is surrounded by different high-altitude mountains, which check the traffic related air pollutants from dispersion and these pollutants remain long time in the urban area and cause different diseases. Further that Table 3 indicated highly significantly correlation between different diseases categories and air pollutants (CO, SO₂, NO₂, and SPM10 & 2.5 μm).

Table 3: Correlation coefficient between air pollutants and data recorded from different Hospitals of Quetta city and Questionnaire about different diseases categories.

| Disease Categories | CO | SO ₂ | NO ₂ | SPM 10 μm | SPM 2.5 μm |
|---|------|-----------------|-----------------|-----------|------------|
| <i>Data recorded from different Hospitals</i> | | | | | |
| Heart Patients | 0.89 | 0.79 | 0.78 | 0.92 | 0.74 |
| Nose/Throat Patients | 0.92 | 0.80 | 0.85 | 0.87 | 0.84 |
| Blood pressure Patients | 0.97 | 0.83 | 0.85 | 0.97 | 0.82 |
| Chest/Lungs/Asthma Patients | 0.88 | 0.91 | 0.88 | 0.93 | 0.86 |
| Eye irritation Patients | 0.98 | 0.87 | 0.88 | 0.98 | 0.86 |
| Headache Patients | 0.87 | 0.91 | 0.88 | 0.92 | 0.86 |
| <i>Data recorded from Questionnaire</i> | | | | | |
| Heart Patients | 0.91 | 0.91 | 0.89 | 0.95 | 0.86 |
| Nose/Throat Patients | 0.98 | 0.93 | 0.93 | 0.99 | 0.90 |
| Blood pressure Patients | 0.83 | 0.87 | 0.84 | 0.89 | 0.81 |
| Chest/Lungs/Asthma Patients | 0.93 | 0.88 | 0.87 | 0.96 | 0.84 |
| Eye irritation Patients | 0.99 | 0.92 | 0.93 | 0.99 | 0.90 |
| Headache Patients | 0.99 | 0.90 | 0.93 | 0.97 | 0.91 |

4. Discussion:

The greater contents of AQI at urban sites might be due to the emission from vehicles and other incomplete combustion of a variety of fuels such as wood, coal, charcoal, oil, paraffin, propane, natural gas and trash. Present consequences were also supported by Yousfzai *et al.* (1970; 1984 & 1987) and Chotani *et al.* (1975) on studies made at the atmosphere of Karachi. This study also indicated that the concentration of CO in urban area and non polluted sites (control areas) was within the permitted limits during spring, summer and winter seasons, while it was more during autumn when compared to WHO, (2006) standards for the developing countries (Fig.1).

High contents of SO₂ in the urban areas with respect to control sites might be due to different reasons such as flaming of coal and fuel oil, vehicle tires and rubbers etc. Therefore, it is tacit that the poisonous gases in ambient air like CO, NO₂ and SO₂ are consequence of vehicular emissions. This idea was also supported by Agbaire & Esiefarienrhe, (2009) and Joshi *et al.* (2009). They indicated that the entire ignitions are accountable for the discharge of gases and particles into the air. SO₂ recorded during this study was within allowable limits as described by WHO, 2006 standard at all the sites and was not hazardous for wellbeing of the study areas. In present study, control site indicated good (G) AQI during all the seasons. Similar observation was also reported by Agbaire & Esiefarienrhe, (2009) and Joshi *et al.* (2009). Presented observations also exhibited that the concentrations of NO₂

estimated from polluted sites were within the permissible limits during spring, summer and winter season, while in autumn season it was more than limit when compared with WHO, (2006), standards.

The concentrations of SPM 2.5 μm estimated from urban sites were more than the WHO's permissible limits in all seasons except spring (Fig. 1). This might be due to high rate of dust fall (Particulate matters) generated by high traffic density, use of Iranian diesel and petrol oils, not proper cleaning of the roads and emissions from tyres, which are the main sources of air pollution particularly atmospheric suspended particulate matter. Similar observation was also reported by Cacciola *et al.* (2002), they found that United Nations estimated that more than 600 million people in urban areas worldwide were exposed to unsafe level of traffic-generated atmospheric particulates. Moreover NEPC, (1998); Schwartz *et al.* (1996) and Borja-Aburto *et al.*, (1998) reported that the atmospheric particulate matters (PM) with aerodynamic diameter $<10 \mu\text{m}$ (PM 10) or $<2.5 \mu\text{m}$ (PM 2.5) are the substantial anxiety for community health. A considerable body of research already supports these assumptions. For example, several studies (Brunekreef *et al.*, 1997; Hoek *et al.*, 2002; Livingstone *et al.*, 1996; Venn *et al.* 2001) have demonstrated higher rates of respiratory illness and symptoms, and reduced lungs function in people living near major roadways and WHO, (2000) reported that about 30 % of the respiratory diseases are related to personal exposure to high level ambient PM concentrations. At global scale, more than 0.5 million deaths per year are due to exposure to ambient PM concentrations (AQEG, 2005). In developed countries, PM emissions are mainly responsible for respiratory health problems (Yang, 2002; Shendell & Naeher, 2002; Wang *et al.*, 2003). They also indicated that the main sources for ambient PM concentrations at urban roadways are vehicle exhausts, emissions from tyre and brake wear and re-suspension of road dust.

5. Conclusion:

In the present study, the seasonal cycle of AQI of CO, SO₂, NO₂, SPM 10 and 2.5 μm concentrations emitted from heterogeneous traffic in Quetta city was investigated near an urban roadway. The data of AQI revealed that the air pollutants in city areas were so high that the life of peoples is in danger, whose belong to sensitive group. Seasonally investigation indicated that autumn remained more polluted than other seasons. Moreover, if individual air pollutant is classified according to their effects and concentration than following categories are formed: (a) CO concentration remained moderate to harmful for the people of sensitive group in city area. (b) SO₂ concentration was found good to moderate but significantly more than control site. (c) NO₂ concentration was reported moderate to harmful for the people of sensitive group. (d) SPM10 μm concentration falls in the categories of very harmful for all the population. (e). SPM 2.5 μm concentration is also considered harmful for very harmful for all the population.

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Impact of Different Shading Color Nets on Growth, Yield and Fruit Quality of Tomato (*Lycopersicum esculentum* L.) in Cold Climatic Conditions of Balochistan

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Abstract

An experiment was conducted to study the influence of various coloured shade-nets on yield and quality of Tomato (*Lycopersicum esculentum* L.). Plants were sown under different coloured shade-nets, viz. red, green, black, and white along with control (without shade net). Various weather parameters and plant development parameters were analyzed at different crop growth stages. Coloured shade net have special optical properties and also effect the microclimate. Plants grown under coloured shade-nets gave satisfactory result in terms of plant height, number of leaves, biomass, leaf area, photosynthetic rate, harvest index etc. compared to control. All the shade-nets decreased heat, light intensity and improved relative humidity. Red and white shade-nets gave higher photosynthetically active radiation (PAR, $\mu\text{mol}/\text{m}^2/\text{s}$) and transmittance than other coloured nets. Plants grown under red and white shade-nets show better result in term of biological and morphological process. The harvest index was better under red shade net. Red and white shade-nets were found best in enhancing plant and weather parameters and hence they can be used in place of the commercially used green shade net for better yield of Tomato.

Keywords: *Lycopersicum esculentum* L.; Color shade net; Leaf area; Transmittance; Quality

1. Introduction:

Color nets represent new agro-technological concept, which no longer only have special optical properties that permit the manipulation of light, but additionally have the gain of influencing the microclimate to which the plant is uncovered and provide functional safety against immoderate radiation, insect pests and environmental adjustments (Shahak *et al.*, 2004). High solar radiation, warmth strain, drought, dehydrating winds and hailstorms are some of the primary environmental barriers to superior productivity and dietary quality of discipline grown

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© 2021 (Accepted for publication in October 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

plants. In conventional vegetable-producing regions, tomato and pepper planting in a secure environment has multiplied to prevent seasonality in the accessibility of fruit. To manage the air temperature, air flow and mist irrigation, shading paint and outside shading nets are generally implemented (López-Marín *et al.*, 2012; Ombódi *et al.*, 2016). This practice is already famous in Europe, particularly in Israel (Fallik *et al.*, 2009; Kong *et al.*, 2013) and different Mediterranean countries (Díaz-Pérez, 2014) as well as in South Africa (Mashabela *et al.*, 2015; Selahle *et al.*, 2015). The use of shading nets has also known to be very popular in Serbia due to the very high (35~forty°C) temperatures within the summer season (Ilić *et al.*, 2011 & 2015; Milenković *et al.*, 2012). The netting is either implemented via itself over net-residence construction, or pinned with greenhouse technologies (Ilić *et al.*, 2015). Temporary shading, carried out only throughout sunny periods, is taken into consideration to be much less deleterious than regular shading. The image selective color netting generation has received popularity throughout the world for many years, because it has the capacity to improve light at the same time as enhancing the healthy crop at cutting. Manipulation of mild quality is currently implemented in horticulture through photograph-selective netting or films to improve great yield, quality and phytochemical components. It is a technology that may be used as an alternative to shield crops from destructive environmental situations excessive sun radiation (Ilić *et al.*, 2011), warmth and drought strain (Meena *et al.*, 2014; Tinyane *et al.*, 2015), wind and hail (Teitel *et al.*, 2008), flying pests (Shahak, 2008), accordingly enhancing crops, yield. This technology has the capacity to prolong shelf existence of produce, thereby lowering postharvest losses. Shade nets are characterized through different shape, by using radiometric and phenotypic properties and mechanical traits (Castanello *et al.*, 2008). Modern coloration nets are made from woven polypropylene or knitted polyethylene substances with various dimensions of fibers and holes to attain specific coloration stages (Castellano, 2008; Appling, 2012). The fraction of light that passes through the holes in the shade cloth sustain its quality, while the light reaching the threads is spectrally changed and spread on exit (Appling, 2012). The combination of light-scattering and spectral management can alter desirable plant growth properties. Along with light, shade nets may alter environmental variables such as temperatures, wind speed, or relative humidity inside the leaf area (Arthurs *et al.*, 2013).

Tomato is a very sensitive plant, and even a mild variation in any of the climate variables could result in notable changes in growth physiology of the crop resulting in extensive yield loss. It is a relative heat season crop. Plants develop well at the temperature range from 19 to 30°C. It additionally needs lots of sunshine, but low humidity, continuous rain in the hot climate will create growth hurdles along with bacterial wilt, blight, rot and Fruit cracking. In order to produce best result with superior productivity, cherry tomato may be grown under colour net houses. The colored net shed population protects the crop from intense climatic conditions. There are several sorts / hybrids found in cherry tomato (Mantur *et al.*, 2014). Fruits grown under pearl nets display better fruit mass, firmness, chlorophyll content, material, ascorbic acid content and antioxidant scavenging activity (Alkalai-Tuvia *et al.*, 2014; Mashabela *et al.*, 2015). The mixed effects result in better crop yields and decrease effect of decay for the period of post-harvest storage compared to traditional black nets (Fallik *et al.*, 2009; Stamps, 2009; Goren *et al.*, 2011; Shahak, 2014). Pearl shade-nets may additionally improve not only yield, but also postharvest shelf-life of the bell pepper fruit (Goren *et al.*, 2011; Kong *et al.*, 2013; Díaz-Pérez, 2014).

Objectives:

Thus, the primary objective of current study was

- To focused how one of a kind environmental control technologies (coloured shade-nets as net-residence).
- To check out its impact on plant growth parameters, yield and excellent tendencies in tomato cultivated in Killi Gawal (district Pishin) Baluchistan Pakistan.

2. Materials and Methods:

2.1. Experimental site:

Experiment was carried out in Gawal region of Khanozai district Pishin Baluchistan during 2019-20 with *Lycopersicon esculentum* L. under colored shade-nets (white, purple, black and green) treatments (Fig. 1). The micro-environment and manufacturing beneath those shade levels had been compared with the out of doors environment (without coloration shade net taken to manipulation as a control). Weather parameters such as temperature and relative humidity were measured by using pocket weather tracker. Light measurement was performed periodically throughout various growth steps, to display the real light situations to which the plant life was exposed. All the measurements were taken on clean days at mid-day. The light depth was measured via digital

light meter (Extech Instruments, 401025). Transmitted photosynthetically active radiation (PAR) as well as intercepted radiation by using plant in each remedy was measured by using the Line Quantum Sensor (LiCOR-3000). While transmittance within the net changed into calculated as the ratio of the PAR radiation spectra inner internet and outdoor. Leaf area temperature was measured using infrared thermometer. Plant height (cm) was taken up to the tip of 3rd leaf the use of well-known scale and number of matured leaves was also counted throughout various growth steps. Leaf readings to show chlorophyll content checked by a chlorophyll meter (modelSPAD-502) with the aid of averaging the 10-15 readings per -plant. The photosynthesis/ CO₂ uptake rate (µmolCO₂/m₂/s), transpiration (µmol H₂O/m₂/s), stomatal conductance (µmol/m₂/s) and PS₂ efficiency were taken by means of LiCor-6400 Leaf Gas Exchange instrument, i.e., Infra-red gas analyzer (IRGA) (Long et al., 1996). Fresh leaf weight was taken, and then leaf canopy was measured by the use of leaf area meter (Licor-3100). Specific leaf area (SLA) was computed by the usage of fresh weight and leaf area. Harvest index (HI) was calculated as percentage of economic yield to the plant yield whereas vase-life was estimated by placing the stem along with leaves in test tube containing distilled water. The trial was laid out in randomized block design (RBD) and the data were checked accordingly.

2.2. Fertilizer Application and Inter-Cultivation:

Recommended dose of N, P, K fertilizers were applied @ 150:80:80 kg per ha⁻¹ in the form of urea, single super phosphate and potash respectively. Half dose of N, K₂O were applied as split doses at 30 and 50 days after sowing. Intercultural operations like staking, weeding and need based plant protection measures were taken up during crop growth period.

2.3. Harvesting:

Tomatoes were harvested at red ripped stage (Bharambe et al., 2016) and TSS, color and lycopene content were estimated according to the method (Ranganna, 2003).

2.4. Statistical Analysis:

Data manifests means of triplicates (n=3) for physicochemical variables. The values of standard deviation are also calculated for each parameter. Correlation coefficients were calculated using Pearson's technique for significant parameters in various cultivars.

3. Results:

In the present investigation all the shade-nets reduced temperature, light intensity and improved humidity. Green shade net manifested the lowest average temperature (30.2) and control highest (37.4). Highest and lowest relative humidity was noted 17.4 & 12.0% under white and green colour net. The light intensity was 46.94, 40.43, 21.29 and 48.92% of control for White, Red, Black and green nets, respectively (Table 1). The average temperature under different coloured shade nets altered and was known to be higher under control as compared to shade net. heat reduction was at peak (30.2) in green shade net followed by red (32.4), white (34.2) and black (35.7), temperature inside the nets was lower compared to control (37.4) (Table 1). Relative humidity was higher under coloured nets even though temperature was low. Relative humidity was highest under white shade net (17.4), which was followed by black (16.32), red (15.21) and green (12.0) as compared to control. Reduction in radiations resulting from netting affects temperature and RH (Stamps, 1994). Wind speed can be decreased from 10.56 m s⁻¹ in the open field (control) to 2.0 – 3.20 m s⁻¹ under different shade nets (Table 1).

Table 1: Average Air temperature, Relative humidity, wind speed and Light intensity under different shading nets during different months of growing season (after planting).

| Parameters | White | Red | Black | Green | Control | P. Value (0.05) |
|-------------------|------------------------|-----------------------|-----------------------|------------------------|------------|-----------------|
| Avg. Temp. | 34.2 ±1.0 | 32.4 ±1.2 | 35.7 ±2.2 | 30.2 ±1.1 | 37.4 ±2.1 | ** |
| Relative humidity | 17.4 ±1.3 | 15.2 ±1.2 | 16.32 ±0.2 | 12.0 ±0.2 | 10.8 ±0.2 | ** |
| Avg. wind speed | 2.0 ±0.04 | 2.1 ±0.04 | 3.20 ±0.02 | 2.5 ±0.06 | 10.6 ±1.0 | *** |
| Light intensity | 39791±1260 (46.94%) | 34274±767 (40.43%) | 18046±551 (21.29%) | 41463±1381 (48.92%) | 84763±1514 | *** |

± = Standard Deviation

The effect changes according to the porosity (knitting density) of different net cover. Colored nets also decrease wind speeds and wind run, which can influence temperatures, relative humidity and gas concentrations resulting from reductions in air mixing. These changes can influence biological activity of plant. The influence on air movement depends on the porosity and physical sight of the netting in relation to the plants and can be influenced by duration of day, season, and other factors. Plants grown in distinct colored nets had varying growth due to their light influence that affects the plant growth. Plants have been seen to be substantially taller by 47.73 cm² in red net which followed by green (40.35 cm²), black (38.19 cm²) and white (36.26 cm²) over manipulate (34.14 cm²) with better range of No of leaves (235.41) below red net (Table 2) which showed highly significant different. Leaf number is a beneficial trait of growth because it interprets the ability of a crop for generating dehydrated matter in term of the usage of intercepted radiation and amount of photosynthesis. Leaf area was calculated, and results display that leaf area became lowest (10.0 cm²) under control, accompanied by using black (12.8), white (13.68), red (13.6) and it became highest (17.31) in green (Table 2) with slightly significant variation. The non-significant variations were also noted in fresh weight of leaves (1.7 – 2.33g). The SPAD reading became observed to be considerably higher under colored net randed (34.7-41.10) as compared to control (30.3) as proven in Table 2. The specific leaf area (SLA) became highest (8.58) in green escorted by white (8.05), red (6.61) and black shade net indicating thinner (5.85) in color net compared to the outdoor (4.0) environment (Table 2) which showed significant difference. Canopy temperature was found low under colour net as compared to outside the net. It was in the range of (24.3 - 27.31°C). Canopy temperature was also noted significantly different (Table 2). Black coloration-nets had lowest canopy temperature (24.3) and red showed highest (27.3) canopy temperature.

Table 2: The effect of different colored shade-nets on plant height, number of leaves, chlorophyll content (SPAD), canopy temperature, leaf canopy, leaves weight, Specific Leaf canopy (SLC).

| Parameters | White | Red | Black | Green | Control | P Value (0.05) |
|-----------------------------------|-------------|-------------|-------------|-------------|-------------|----------------|
| Plant height | 36.26 ±12.3 | 47.73 ±10.4 | 38.19 ±10.5 | 40.35 ±11.7 | 34.14 ±9.5 | ** |
| No of leaves | 200.2 ±44.7 | 235.4 ±42.2 | 210.3 ±55.4 | 187.3 ±42.3 | 180.3 ±47.3 | *** |
| Leaf area (cm²) | 13.68 ±4.5 | 13.6 ±5.3 | 12.8 ±3.4 | 17.31 ±5.1 | 10.0 ±1.4 | * |
| Fresh leaf wt. (g) | 1.7 ±0.7 | 2.33 ±0.5 | 1.93 ±0.2 | 2.02 ±0.4 | 1.50 ±0.05 | Ns |
| SPAD reading | 41.1 ±2.04 | 38.4 ±2.5 | 37.0 ±3.2 | 34.7 ±2.5 | 30.3 ±1.3 | * |
| Canopy temp. (°C) | 27.0 ±2.31 | 27.3 ±2.7 | 24.3 ±1.0 | 25.12 ±1.2 | 30.12 ±2.3 | ** |
| SLA (cm² /g) | 8.05 | 5.85 | 6.61 | 8.58 | 4.0 | ** |

Dry leaf weight for *Lycopersicon esculentum* L. was found to be significantly higher (4.11 g) under red net and lowest (3.0 g) under control (Table 3). In accordance with study carried by Crowley, (2007) in his investigation fresh and dry weight of leaves were highest under red shade net. Plants grown under red shade nets have maximum harvest index (74.32%) which followed by white and black (70.0 & 67.45%), while control confirmed minimal (62%) harvest index (Fig. 1).

Table 3: Leaf dry weight, gas exchange characteristics and vase-life of *Lycopersicon esculentum* L.

| Parameters | White | Red | Black | Green | Control | P Value (0.05) | |
|-------------------------------------|--|--------------|--------------|--------------|--------------|----------------|----|
| Dry leaf wt.(g) | 4.00 ±0.2 | 4.11 ±0.4 | 3.10±0.4 | 4.0 ±0.2 | 3.00±0.5 | * | |
| Gas exchange characteristics | Photosynthesis (days) rate (µmol CO ₂ /m ² /s) | 7.23±0.4 | 4.31 ±0.3 | 3.12±0.4 | 2.4 ±0.3 | 5.00±0.4 | ** |
| | Stomatal conductance (µmol H ₂ O/m ² /s) | 0.025 ±0.001 | 0.12 ±0.003 | 0.007 ±0.000 | 0.006 ±0.001 | 0.008 ±0.002 | ** |
| | Efficiency of photo system 2 | 0.202 ±0.002 | 0.195 ±0.004 | 0.067 ±0.004 | 0.170 ±0.005 | 0.210 ±0.004 | ** |
| | Transpiration rate (µmol H ₂ O/m ² /s) | 1.10 ±0.02 | 0.62 ±0.04 | 0.35 ±0.01 | 0.34 ±0.02 | 0.42 ±0.01 | ** |
| Vase-life (days) | 20 ±0.12 | 13 ±0.14 | 18 ±0.12 | 12 ±0.15 | 11 ±0.4 | *** | |

Red and white coloration-nets gave higher PAR and transmittance than other shade nets (Table 4). Red and white colored nets have been found to be advanced in improving maximum of the plant characteristics as compared

to green net, black net and control. Red color net has been discovered to be powerful for enhancing plant height, number of leaves, leaf place and Harvest Index (Fig. 1), while other crucial traits have been advanced underneath white.

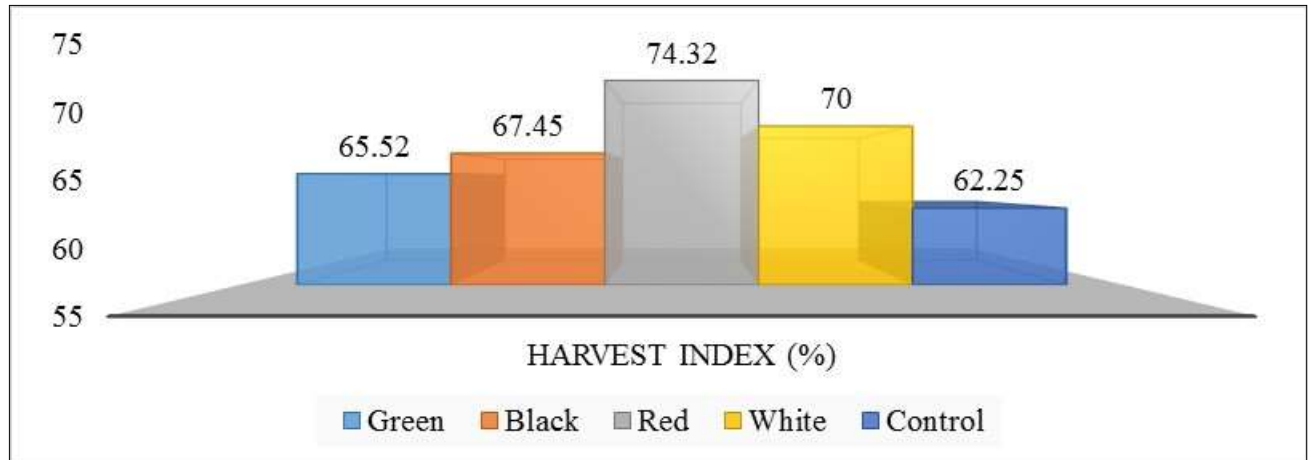


Fig. 1: The effect of different colored shade-nets on the harvest index of *Lycopersicum esculentum* L.

Hence, it is able to be advocated to use red and white nets for commercial production of dracaena in area of typically used green net.

Table 4: Photosynthetically active radiation (PAR - $\mu\text{mol m}^{-2} \text{s}^{-1}$), Solar radiation (W m^{-2}) over lettuce canopy measured and Transmitted PAR ($\mu\text{mol/m}^2/\text{s}$) under different coloured nets

| Treatment | B-Photosynthetically active radiation (PAR - $\mu\text{mol m}^{-2} \text{s}^{-1}$) | Solar radiation (W m^{-2}) over lettuce canopy | Transmitted PAR ($\mu\text{mol/m}^2/\text{s}$) |
|-----------|---|---|--|
| White | 1537 \pm 471.9 | 457 \pm 228 | 580.18 \pm 214 |
| Red | 0700 \pm 248.5 | 310 \pm 212 | 238.44 \pm 103 |
| Black | 0642 \pm 231.1 | 261 \pm 179 | 100.31 \pm 27.6 |
| Green | 0595 \pm 221.5 | 279 \pm 193 | 325.35 \pm 108 |
| Control | 1588 \pm 460.4 | 523 \pm 271 | 1342.54 \pm |
| LCD | *** | *** | *** |

Vase-life manage the worthwhile well worth of cut vegetables and higher value is always desired in trade. Vase-existence turned into referred to most (20 days) in white color net, it become well through 81.81% while as compared to control (Fig. 3 and Table 3).

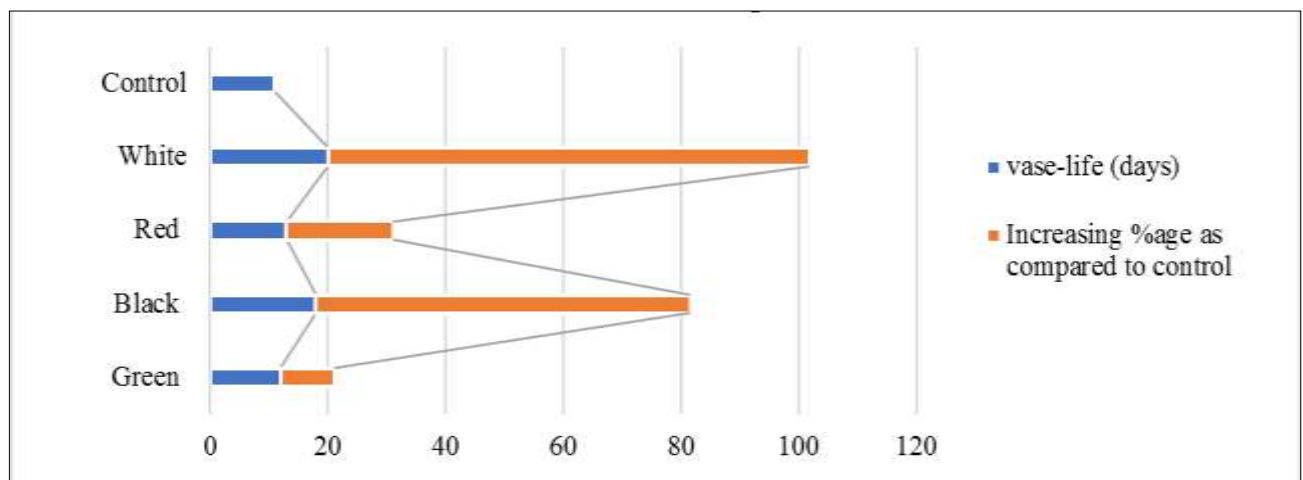


Fig. 3: Influence of colored shade-nets on vase-life of *Lycopersicum esculentum* L. cut foliage

This may be because of higher safety of the leaves by way of those nets from high light intensity and thereby improved quality of cut foliage. PAR and transmittance levels were notably lower under different color shade nets. White and Red shade-nets show higher B-Photosynthetically active radiation PAR (1537 & 700 PAR $\mu\text{mol m}^{-2} \text{s}^{-1}$) and transmittance PAR (580 & 238 $\mu\text{mol/m}^2/\text{s}$) than other color nets, while it was lowest under black net (Table 4). The solar radiation (W m^{-2}) over lettuce canopy was note in the ranged of 261-457) and at control it was (523). Statistical analysis indicated there was highly significant variation in B-Photosynthetically active radiation, solar radiation (W m^{-2}) over lettuce canopy and Transmitted PAR between the different colour net (Table 4).

Tomato fruit mass plays an important role in consumer preference as well as in processing industry. The present study show that tomato had an enormous and significant mobility in fruit mass among the different net color treatment. The mean mass of the fruit is from 34.4 g to 26.5 g and the maximum being in red color, whereas the minimum was found in green color net (Table 5).

Table 5: Fruit quality as influenced by shade-nets

| Treatment | Average fruit weight (g) | Total soluble solids (TSS) % | Total acidity (TA) % | Vitamin C (mg 100 g-1) |
|-----------------------|--------------------------|------------------------------|----------------------|------------------------|
| White | 29.6 | 8.5 | 0.19 | 171.24 |
| Red | 34.4 | 7.5 | 0.24 | 150.35 |
| Black | 31.7 | 8.2 | 0.20 | 170.41 |
| Green | 26.5 | 7.0 | 0.25 | 165.12 |
| Control | 30.4 | 9.7 | 0.19 | 175.30 |
| P-Value (0.05) | *** | * | ** | *** |

Where, Fruit weight is the average of 100 fruits

Significant ($P \leq 0.05$) difference was observed in TSS (Total soluble solids) content in different color net (Table 5). At harvest, total soluble solid (TSS) was noted was from 7.0-8.5 %. The TSS content of White net was highest while that of green net was the least. The peak concentration of TSS (9.7%) was found in fruits planted under open field conditions. Total acidity % age and Vitamin C mg 100 g^{-1} was found in the range of; (0.19 – 0.25% and 150-171 mg 100 g^{-1}) respectively and there were significant variations between the colour treatment (Table 5).

4. Discussion:

Light is the major mode of energy transmission between plant and environment. Sun light provides the main energy source to plants, with much of this energy being converted to heat and driving other radiation exchanges and biological processes, as well as being involved in showing tissue temperatures with consequences for rates of biochemical processes and the balance between them (Jones, 1992). The major weather variables affected by shade net is sun light, which relies upon type of shade net and density. The colored shade cloth is made to modify light in either the ultra-violet, visible, or far-red spectral regions; the cloth also increases the relative content of scattered vs. direct light and absorbs infrared radiation (Shahak *et al.*, 2004). Reduced air temperature was in accordance with the result of Campanha *et al.* (2005). The present study was in accordance with previous studies that the temperatures reduced by 2-3°C under color net and this in turn influenced plant processes (Smith *et al.* 1984).

Similar results of decrease in wind speeds were also shown by Gaurav *et al.* (2016). The effect changes according to the porosity (knitting density) of different net cover. The present study of plant growth parameters and their influence factors were turned into accordance to Kawabata *et al.* (2007). Similar results of leaves area and its pigmentation were also mentioned by Gaurav *et al.* (2016). In present study, the non-significant variations were also noted in fresh weight of leaves. Observation noted by Gaurav *et al.*, (2016) were contradict to our results. Similar observations of Canopy temperature were also noted by Smith *et al.* (1984).

Red and white coloration-nets gave higher PAR and transmittance than other shade nets. Similar results had been shown by means of Gaurav *et al.* (2016). Abhay *et al.* (2016) reported that plants grown under red and white shade nets exhibited better plant height, leaf number, leaf chlorophyll content, leaf area, fresh weight and dry weight, photosynthetic rate and transpiration and thus HI. In the existing observation, vase-existence turned into observed to be larger in white and black colored (18 days) nets, that is just like the statement stated by Gaurav *et al.* (2016), while it changed into in contradiction to earlier reviews (Stamps and Chandler, 2008). The proportion of diffused to direct PAR was notably higher under green shade net compared to black (Oren-Shamir *et al.* 2001). These findings of fruit quality as influenced by shade nets are similar with reported value of Gaurav *et al.* (2016), Madhumathi & Sadarunnisa, (2013) in cultivar Pusa Ruby (35.27 g). The higher values of TSS content in field condition was also

published by Madhumathi & Sadarunnisa (2013).

Starch is gathered in green tomatoes that start to fall with the start of ripening. This reduction in starch is followed by rising soluble solids (Eskin, 2000). It has been also reported that total soluble solids enhanced with color and maturity (Tigist *et al.* 2013). Total soluble solids content is one of the most significant quality variables in continuing tomato cultivars, having higher TSS content are better suited for the preparation of processed products like tomato powder, canned products, ketchup, sauce and chutney (Singh *et al.* 2014). High TSS is suited to yield higher recovery of processed products. Purkayastha, (2011) also notified that the total soluble solids content is from 3.60 to 5.40° Brix in five different cultivars of North-eastern Hill region.

4. Conclusion:

Red and white colored color-nets have been observed to be superior in improving most of the plant canopy in comparison to green net, black net and manage. Red coloration net has been observed to be powerful for enhancing plant height, variety of leaves, leaf region and Harvest Index, whilst different crucial traits were superior below white. Hence, it may be encouraged to use red and white nets for commercial production of dracaena in place of usually used green net.

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Exploitation of Phytoremediation Potential in Different Plants for Reducing Heavy Metals Burden on Contaminated Soils of Gujranwala District, Punjab-Pakistan

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Abstract

The current study was conducted for determination of pollutants absorption capacity in local plants growing in the contaminated sites. The four sites were selected randomly from Gujranwala District (Punjab) for determining their pollution status of the soils and uptake level of different heavy metals in the growing vegetation. The plant samples (roots, shoots and leaves) were subjected to acid digestion. The conc. of Ni, Zn, Cd, Mn, Fe, and Cu metals was determined by atomic absorptions spectrometry. The selected soil samples were analyzed for the determination of heavy metals. The Cu, Ni, Fe, Mn & Cd were found relatively at higher concentrations at the soil surface in most of the samples, however, Fe, Ni, Mn, and Cu were reported to be higher below 6-12 inches in some soil samples. Plants samples were analyzed of *Achryanthes aspera*, *Amaranthus spinosus*, *Eichornia crassipes*, *Typha latifolia*, *Polygonum glabrum* and *Trianthema portulacastrum* species for heavy metals absorption. It was recorded that almost all the selected plants absorbed relatively higher concentration of the metals as compared to the respective habitat soil. Nevertheless, different plants showed variable performance on different conc. of metals exposure. It was interesting to note that shoots and leaves accumulated relatively higher concentrations of certain heavy metals than those of the roots. In a nutshell, the preliminary study of the selected sites divulges that further scrutiny of these plants may be helpful to tag some candidate plants to be used for controlling heavy metals pollution.

Keywords: Gujranwala soil; Heavy Metals; Phytoaccumulation; Phytoemmediation; Pollution Status

1. Introduction:

Metal elements are of atomic number 20 and more show characteristic luster, ductility and conductivity of heat and electricity. The Earth's crust has numerous metals and related pollutants invade environment though fossil fuels burning, mining and smelting, applying pesticides, fertilizers, and sewage and municipal wastes. Thus, these

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© 2021 (Accepted for publication in September 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

eventually render soil and water unfit for agriculture and other activities. Escalating evidence support that small amounts of different metals are necessary for the integrity of a cell, but their higher concentrations are toxic to plants, animals and microbes (Hemen, 2011; *Fasih et al.*, 2021). They even cause serious diseases and impairment in physiology and DNA damage (*Rafique et al.*, 2010).

Therefore, it is necessary to alleviate heavy metals pollution burden form the environment for sustainability. Traditional approaches for mitigating metal contamination in soils include isolation, extraction, immobilization, and toxicity reduction methods. More often used are physical barrier, chemo-stabilization, soil washing, and pumping and treatment systems (*Mulligan et al.*, 2001). Accumulating evidence suggest the mechanisms of pollutants conversion into less toxic through phytoremediation (*Negri et al.*, 2003).

Phytoremediation is the utilization of common plants and associated organisms, water usage and enzymes to extract and degrade pollutants from wastewater and contaminated soil. Therefore, plants absorb and degrade organic and inorganic pollutants effectively from soil, sediment, sludge and wastewater (*Susarla et al.*, 2002). Moreover, phytoremediation approach is cost-effective, solar powered; eco-friendly and a substitute for extraction, pumping and treatment systems (*Zhu & Rosen*, 2009; *Yan et al.*, 2020). Usually metals are non-biodegradable; therefore, metals phytoremediation occurs by using mechanisms of phytoextraction, phytovolatilization and phytostabilization (EPA, 2000; *Pivetz*, 2001). Metal-hyperaccumulating plants were characterized in 1970s, and their morphological features and geographical characteristics were reported for over 400 plant species (*Banuelos*, 2001). However, most plants reported to be restricted to extremely metaliferous soils and humid-hot climate with sluggish growth and less biomass (*Pulford & Watson*, 2003; *Reeves*, 2003).

Gujranwala Division comprising of Gujranwala, Gujrat and Sialkot districts constitutes a golden triangle of industrial set-up. It earns a lion's share of foreign reserves through export of industrial products. It releases unwise and untreated effluents directly into the water bodies. Thus, it is continuously adding heavy metal pollutants into soil, water that eventually render unfit for growing crops to meet growing demand for food provision to ever increasing human population.

1.1. Objectives:

- To investigate the pollution status of the selected sites.
- To determine the potential of some of the plants grown at that area (Gujranwala).

2. Materials and Methods:

2.1. Site Selection:

Randomly four sites of pollution were selected where the wastewater water discharge was flowing form Gujranwala District. The Site# 1: Samman abad, G.T Road, bypass Gujranwala; Site #2: Steel Furnace Mill Area, Saman Abad Chungi, Gujranwala, Site #3: Ganda Nala near Coca Cola Factory Khiali, G.T. Road Gujranwala and Site # 4: Leather Field, Sambrial Road, Gujranwala.

2.2. Plant Selection and Sampling:

The whole plants were collected from the selected contaminated sites representing the growing vegetation of the selected sites. The plants were put in polythene bags, air tightened and labelled, accordingly.

2.3. Soil Samples:

The soil samples were collected from randomly selected four sites of Gujranwala district. The samples were taken from soil surface (*Mulligan et al.*, 2001; *Susarla et al.*, 2002; *Negri et al.*, 2003; *Zhu & Rosen*, 2009; *Rafique et al.*, 2010) and below surface (EPA, 2000; *Pivetz*, 2001; *Reeves*, 2003; *Pulford & Watson*, 2003; *Banuelos*, 2006; *Jain et al.*, 2010; *Hemen*, 2011) inches depths separately. Each sample was homogenized by mixing thoroughly, put in polythene bags, air-tight and labeled.

2.4. Samples Preparation:

The plants were washed with clean water twice and air-dried in shadow. The plants were oven dried at 70 °C for 72 hours. The roots and aerial parts (stem and leaves) were separately ground in a pestle mortar. 0.1 gram of the representative sample was taken in 250 ml glass flask, added 2 ml H₂O₂, 2ml H₂SO₄ and digested twice at hot plate till the clear solution obtained. The samples were diluted at 1:50 ratio. The soil samples were oven dried and took thoroughly mixed ground soil 0.1 gram of the represented sample was taken in 250 ml glass flask, added 2 ml H₂O₂ and 2ml H₂SO₄ and digested twice at hot plate till the clear solution obtained. The samples were diluting 10 times for analysis.

2.5. Analysis of Heavy Metals in Samples:

The analysis of prepared samples was made through atomic absorption spectrophotometer (AAS) Perkin-Elmer, USA, Aanalyst-6000). The instrument was calibrated using internal standard of respective element. Thereafter the samples of soil and plants were run on the atomic absorption spectrophotometer. In this analysis by AAS, quality control was determined using 5% sample blanks and 5% sample replicates in each set of sample analysis.

2.6. Statistical Analysis:

The data was subjected to statistical analysis using MS Excel 2004.

3. Results:

The current study was conducted for exploration of potential for absorption of phytoremediation in the indigenous plants. Our environment is depleting gradually by anthropogenic and industrialization activities in order to uplift living standards and economic growth. In Gujranwala District, four sites were randomly selected for the evaluation of their pollution status. Soil samples at surface (1-6 inches) and below surface (7-12 inches) were taken and subsequently analysed. At these sites different plants growing were sampled. The representative samples were analysed on AAS.

3.1. Plants Samples Analysis:

At site 1, Ni, Cd and Mn were accumulated at 0.15, 0.48, and 7.6 ppm, respectively in the shoot/leaves of *Amaranthus* sp. highest than rest of the plant species. However, *Eichornia* accumulated Fe 9.8 ppm in aerial parts, which is more than the other plants. Nonetheless, *Parthenium* accumulated 0.18 ppm Cu in shoots/leaves that is higher than rest of the plants. While, *Achryanthes* accumulated 10.8 ppm in shoot/leaves that is higher than that of the remaining plants. At the same site, roots of the selected plants accumulated different metals at variable concentrations. *Achryanthes* roots accumulated Cd and Fe at the conc. of 0.5 and 2.1 ppm and *Parthenium* roots gathered Ni and Zn 0.2 and 8.5 ppm, respectively higher the other plants. However, 0.82 ppm Mn in *Amaranthus* roots and 0.2 ppm Cu in *Eichornia* roots were recorded higher than rest of the plants (Fig. 1).

At site 2, *Typha latifolia* shoot accumulated Ni 0.2 ppm while, Cd (0.41), Mn (0.54), Zn (9.63), Fe (1.43) and Cu (0.71) ppm in the roots of *Polygonum glabrum*. However, Cd (0.43) and Cu (0.38) ppm were gathered in the roots of *Typha latifolia*, but *Polygonum glabrum* roots absorbed Ni (0.46), Zn (12.71) Mn (0.98) and Fe (2.13) ppm greater than *Typha* (Fig. 1).

At site 3, only *Amaranthus* was found to grow that accumulated Ni, Cd, Zn, Fe, Mn, and Cu in the roots and shoots at relatively low concentration of metals (Data not shown). At site 4, *Trianthema* shoots and leaves accumulated Zn (10.16), Mn (0.10) and Cu (0.8) ppm while, aerial parts of *Portulaca* absorbed Ni (0.6) and Fe (0.4) ppm and equal conc. of Cd (0.44) ppm was absorbed by both the plant species (Fig. 1).

3.2. Plants Samples Analysis:

At the site #1, the surface soil contained Ni (0.31), Cd (0.35), Zn (9.4) and Cu (0.04) and below surface soil has Mn (9.6) and Fe (26.3) ppm. At the site# 2, the surface soil contained Cd (0.35), Zn (12.9), Mn (10.5), Fe (0.68) and Cu (1.5) ppm and below surface soil has Ni (0.72) ppm. At the site# 3, the surface soil contained Ni (0.31), Cd (0.34), Zn (11.21), Mn (6.8), Fe (0.68) and Cu (3.6) ppm that was greater than at the below surface soil (Fig. 2).

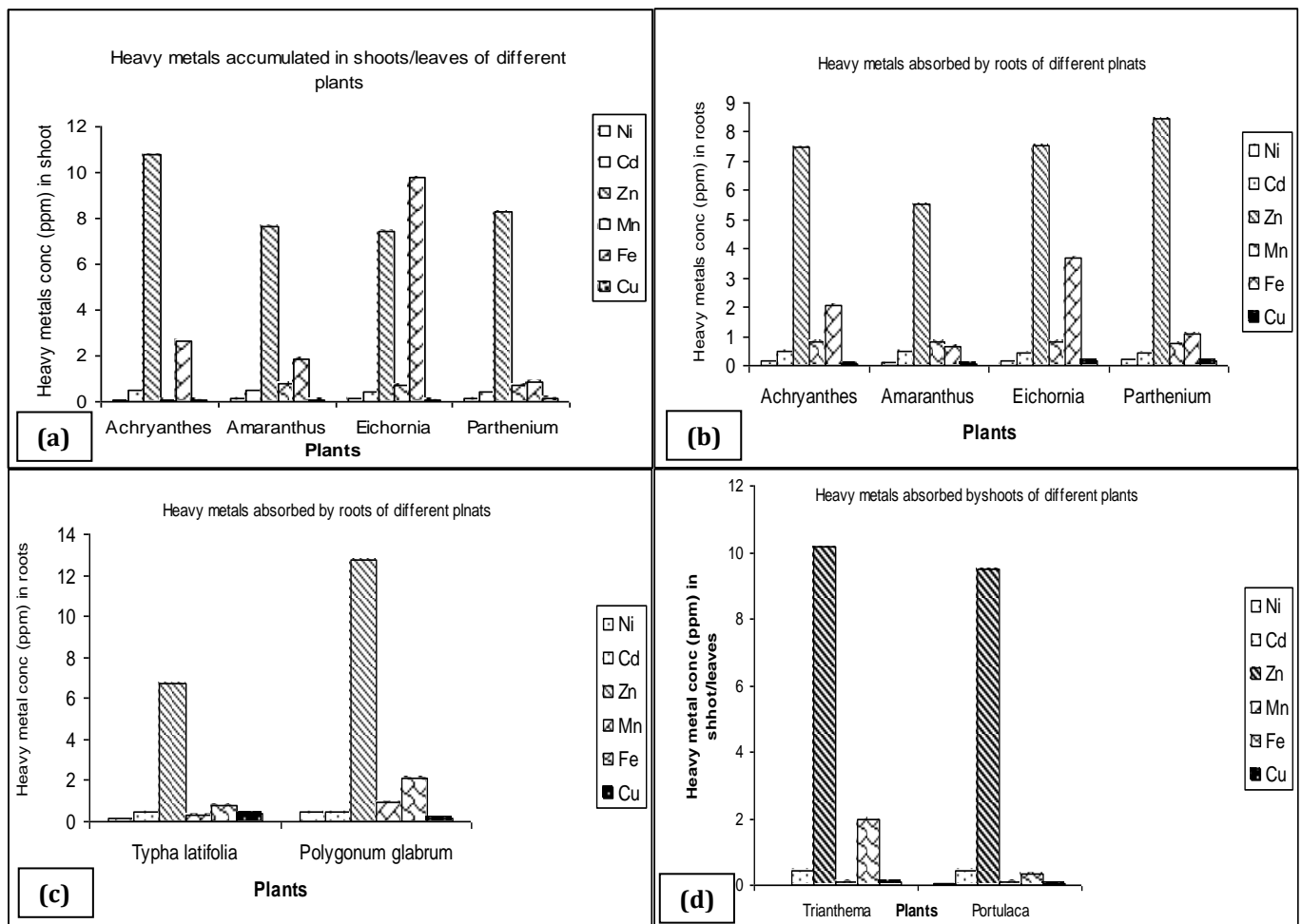
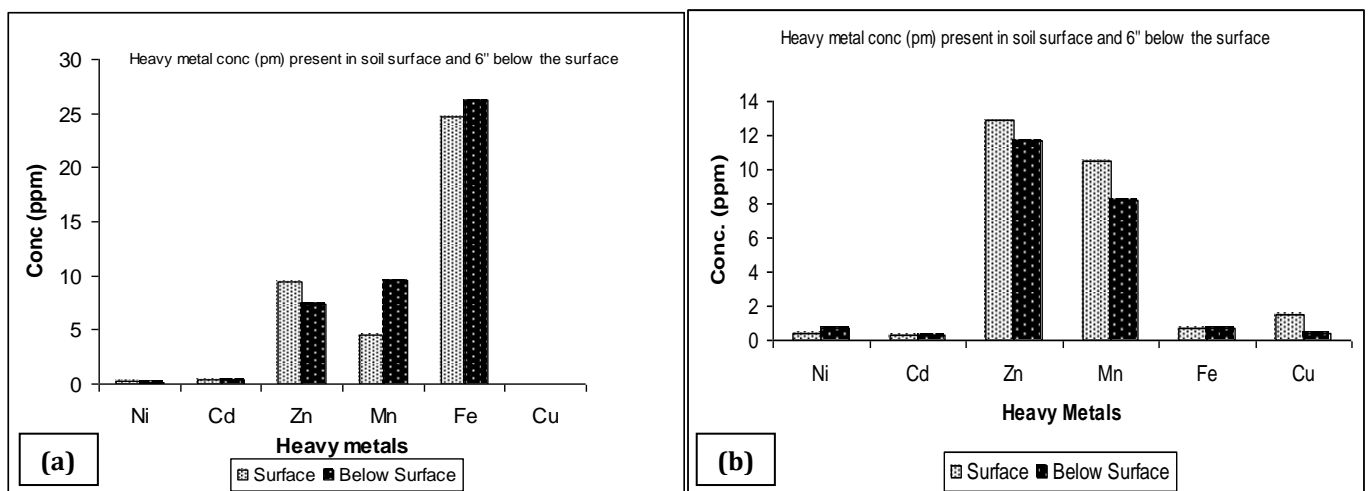


Fig. 1: Showing the uptake of heavy metals in the (a) aerial parts, (b) roots of the randomly selected plants at site #1, (c) in roots of the representative plants at site# 2, and (d) roots of the representative plants at site# 3 and 4.

At the site # 4, in the surface soil, concentration of Ni (0.43), Zn (8.6), Mn (5.3) and Fe (0.69) ppm that was greater than at below surface soil. However, Cd (0.34) and Cu (3.6) ppm were found more in below surface soil (Fig. 2).

In a nutshell, environment is being deteriorated persistently by anthropocentric and industrialization for improving living standards and economy. So far, very little importance has been given towards keeping ecosystem balanced and environment healthy for sustainable development. Therefore, to tag biological systems such as indigenous plants seem promising approach to launch comprehensive plan for controlling pollution.



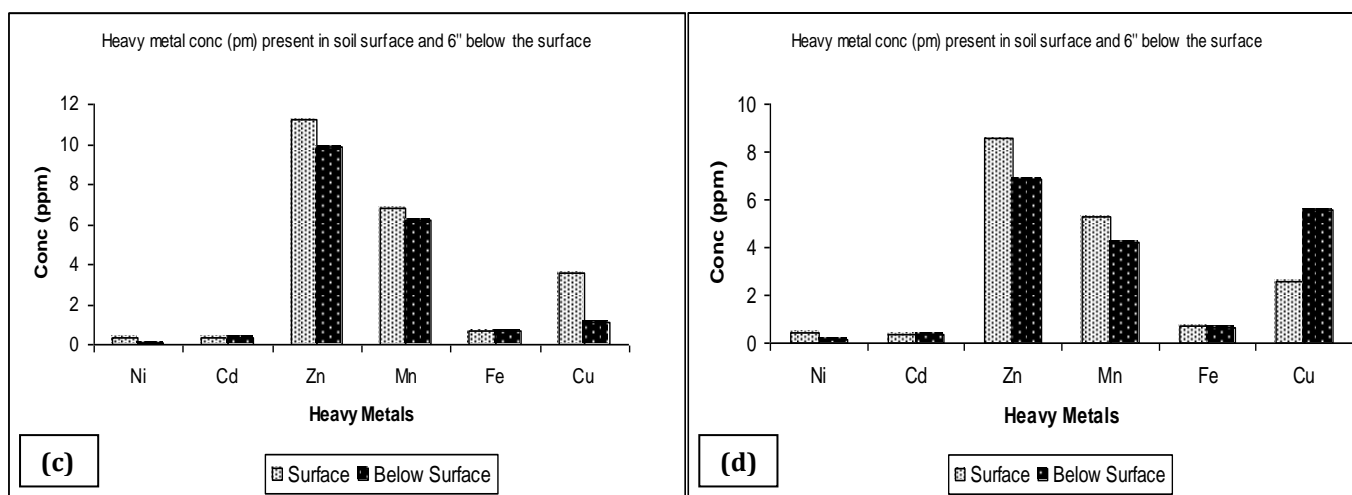


Fig. 2: Showing the heavy metals concentration in the top and subsoil in the representative samples at (a) site# 1, (b) site# 2, (c) site# 3 and (d) site# 4.

4. Discussion:

The soil and water resources are continuously contaminated by un-judicious release of untreated effluents leave unfit to be used for cultivation (Abida *et al.*, 2009). Thus, continuous efforts are needed in searching for remediation potential in the plants owing to enhanced absorption, hyper-accumulation, greater evapo-transpiration and better phytoextraction capabilities. Thus, amalgamation of conventional and transgenic approaches may be a promising approach to curtail pollution burden on the contaminated soil and wastewater to make re-usable for cultivation and irrigation (Rufus *et al.*, 2007; Suman *et al.*, 2018).

Our soil and water resources have been continuously contaminated by unwise and untreated release of effluents that render unfit for cultivation (Jain *et al.*, 2010; Ashraf *et al.*, 2019). Thus, continuous strides are needed for investigation of remediation potential in selected plants owing to enhanced absorption, hyper-accumulation, greater evapo-transpiration and better phytoextraction capabilities (Green & Hoffnagle, 2004). It is common practice that industries discharge untreated wastewater and sewage wastes, which make their passage into water bodies (Nawaz *et al.*, 2010). This eventually leads to enter into food chain through wastewater application for agriculture and growing fish to meet protein demand. These pollutants including heavy metals cause various ailments, as well (Chen *et al.*, 2016). The higher concentration of metals in the below surface soil may be due leaching effect (Rehman *et al.*, 2008; Ali *et al.*, 2013). The higher concentration of certain metals at the top soil surface might be due to adsorption at the soil particles (Khan *et al.*, 2005; Sarwar *et al.*, 2017). Therefore, the higher concentration of the metals at the topsoil surface may be attributed to adhesion with the soil particles (Khan *et al.*, 2005) and higher concentration of the metals at the topsoil surface may be attributed to greater affinity towards soil particles (Khan *et al.*, 2005).

5. Conclusion:

Pollutants adversely affect microorganisms, animals, plants and the living environments. They also pose threat to the human health. In past, various technologies have been used for reclamation of contaminated soils, and wastewater rendering fit for cultivation and irrigation, but the success claimed was quite low. Phytoremediation alone and/or integrated with conventional techniques may be promising solution for remediation of contaminated soils and wastewater. Moreover, phytoremediation is an up-and-coming green technology that gained recognition industry-wide. Thus, incessant efforts are necessary to mark plants having phytoremediation potential for pollution control.

6. Acknowledgment:

The present study is a part of the research project on "use of indigenous technologies to control pollution" that was financed by EPD-Lahore and executed by Department of Botany, University of Gujrat, Hafiz Hayat Campus-Gujrat, Pakistan.

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Efficiency of Gypsum and Compost for Restoration of Wastewater Irrigated agricultural Soil and Spinach Growth

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Abstract

Cadmium (Cd) contamination in wastewater irrigated agricultural soils has become a serious environment concern due to its toxic effects on organism and the food chain. Possible eco-friendly solutions for Cd immobilization were required to reduce its mobility through biochar. This study evaluated the comparative efficiency of vegetable waste induced compost and on Cd mobility and its accumulation in spinach which is highly Cd accumulating crop. Results showed that the soil chemical properties pH, EC and bioavailable Cd concentration were significantly altered after these amendments. Concentration of Cd decreased in CaCl₂ extract by 57.03% and 55.74% when gypsum and compost were applied at 3% application rate respectively, relative to control. Moreover, the Cd contents in the spinach shoots and roots were significantly decreased and thereby increased spinach shoot and root dry biomass, Furthermore, incorporation of gypsum and compost has prominent effect on chlorophyll contents. Overall, compost and gypsum at 3% application rate demonstrated positive results as soil amendments for Cd immobilization and thereby, reducing its bioavailability in the Cd contaminated soil to mitigate food security risks.

Keywords: Gypsum; Heavy Metals; Wastewater; Compost; Spinach; Cadmium (Cd)

1. Introduction:

The presence of cadmium (Cd) in wastewater poses serious health risks which could cause liver disease, cancer and abdominal pain. It has persistent and non-degradable nature and can easily uptake through plants. Accumulating of Cd in agricultural soil can hinder the plant growth by disturbing its physiological and morphological disorder and ultimately reduce crops yields. Thus, Cadmium pollution control and restoration of polluted soil have considered an emerging task and attained much attention from researchers (Hu *et al.*, 2014). The rapid industrialization and rising urbanization the release of potential toxic elements such as pesticides, pollutants and heavy metal have been increasing that directly or indirectly pose threat to the soil ecosystem (Bashir *et al.*, 2018; Ahmad *et al.*, 2014a; Xu *et al.*, 2013). The improper discharge of toxic elements causes serious hazardous effect on plants, animals and food security and environment (Ahmad *et al.*, 2014b). Heavy metals accumulating in agriculture sector in one of the serious environmental problem. The excessive use of contaminated water increasing the concentration of heavy metals such as cadmium in the soil. Cadmium is one of the heavy metal that has toxicological

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© 2021 (Accepted for publication in September 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

effect on soil ecosystem. Cadmium is more persistent because it cannot be removed if once introduced into the soil ecosystem. Cd as a transition heavy metal is highly toxic even at less than $<1\text{mg kg}^{-1}$ concentration in soil (Adriano, 2001). The origin of cadmium is from mine tailing, fuel combustion, phosphate product and industrial sludge (Lu *et al.*, 2014). The agricultural crops get high concentration of cadmium when soil is irrigated with wastewater and then it enters the food chain (Chaney *et al.*, 2005). In Pakistan, Cd content ranges from 0.24 to 2.1 mg/kg in all vegetables but it is highest in spinach. Vegetables are considered as the most important component of human diet because these are the rich source of vitamins, fibers and minerals that are essential constituent for human proper growth. However, contaminated vegetables pose a risk to food chain and human health (Parveen *et al.*, 2015). Heavy metals are taken up and accumulate in edible and non-edible parts of vegetables and their quantities are enough to cause diseases in both humans and animals. Some metals like cadmium (Cd), lead (Pb), arsenic (As) and chromium (Cr) adversely affect the human's life. Vegetables uptake heavy metals from soil by different mechanism such as absorption. Ion exchange, redox reactions etc. (Zabalawy *et al.*, 2015).

Spinach (*Spinacia oleracea* L.) is one of the common leafy winter nutritious vegetables that is grown in Pakistan on large scale. It is also used as herbal medicine in southeast part of Asia especially in Pakistan and China (Gothberg *et al.*, 2002). Pakistan is producing 95.5 thousand tons of spinach with 1.2 tons / ha in yield. It consists of various vitamins like vitamin A, B, C and K and also has high content of essential nutrients such as calcium, magnesium, phosphorous and potassium which are important in human's diet and good for health (Single *et al.*, 2010). It has strong ability to absorb Cd in its edible parts that are toxic to human body and may cause serious health disorders. It has been confirmed that the ability of spinach to uptake Cd is about 0.65 mgkg^{-1} on fresh biomass basis which is too high from proposed value of Cd (0.2mgkg^{-1}) by FAO and WHO (Zhuang *et al.*, 2009). So, the application of amendments in metal contaminated soil could improve soil properties and enhance the plant growth. (Bolan *et al.*, 2014). Some decades back many conventional techniques were introduced including land filling, excavation and soil washing but recently due to their negative effects on soil properties, these techniques were ignored. *In situ* metal immobilization has attracted substantial attention because of inexpensive and environmentally friendly technology. (Ahmad *et al.*, 2014a; Xu *et al.*, 2013). The use of organic and inorganic amendments in metal polluted soils can play significant role in mobility of heavy metals in polluted soil and ensure food risks (Kumpiene *et al.*, 2008; Shaheen & Rinklebe, 2015). The detoxification of heavy metals on soil-plant system by using organic amendments is highly effective method as they derived from the raw biological waste and before its application little pre-treatment is needed (Park *et al.*, 2011; Yin *et al.*, 2016).

Compost is one of the valuable soil amendment which improves the quality of soil and enhance plant growth (Rouse *et al.*, 2008). Compost consists of stabilized decomposed organic matters that are the result of biological degradation of organic matter under controlled aerobic environment (Paulin & Peter, 2008). Plant waste composts, such as garden grasses, tree leaves, and tree barks, make ideal amendments because of high carbon and nitrogen and low heavy metals contents. It is reported that the compost addition increased cation exchange capacity (Hseu *et al.*, 2013), which is most important for Zn and Cd immobilization in contaminated soils. It is found that the mushroom compost addition to a clay loam soil in Ankara (Turkey), clearly reduced Cu availability from 2.20 to 1.90 mg Kg^{-1} and Cd from 0.057 to 0.005 mg Kg^{-1} (Karaca, 2004). Clemente *et al.* (2006) stated that the Cu availability decreased after the olive leaf compost incorporation into the soil and effectively immobilized both Pb and Zn. It is resulted that in compost amended soils, the dominance of Fe-MN oxides and exchangeable forms of Cd and Zn are found in Cd, Zn co-contaminated soils. Gypsum is often used for the regulation of soil reaction (pH) which has substantial effect on nutrient availability of soil. The mineral composed of soft sulphate compound of calcium sulphate dehydrate having formula of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ is known as Gypsum (Klein & Hurbur, 1985). Gypsum has detrimental role in maintaining soil structure (Shainberg *et al.*, 1989) due to the supply of sulphate (for plant nutrition and excess of sodium leaching) and calcium (for flocculation). The use of gypsum as soil amendment increases the ionic strength and CA concentration in the soil solution (Aura *et al.*, 2006; Pietola, 2008) and it also reduces the soil erosion. Infiltration increases if soil physical condition is changed, and soil erosion may be further reduced (Cox *et al.*, 2005). Currently, gypsum is also a popular soil amendment. Previous research stated that gypsum rich products and industrial by-product have the ability to reduce the heavy metals (Cd, Pb, and Cu) concentration in water (Illera *et al.*, 2004). The addition gypsum by-product to an acidic soil decreased these metals concentration through the formation of anglesite (PbSO_4); as it increased sorption capacity of the soil with the formation of Al-hydroxyl polymers (Illera *et al.*, 2004). Additionally, under anoxic conditions phosphogypsum has been shown to reduce Cd and Ni in water, due to their precipitation as sulphate (Maher *et al.*, 2016). It is reported that red gypsum can be used as an amendment for metals contaminated soils, as it has the Fe oxides materials with a good sorption capacity (Peacock & Rimmer, 2000). Several types of organic and inorganic amendments have been studied for Cd immobilization in contaminated soils (Bashir *et al.*, 2018; Sun *et al.*, 2013). However, a limited knowledge has been reported regarding the incorporation of vegetable compost and gypsum mineral in wastewater irrigated agricultural

soils for Cd immobilization and phytoavailability to spinach in contaminated soil. Thus, the primary objectives of the current study were to assess the comparative effectiveness of compost and gypsum and its surface properties on Cd polluted soil restoration. Moreover, their impact on spinach growth in wastewater irrigated contaminated soils.

1.1. Objectives:

- To evaluate the comparative effects of amendments on Cd solubility and mobility in contaminated soil.
- To sort out the potential amendment and its application level to increase Cd immobilization in contaminated soil.
- To evaluate the consequent amendments effects on the phytoavailability of Cd on spinach growth.

2. Materials and Methods:

2.1. Soil Characterization:

The soil samples were collected from 0-15cm from wastewater irrigated agricultural fields of DG Khan, Punjab province, Pakistan. After that, soil samples were transferred to the laboratory and then air dried and sieved from 2 mm plastic sieve before pot study. The basic chemical properties of soil such as pH and EC were measured with soil to water ratio (1:2.5) and 1:5 (w/v) with automated pH and EC meter respectively. Pipette method was used to determine soil texture, whereas total nitrogen and total phosphorus contents of soil were measured according to Lu, (1999). Cation exchange capacity (CEC) of soil was measured according to the ammonium acetate method on pH 7.0. The determination of soil cadmium concentration was determined by using atomic absorption spectrophotometer (AAS) after digesting soil with acids mixture (HCL-HNO₃-HClO₄).

2.2. Pot Experiment:

Pot experiment was conducted to evaluate the effect of gypsum and compost on the bioavailability of metal contaminated soil. The experiment was statistically arranged with seven treatments with three replicates, as follow: (1) control (CK); (2) gypsum (G) 0.5%, 1% and 3% and compost (c) (containing soil, manure and rotten leaf) 0.5%, 1% and 3% respectively. Pots were filled with 2 Kg of air-dried soil. The experimental soil was homogeneously amended with gypsum and compost with their application rates. Before seed sowing, the amended soil pots were moistened at 65% of their water holding capacity and leave for 2 weeks. Seven healthy spinach seeds were selected for sowing by making 3-5 cm deep holes. After one month of seed germination, the plants were keeping 3 plants per pot. After a period of 2 months, the aboveground biomass of the spinach was harvested. The shoots of plants were thoroughly washed with tap water and after that with deionized water. After that plant samples were dried in oven at 70°C in paper bags for 48 hrs. The oven dried sample were ground with a stainless-steel mill and were stored for chemical analysis. The soil sample from each pot was air-dried and thoroughly mixed. In order to remove any kind of root debris then air-dried soil was passed through a 0.54-mm sieve for further chemical analysis.

2.3. Heavy metal determination:

After harvesting spinach plants, the heavy metal bioavailability was determined according to the procedure of Houben *et al.* (2013). Samples containing 2 g of soil was shaken with 25 ml of 0.01M CaCl₂ for 2 h at 3000 rpm for 15 min. The dried spinach roots and shoots were ground to powder with the help of electric mill. The roots and shoots samples (0.2 g) were digested by using the mixture of HSO/HClO at a ratio of (3:1 v/v) for determination of heavy metal concentration using the AAS.

2.4. Plant Growth Parameters:

After crop harvest, plant growth parameters such as plant height, number of leaves, plant root, shoot fresh and dry biomass, plant chlorophyll contents by using chlorophyll meter.

2.5. Statistical Analysis:

The data were subjected to Analysis of Variance (ANOVA) using Statistic 8.1 and LSD tests ($p < 0.05$) were used to test the mean significance of all treatments. Statistical variations of the data were expressed as standard deviation and significance of the data was calculated at the $p < 0.05$.

3. Results:

The application of amendments such as gypsum and compost with three application rates effectively altered the soil pH (Fig. 1). The application of gypsum at 0.5%, 1% and 3% showed ($p < 0.05$). The significant alteration in soil pH. As compared to control the decrease in soil pH was observed by 0.24 units when gypsum was applied at 3% application rate, respectively. Similarly, addition of compost at 3% significantly decreased soil pH by 0.22 units.

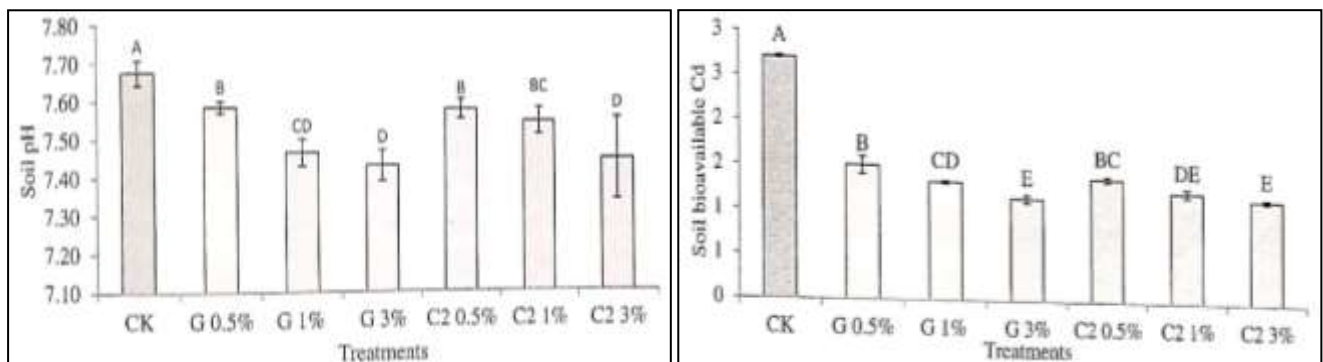


Fig. 1: Soil pH, Soil Cd bioavailability changes after treated with various amendments.

Treatments: control (CK). Gypsum (G), and Compost (C2). Error bars are the SD of the means ($n = 3$) and different letters indicate the values are significantly different $p < 0.05$

3.1. Effect of amendments on Cd solubility:

The immobilization of Cd was quantified by using CaCl_2 extraction technique, showed the decreasing trend when gypsum and compost were incorporated into wastewater irrigated agricultural soils. The maximum reduction was occurred in CaCl_2 extractable Cd in soil when gypsum was added at 0.5%, 1% and 3% by 44.4%, 50.74% and 57.03%. Similarly, incorporation of compost at 0.5%, 1% and 3% prominently reduced the Cd concentration in soil by 48.14%, 53.14% and 55.74% respectively at rate of 0.5%, 1% and 3% application rate.

Application of gypsum and compost significantly ($p < 0.05$) reduced the Cd uptake in spinach plant tissues (shoot and root) (Fig 2 and 3). By using gypsum and compost as amendments, the roots and shoot of spinach plant showed various trends of Cd accumulation concentration.

The maximum reduction was observed by 50.7% in root while in shoot by 51.7%, respectively after the addition of gypsum at 3% rate. The maximum decrease in Cd uptake by plant shoot was 55.9% and, in a root, it was 56.5%, respectively. The maximum decrease in translocation factor was observed by 15% and 18.8 %, when gypsum and compost were applied at 3% application rate, respectively.

The data regarding the effect of gypsum and compost on spinach plant growth in Cd contaminated soil is shown in Figure. The addition of gypsum at 1% and 3% rate increased the root length by 28.7% - 45.1% while maximum increase in shoot length was observed by 25.1% - 33.1%, respectively as compared to control.

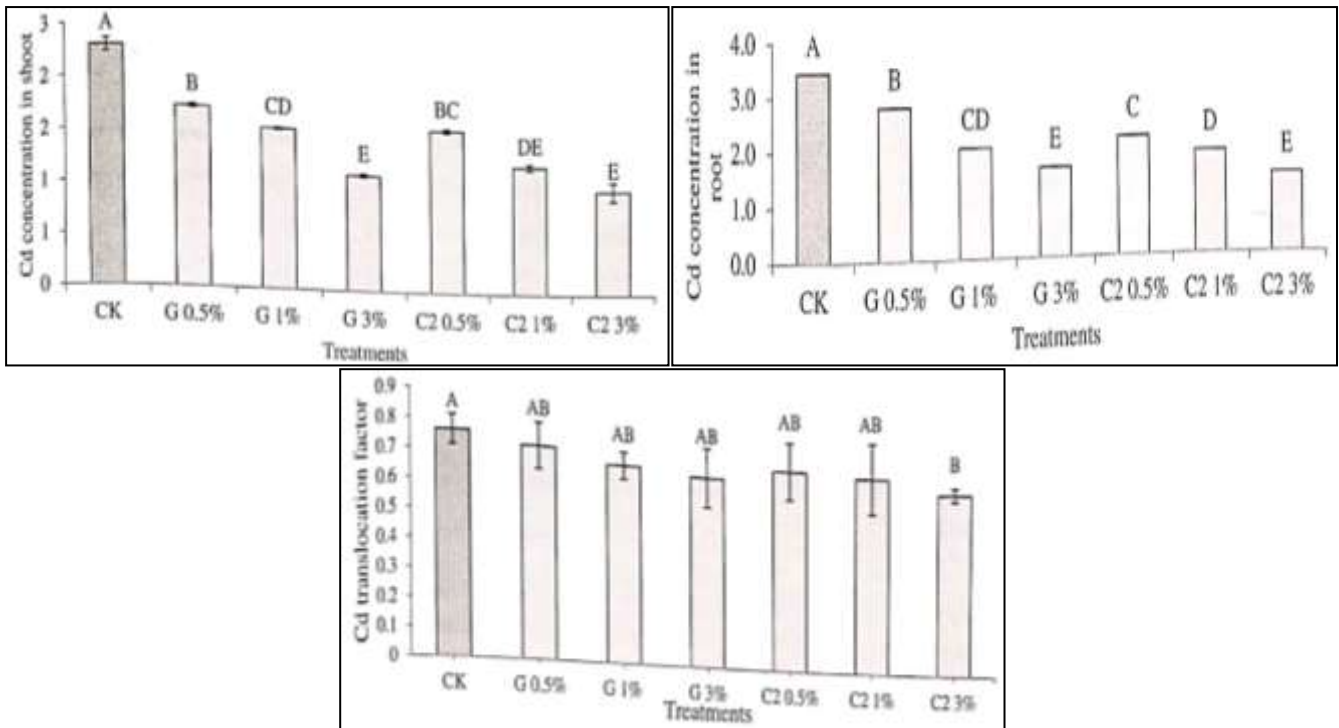


Fig. 2: Cd concentration in plant shoot, root and Cd translocation factor from root to shoot changes after treated with various amendment.

Treatments: control (CK), Gypsum (G) and Compost (C). Error bars are the SD of means ($n = 3$), and different letters indicate that values are significantly different $p < 0.05$.

Whereas compost application significantly increased the root length by 49.5% - 50% and shoot length increased by 12.2% - 39.5% at rate of 1% and 3% respectively. It is showed that the application of amendments like gypsum and compost have positive effect on growth of spinach plant in Cd contaminated soil. The use of gypsum and compost significantly influenced the plant nutrient uptake as well as enhanced the chlorophyll concentration in leaves as compared to the contaminated soil without treatment amended soil. Among amendments, addition of gypsum at the rate of 1% and 3% enhanced the chlorophyll content by 41.1% - 46.4% likewise maximum chlorophyll content was observed by 45.5% - 49.3% observed after the addition of compost at 1% and 3% respectively, as compared to the untreated soil. The application of amendment also influenced the leaves production in spinach plant. The use of gypsum at rate of 1% and 3% significantly increased the number of leaves by 22% - 27%. The maximum leaves production was observed by 35% - 44% when compost was applied at 1% and 3% respectively, as compared to untreated soil. The spinach plant showed better response with the application of all amendments. The application of gypsum significantly influenced the growth of spinach as addition of 1% and 3% gypsum to contaminated soil. The maximum shoot fresh weight was measured by 24.5 - 46.9% while root fresh weight by 24.5 - 36.1%. Compost addition at rate of 1% and 3% to polluted soil increase the shoot fresh weight by 51.1% - 53.77%, while increase in root fresh weight was by 39.4% - 44.5% respectively. Result revealed that the application of gypsum at rate of 1% and 3% enhanced the shoot dry weight by 24.5% - 72.3%.

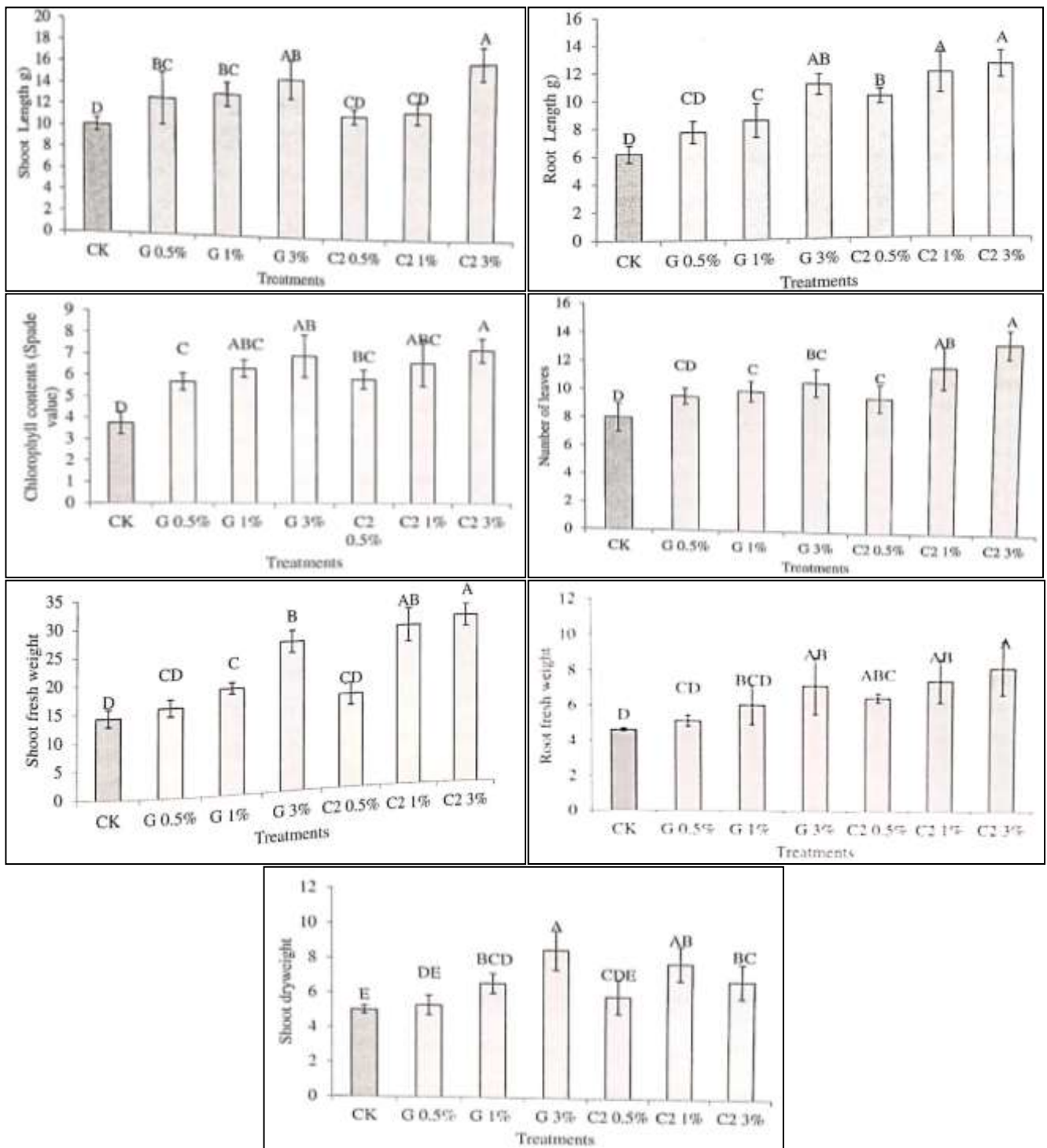


Fig. 3: Spinach shoot, root length, No. of leaves, shoot and root fresh and dry weight changes after treated with various amendments.

Treatments: Control (CK), Gypsum (G) and Compost (C). Error bars are the SD of the means (n = 3) and different letters indicate that values are significantly different p < 0.05.

4. Discussion:

The application of chemical and organic amendments to metal polluted soils is considered an emerging solution for polluted soil remediation and restoration. The purpose of this study was to assess the efficiency of different amendments including vegetable compost and gypsum, in order to quantify their effectiveness on the

immobilization of Cd in soil and also bioavailability towards leafy vegetable (spinach). The soil pH has the strong effects on solubility and mobility of metals, so pH is viewed as the most important factor for metal in environment (Harter, 1983; Wilkins *et al.*, 1998; Wang *et al.*, 2013b, 2015a). The solubility and mobility of metals have the significant negative correlation with soil pH, which means metal ions are easier to solubilize and mobilize in soil and be uptake by biology under low pH (Huang *et al.*, 2014; Zeng *et al.*, 2011). The present study confirmed that soil pH is the most suitable indicator (Schhoeneau, 2005) for soil remediation and metals mobility (Ramos, 2005). Relatively high value of soil pH could favour to decline heavy metals mobility in acidic soils, providing new bindings sites for heavy metals precipitation and adsorption. The other reason for immobilization of heavy metals is the precipitation of metals with humic substances (Petruzzelli, 1989; Godfrin & Van Bladel, 1990; Pigozzo *et al.*, 2006). However, the addition of vegetable waste compost and gypsum significantly lowers the soil pH might be due to the prominent increase in CO₂ level after the decomposition and mineralization of the organic matter. Similarly, Zinati *et al.* (2001) reported that incorporation of organic amendments such as compost could reduce alkalinity of soil and thereby reduced soil pH. On the contrary, several studies reported that slight increase in soil pH was occurred after the addition of organic amendments (Geeblen *et al.* 2003; Mendoza *et al.*, 2006). The mineralization of carbon and successive production of OH⁻ ions by ligand exchange with the basic cation such as Ca⁺², K⁺ Mg⁺² also alters soil pH (Mkhabela & Warman, 2005). The additions of organic matter in soil maximize the number of negative charges in acidic and organic matter poor soils. After that it absorbs protons from the soil and result in increase in soil pH (Jordão *et al.*, 2006). The maximum use of organic amendment decreases the heavy content by fixing them in soil by the production of insoluble organo metallic complexes and thus results in immobility and decrease in phytotoxicity of heavy metals (Pare *et al.*, 1999; Udom *et al.*, 2004). Soluble metal complexes are also formed from organic ligands that are low to medium weight i.e., carboxylic acid, amino acid and fluvic acid because of metal desorption from soil (Alvim Ferraz & Lourenco, 2000; Kalbitz & Wennrich, 1998). It has been reported that various composition of dissolved organic matter in soil result the affinities for metal species because of the presence of dissolved organic matters (Kalbitz & Wennrich, 1998). The uptake of Cd by plant tissues showed a different behaviour among all amendments. The organic (compost) and chemical (gypsum) amendments may have highest adsorption capacity because it has greater chemical and biological properties. This suggests that addition of amendments could enhance Cd adsorption on treatment amended soil, which might be one of the reasons for reducing Cd bioavailability to spinach as compared to control. The lowest values of Cd solubility and leachability in soil amended with increasing rate of gypsum and compost might be another important reason to justify the minimum adsorption of Cd by plant and shoot.

The present study confirmed that the soil treated with compost as an amendment improves the soil subsurface layer and helps in improving the root growth of plant by stabilizing soil texture and thus provide the long-lasting soil restoration (Bradshaw, 1997). Therefore, a positive effect after the addition of various compost can be anticipated in the light of altering the soil pH which could decrease CaCl₂ extractable Cd. Furthermore, available Cd in soil was also correlated with TOC, indicating total organic carbon could increase Cd retention by organic amendments applied (Beesley *et al.*, 2010). Moreover, study performed by Clemente & Bernal (2006) observed that compost has high organic matter content which might play role to increase Cd distribution in exchangeable and carbonate bound fractions. The results indicated the bound force of Cd to soil, and amendments was weak as the result of active nature of Cd, which was consistent with other studies (Yin *et al.*, 2016; Park *et al.*, 2011). The addition of organic amendments to soil can contribute to metal immobilization through formation of stable complexes with OH or COOH groups on the solid surfaces of the organic polymers (Madrid *et al.*, 2007). Udom *et al.* (2004) announced that the increase of organic matter content contributes to fix the heavy metals in soil by the formation of organo metallic complexes and therefore, decreases their mobility and their phytotoxicity. However, fresh manure and immature compost, with relatively high soluble organic matter content, could increase the metal mobility (Madrid *et al.*, 2007). Added to soils, the organic amendments have different effect on metal availability, depending upon the particular metal, the soil and the characteristics of organic matter added, particularly the degree of humification (Walker *et al.*, 2004). The phytoavailability of heavy metals such as As and Cu has been decreased as these heavy metals bound strongly with organic matter applied to the soil (O'Neill, 1990; Baker, 1990; Pérez-de-Mora *et al.*, 2005). The municipal solid waste compost (MSWC) and phosphate fertilizer encourages the level of Cd metal in tissues of crops (Warman, 2001; Oliver *et al.*, 1997, Grant & Bailey, 1998). It is reported that plants only get those metal elements from soil which are soluble, chelated and exchangeable in soil (Kabata-Pendias, 1993). The use of municipal solid waste compost (MSW) that is a good source of essential nutrient element for plant helps in improving plant growth (Farrell & Jones, 2009). It is reported that leaf-k and Mg content was maintained in specific limit in plant tissues by the application of compost as an amendment to the soil for plant proper growth (Bumgarner *et al.*, 2011; Campbell *et al.*, 2000; Gent *et al.*, 2002).

5. Conclusion:

Based on our studies, we concluded that among all applied amendments, vegetable waste derived compost performed better and has ability to adsorbed Cd by changing the nature/behavior of Cd in soil by reducing their (bio) availability, leachability. Our studies markedly have shown that the application of compost at 3% to Cd-contaminated soil has potential to immobilize, thereby reducing Cd bioavailability to plants by enhancing agronomic values.

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Pigment Contents in Seventy-Three Wild Medicinal Plant Species of Juniper Forest Ecosystem at Ziarat Balochistan, Pakistan

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Abstract

This study was conducted to determine the pigments (chlorophyll a, b and carotenoid) contents in seventy-three wild medicinal plants species, through spectrophotometer methods. Leaf samples were collected from 73 wild medicinal plant species (including trees, shrubs and herbs), grown in the Juniper Forest ecosystem under same environmental condition of district Ziarat Balochistan Pakistan. Significant variation in the leaf contents (chlorophyll a, b and carotenoid) was noted among the 73 wild medicinal plant species. Lowest level of chlorophyll a (0.4 mg/g fw) was noted in *Achillea welhelmsii* L. and *Alhagi maurorum* Medic and highest content were in *Perovskia abrotanoides* Karel. and *P. atriplicifolia* Benth. (Around 2.0 mg/g fw). Chlorophyll b was minimum in *Caragana ambigua* Stocks., *Daphne mucronata* Scherb and *Allium ascalonicum* L. (0.1 & 0.15 mg/g fw), respectively and maximum (0.48 mg/g fw) was found in *Pistacia atlantica* Roch. F. and *Serriphedidium quettense*. Carotenoid content was minimum (around 0.1 mg/g fw) in *Arnebia griffithii* Boiss. *Allium ascalonicum* L. and *Asparagus neglectus* Kar. & Kir., while the maximum level (0.8 mg/g fw) was observed in *Berberis baluchistanica* Ahrendt. Lowest value of total chlorophyll contents was established for *Alhagi maurorum* Medic. and *Achillea welhelmsii* L. (0.6 & 0.65 mg/g fw), but *Perovskia abrotanoides*, Karel. showed highest value (2.34 mg/g fw). Maximum content of chlorophyll a/b ratio (9.0) was reported in *Caragana ambigua* Stocks. and minimum was found in *Achillea welhelmsii* L. (1.6). The ratio of total chlorophyll/carotenoids was maximum in *Arnebia griffithii*. (9.6), while minimum value was observed in *Berberis baluchistanica* Ahrendt. (1.16).

Keywords: Chlorophyll a & b; Carotenoids; Wild Medicinal Plants; Juniper Ecosystem; Ziarat Balochistan

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© 2021 (Accepted for publication in September 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

1. Introduction:

Plants have a significant position in our lives for the reason that they accomplish our basic needs for food, shelter, clothing, fuel, ornamentals, flavoring and medicines (Cynthia *et al.*, 2011). It seems the need of the time that we should know all about (Physio and chemical characteristic) the plants especially medicinal flora. In plant leaf cells certain pigments including chlorophyll a (Chl. a), chlorophyll b (Chl. b), flavonoid, carotenoids and xanthophyll are present. These colors show a significant character in plants metabolism and other physiological process (Sadaoka *et al.*, 2011). Chlorophyll is the green molecule in the chloroplast, which carried out the process of energy fixation photosynthesis in plant cell. Sunlight is absorbed by chlorophyll during the process of photosynthesis in plant cell chloroplast. This chloroplast this one is not an only particle, it includes the group of similar molecules such as chlorophyll a, b, c and d (Signal *et al.*, 2008). Chlorophyll have not only significant role in photosynthesis system but also have responsibility for green color of fruit and leaves, consequently it has a vital character in growth and plant productivity as stated by Peng *et al.* (2013). Carotenoid along other pigments likewise perform a dynamic part photosystem process, they are being normal fat-soluble pigment not only present in plant and algae also in photosynthetic bacteria. These pigments too help in defensive purpose in contradiction of damage by light and oxygen in some non-photosynthetic bacteria as reported by Biswal, (1995) and Gitelson *et al.* (1999). In addition, by their role as an attracting pollinators and seed dispersal, they carry out very imperative functions in plant reproduction (Yeum & Russell 2002). Different environmental factors such as seasonal variation and climatic condition may effect on pigments contents. Saucedo *et al.* (2008) described seasonal disparities in chlorophyll (a & b) and carotenoid in the natural shrubs of Mexico. They reported that there were extraordinary variations in the contents of these pigments between the years and also among the species. Therefore, they designated that the climate has an inspiration on pigment manufacture in plants. Some cultivated and wild plant species of Lamiaceae were studied by Castrillo *et al.* (2001) for their chlorophyll content and they found variation in pigment contents from species to species. Gonzalez -Rodriguez *et al.* (2015) stated seasonal variation effect on the leaf pigments, they found difference in leaf pigments during two different seasons (Summer & Winter) in 2015.

The study area Juniper Forest ecosystem of district Ziarat has an extensive diversity of remedial plant species. Several species of these plants are being utilized in native medication. District Ziarat is located in the northern Baluchistan. This region is actual rough and fragmented containing High Mountain, bottomless valleys by rash grades and having summary watercourses. The climate of the area is drying temperate, with exciting cold throughout winter and pleasant and cool summer. Snow falls occur among December to march and mean annual precipitation is about 282 mm/yr (Ahmed *et al.*, 1990). There is no information about the pigment concentration of wild medicinal plant species of district Ziarat Baluchistan.

1.1. Objectives:

- To estimate the pigments Chlorophyll a, b & Carotenoid level in wild Seventy-Three medicinal plant species of juniper forest ecosystem.
- To determine the folk or traditional rich values, which are frequently used by the local communities for medicinal purpose in district Ziarat.

2. Materials and Methods:

2.1. Collection of Plant Material:

This research has been performed in district Ziarat Balochistan Pakistan. Fresh leaf sample from seventy-three wild medicinal plant species *viz.*, *Achillea wilhemsii* L., *Acantholimon longiflorum* Boiss., *Alhagi maurorum* Medic., *Arnebia griffithii*, *Allium ascalonicum* L., *Asparagus neglectus* Kar. & Kir., *Berberis baluchistanica* Ahrendt., *Bunium persicum* B. Fedtsch., *Chenopodium album* L., *Coriandrum sativum* L., *Cannabis sativa*, *Centurea iberica* Pall., *Centurea cynous* L., *Citrullus colocythis* L., *Caragana ambigua* Stocks, *Cotoneaster afghanica* Klotz., *Daphne mucronata* Scherb., *Datura stramonium* L., *Descurainia sophia* (L.) Webb & Berth., *Ephedra intermedia*, *E. procera* Fisch & Mey., *Elaeagnus angustifolia.*, *Eremurs persicus* (Jamb & Spach) Boiss., *E. stenophyllus.*, *Euphorbia osyridea.*, *Fraxinus xanthoxyloides* Dc., *Ferula ovina.*, *F. oopoda.*, *Foeniculum vulgare.*, *Fumaria indica*, *Hertia intermedia*, Boiss., *Hymenocrater sessilifolius* Benth., *Hyssopus officinalis* L., *Haloxylon griffithii* Moq., *Iris stockii* Hemssley., *Juniperus excelsa* M.Bieb., *Malcolmia Africana*, *Malva neglecta*, *Morus nigra*, *M. alba* L., *Nicotiana tobacum*, *N. rustica* L., *Nepeta preatervisa*, *Olea ferruginea* Royle., *Peganum harmala* L., *Plantago lanceolata* L., *P. major* L., *Punica granatum* L.,

Prunus eburnea, *P. microcarpa*, *P. domestica*, *Perovskia abrotanoides* Karel., *P. atriplicifolia* Bth., *Pistacia atlantica* Roch. F., *Robinia pseudoacacia* L., *Ricinus communis*, *Rosa beggeriana* Schrenk., *Rosa sp.* (ver. Name: Gulab), *Salvia cabulica* Bth., *S. bucharica* M. Pop., *Sisymbrium irio* L., *Seriphidium quettense*, *S. onobrychis*, *Solanum tuberosum* L., *Sophora mollis*, *Tribulus terrestris* L., *Tamarix indica*, *Teucrium stocksianum* Hedge & Lamond., *Thymus linearis* Benth., *Taraxicum officinale* Wigg., *Vitis vinifera* L., *Ziziphora tenuior* L. and *Z. clinopodioides* Lam., were collected from different area of Juniper ecosystem of district Ziarat during summer season (April to June). For chemical analysis adequate amounts of leaf samples from Seventy-Three wild medicinal plant species were saved in labeled plastic bags and stored in ice box under dark conditions. Cooled samples were transferred to the laboratory and analyses were performed with in 12 h after the collection.

2.2. Chemical Analysis:

Chlorophyll a, b and carotenoid pigments were estimated from leaves extract of all investigated plant species by the procedure given by Lichtenthaler & Wellburn, (1983). For the soaking of 1 gm fresh leaf sample 10 ml of 80 % (v/v) acetone was utilized. The samples were centrifuged at 1000 rpms for 10 minutes. After centrifuge the clear suspension supernatant were utilized to analyze the chlorophyll and carotenoids level. For chlorophyll a, b and carotenoid concentration from leaves only 1 ml solution (for each) was used in spectrophotometer. Absorbance of extract on spectrophotometer was noted at different wavelengths such as 663 & 645 for chlorophyll a and b and 470 nm for carotenoid against blank sample of 80 % (v/v) acetone and the calculation was made by the following formula.

$$\text{Chlorophyll a (mg/g fw)} = [(12.3D_{663} - 0.861D_{645}) \div (L \times 1000 \times W)] \times V$$

$$\text{Chlorophyll b (mg/g fw)} = [(19.3D_{645} - 3.60D_{663}) \div (L \times 1000 \times W)] \times V$$

D_{663} = absorbance at 663 nm, D_{645} = absorbance at 645 nm, W = weight of fresh leaf sample, L = length of light path in cm and V = volume of leaf extract

$$\text{Total Chlorophyll contents (mg/g fw)} = \text{Average of chlorophyll a} + \text{Average of chlorophyll b}$$

Ratio among chlorophyll a to b and total chlorophyll to carotenoid were also determined as follow;

$$\text{Ratio between Chlorophyll a to b} = \frac{\text{Chlorophyll a}}{\text{Chlorophyll b}}$$

$$\text{Ratio between Total Chlorophyll to Carotenoids} = \frac{\text{Chlorophyll a}}{\text{Chlorophyll b}}$$

2.3. Statistical Analysis:

The data of plants pigment concentration were not normally distributed, and the variances were not homogenous (Levene test), therefore plant pigments contents were subjected to the Kruskal-Wallis non parametric test (Brown & Forsythe, 1974; Steel & Torrie, 1980; Ott, 1993) were used. All applied statistical parameter were done according to the SPSS (statistical Package for the social sciences) software package version 16.0.

3. Results:

The results indicated that there were significant variances among the Seventy-Three wild medicinal plant species investigated for various pigment contents (Table 1). Meanwhile there are several alterations amongst plant species for individually examined pigment, it is fair wise to reference the maximum and minimum value developed in individual species. In addition, it will be needed to indicate the overall normal plant pigment concentration between all the deliberated medicinal plant species. The chl. a, chl. b, carotenoid, Total chlorophyll contents, chlorophyll a ratio b, total chlorophyll ratio carotenoid in various Seventy-Three wild medicinal plant species are exposed in Fig 1, 2, 3, 4 and 5, respectively.

Table 1: Statistical test to determine the significant variances in pigmentation among Seventy-Three different wild medicinal plant species of Ziarat

| Statistic | Plant Pigments | | | | | |
|----------------|----------------|---------|---------|------------|----------------------|---|
| | Chl. a | Chl. b | Car. | Total Chl. | Chl. a ratio b (a/b) | Total Chl. ratio Car. (Total Chl. / Car.) |
| x ² | 103.246 | 101.458 | 125.457 | 102.673 | 114.785 | 111.354 |
| P-Value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Where, **Chl** =Chlorophyll, **Car** = Carotenoid

3.1. Chlorophyll a & b Contents:

The Seventy-Three wild medicinal plant species exhibited variation in the contents of different pigments during summer season. Among these species *Achillea welhemsii* L. and *Alhagi maurorum* Medic had minimum chl. a content (0.4mg/g fw), while *Perovskia abrotanoides* Karel. and *P. atriplicifolia* Bth. had maximum (around 2.0 mg/g fw). Some species like *Robinia pseudoacacia* L., *Seriphedum quettense*, *Pistacia atlantica* Roch. F. and *Hyssopus officinalis* L. exhibited moderate values of chl a (1.7 to1.8 mg/g fw).

With respect to chlorophyll b the pigment content were in the range of (0.1 to 0.48 mg/g fw). Minimum chl. b content was found in *Caragana ambigua* Stocks, *Daphne mucronata* Scherb and *Allium ascalonicum* L. (0.1 to 0.15 mg/g fw). *Pistacia atlantica* Roch. F. and *Seriphedum quettense* contained maximum (0.48 mg/g fw) chl. b, which followed by *Sisymbrium irio* L., *Taraxacum officinale* Wigg (0.46 mg/g fw for each) and *Robinia pseudoacacia* L. (0.44 mg/g fw) among others (Fig. 1).

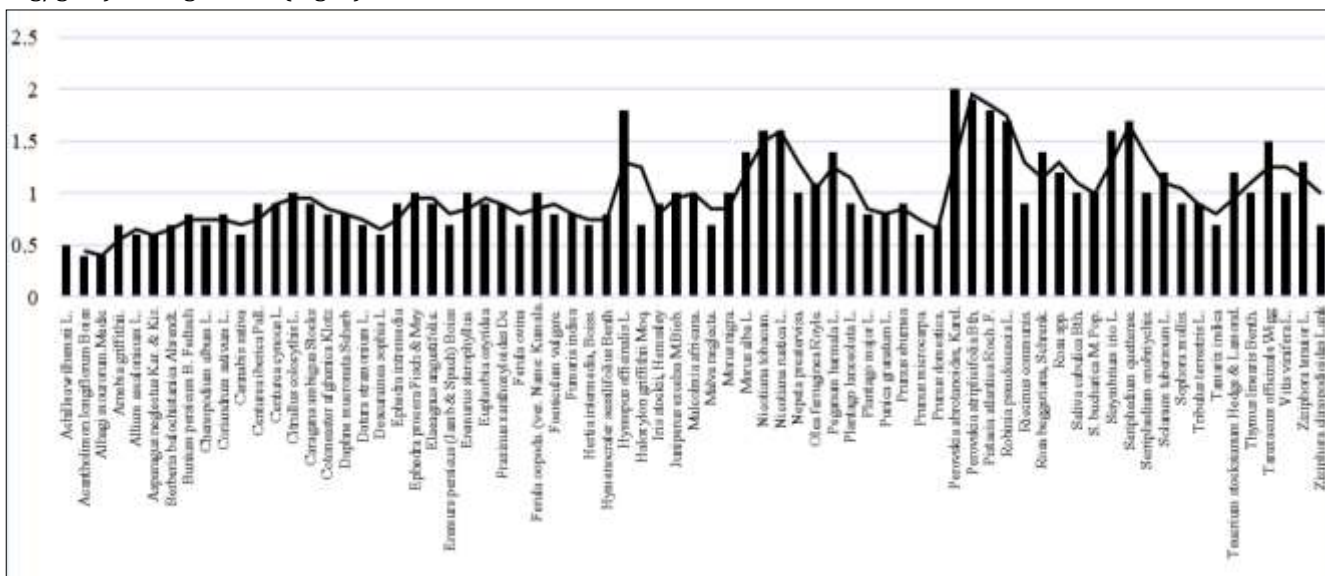


Fig. 1: Chlorophyll a (mg/g fw) contents in Seventy-Three wild medicinal plant species of District Ziarat

3.2. Carotenoid Contents:

With respect to the carotenoid its contents (Fig. 2) were found in the range (0.1 to 0.8 mg/g fw). The maximum carotenoid values (0.8 & 0.72 mg/g fw) were noted for *Berberis balochistanica* Ahrendt and *Taraxacum officinale* Wigg, which followed by *Seriphedum onobrychis* (0.6 mg/g fw), *Seriphedum quettense* and *Nicotiana rustica* L. (0.4 mg/g fw) but the minimum values (around 0.1 mg/g fw) was observed in *Arnebia griffithii*, *Allium ascalonicum* L. and *Asparagus neglectus* Kar. & Kir.

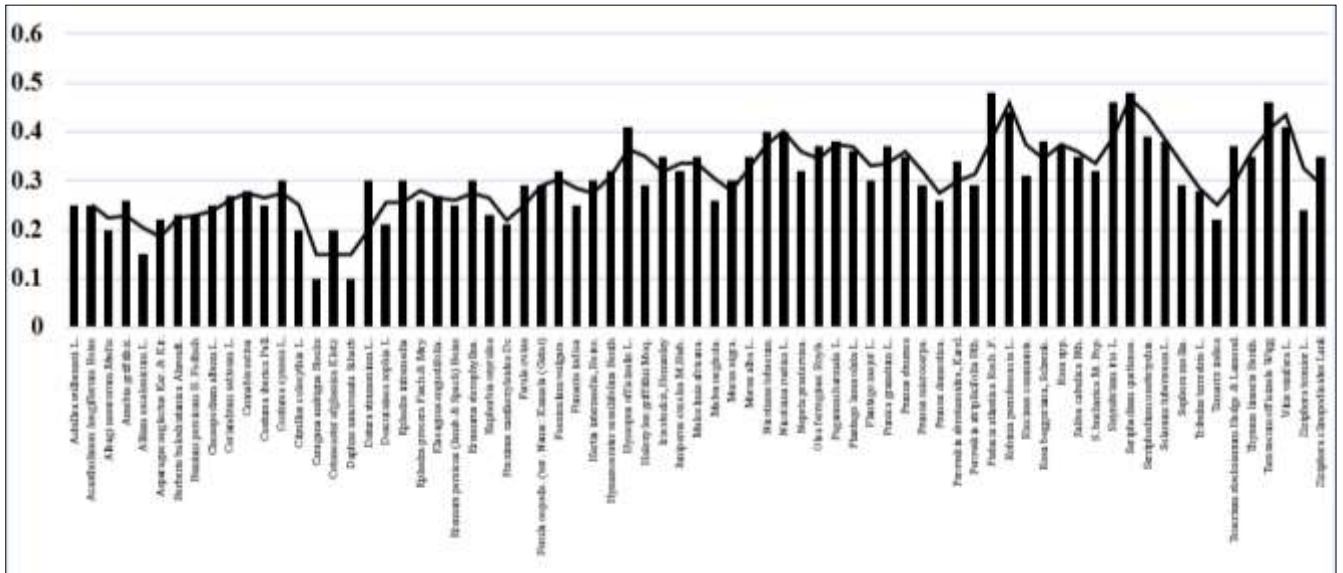


Fig. 2: Chlorophyll b (mg/g fw) contents in Seventy-Three wild medicinal plant species of District Ziarat

3.3. Total Chlorophyll Contents:

The total chlorophyll contents were found in the range of 0.6 to 2.34mg/g fw (Fig. 3). Maximum values of total chlorophyll (a + b) were obtained in *Perovskia abrotanoides*, Karel., which followed by *Pistacia atlantica* Roch. F. and *Hysopus officinalis* L. (2.28 & 2.21 mg/g fw), respectively, while the minimum contents was obtained in *Alhagi mourorum*. Medic (0.6 mg/g fw) and *Achillea welhelmsii* L. (0.65 mg/g fw).

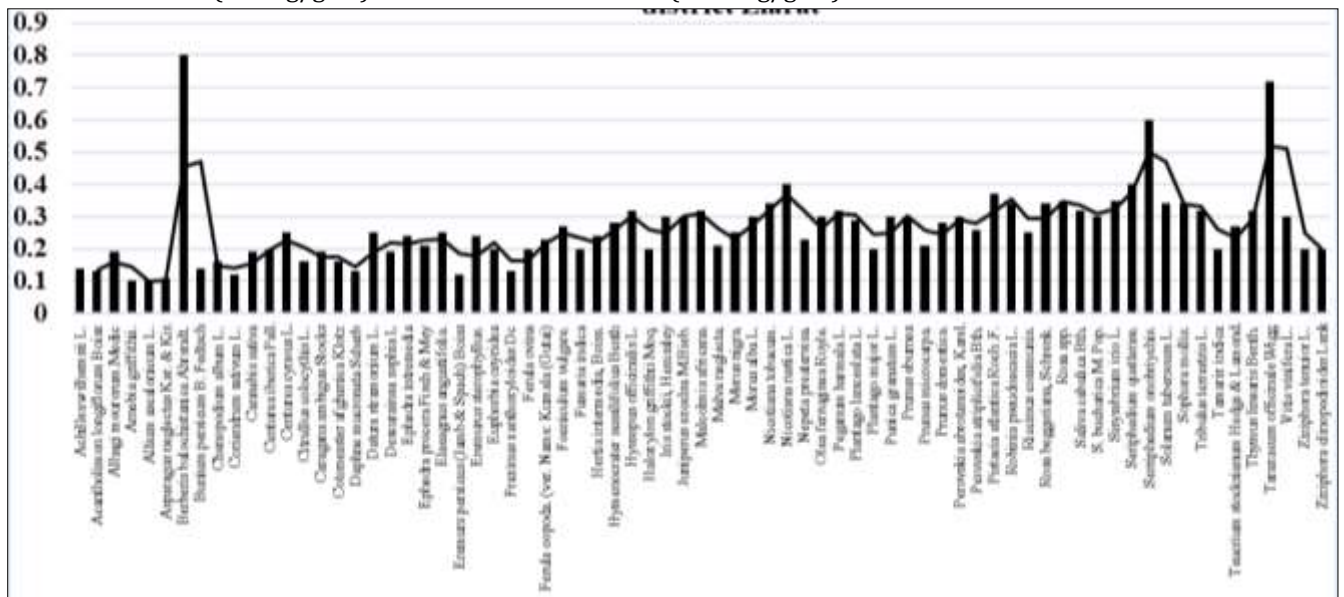


Fig. 3: Carotenoids (mg/g fw) contents in Seventy-Three wild medicinal plant species of District Ziarat

3.4. Ratio Among Chlorophyll a, b and Total Chlorophyll to Carotenoid:

Ratio between chlorophyll a to b (a/b) was in the range of 1.6 - 9.0 mg/g fw (Fig. 4). Maximum ratio was noted in *Caragana ambigua* Stocks (9.0 mg/g fw), which followed by *Daphne mucronata* Scherb (8.0 mg/g fw), *Perovskia atriplicifolia* Bth. (6.55 mg/g fw) and *P. abrotanoides*, Karel. (5.88 mg/g fw) and the minimum was (1.6 mg/g fw) for *Achillea welhelmsii* L.,

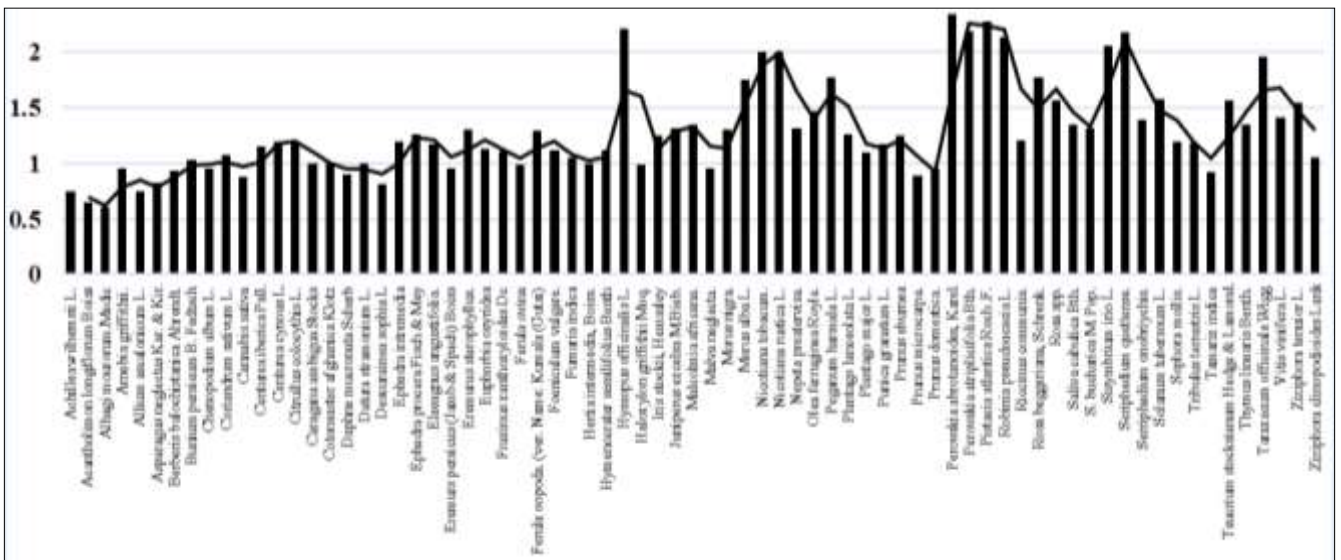


Fig. 4: Total Chlorophyll a+b contents (mg/g fw) in Seventy-Three wild medicinal plant species of District Ziarat

Further that the total chlorophyll (a + b)/carotenoids ratio was found between 1.16 to 9.6 mg/g fw (Fig. 5). Maximum ratio was noted in *Arnebia griffithii* (9.6 mg/g fw), which followed by *Coriandrum sativum* L. (8.92 mg/g fw), *Fraxinus xanthoxyloides* Dc (8.54 mg/g fw) and *Perovskia atriplicifolia* Bth. (8.42 mg/g fw), while minimum ratio was reported (1.16 mg/g fw) in *Berberis balochistanica* Ahrendt.

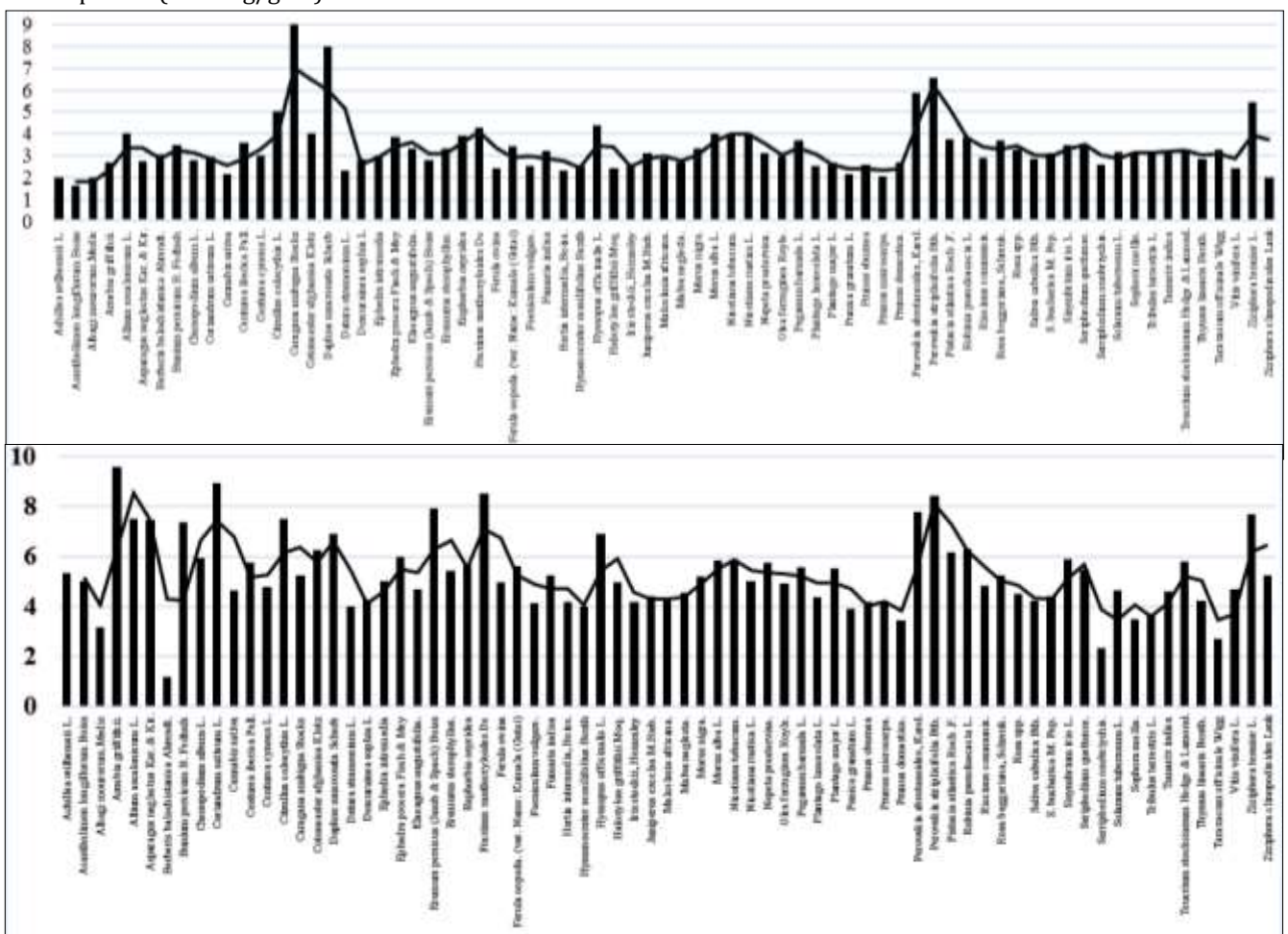


Fig. 5: Chlorophyll a/b & Total Chlorophyll (a+b)/Carotenoids ratio in Seventy-Three wild medicinal plant species of District Ziarat

4. Discussion:

On earth the chlorophyll is one of the greatest organic molecules as stated by Reinbothe *et al.* (2010). The consequences of this study disclose that the different wild medicinal plant species (Seventy-Three species) showed variation in the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. This conclusion authorizes the significance of the plant species in their ability for the construction different stain to control photosynthesis process in the leaves and their likely standards. Plant pigments play very important function in the ecosystem productivity (flower development, phenology, periodicity, fruiting, leaf senescence, leaf surface wax characteristic, biomass production, seed germination, seedling growth, physio-biochemical characteristics and plant growth), but it is fact the productivity is also influenced by environmental condition such as air pollution, extreme temperature and drought that predominant through winter and summer seasons (Iqbal & Siddiqui, 1996; Iqbal & Shafiq, 1997; Aksoy & Sahl, 1999; Iqbal & Shafiq, 1999; Gonzalez *et al.*, 2000; Aksoy *et al.*, 2000; Wagh *et al.*, 2006; Prajapati & Tripathi, 2008; Shafiq & Iqbal, 2007; 2012; Honour *et al.*, 2009; Narwaria & Kush, 2012; Leghari *et al.*, 2013a; Parveen *et al.*, 2014). It is important that the output of any plant is judged through the process of photosynthesis in green leaves and their variation adopted by leaves (Valladares & Pearcy, 1997; Valladares *et al.*, 2000; Gonzalez-Rodriguez *et al.*, 2004; Boutraa *et al.*, 2015; Khalequzzaman, 2015). The little temperature in winter affects the growth of plant species, such situation reflect in the form of possibly insufficient production of photosynthetic pigments. Castrillo *et al.* (2001) indicated that dominant shade characteristics also affect the chlorophyll level. Present study area is thickly covered by the Juniper Forest, which shaded over the wild medicinal plant species which may cause high chlorophyll contents in most shade wild plant species. Valladares *et al.* (2000) reported the chlorophyll concentration was maximum in shade leaves, while the carotenoids and non-photochemical increased with light. Similar observation was reported by Demmig-Adams and Adams III, (1992). They also reported that the sun leaves of plants enclosed greater quantity of carotenoid and of xanthophyll, while the lessening of chlorophyll contents is not due to the adaptation against drought disorder during summer, which may be appropriate in the region of District Ziarat Balochistan Pakistan. These leaves stains concentration might be due to the variation in leaf structural parameters indices for the production. In 2000 Sims and Gamon, investigated the relationship between the level of leaf pigments and spectral reflectance. Daniel & Gamon, (2002) studied the relationship between leaf pigment content and spectral reflectance and exposed the environmental effects on pigment contents. Leghari *et al.* (2013b) reported that the all the investigated plant species were under stress and showed variation in their chlorophyll contents which might be due to the environmental problems and seasonal variation. Several investigators (Jiang & Han, 2008; Kalituhu *et al.*, 2007) reported that the fresh leaves produced considerably greater contents of chlorophyll and perform photosynthesis process better as compared to the completely extended leaves further that the leaf direction, photorespiration and xanthophylls cycle photorespiration and xanthophylls cycle shelter to the young seedling in contradiction of high irradiation in field. Giufrida *et al.* (2006) investigated chlorophyll and chlorophyll derived components in *Pistacia vera* L. and reported Thirteen different compounds. Goncalves *et al.* (2001) examined the environmental light effect on photosynthetic pigments and chlorophyll fluorescence in *Switinnia macrophylla* Kung, (Mahogany) and *Dipteroryx odorata* (Tonka bean) and found grater contents of chlorophyll in shade leaves then that of sun leaves. Kyparissis *et al.* (2000) investigated eight Mediterranean plant species belonging to different growth forms and found seasonal variations in xanthophyll cycle (photo-protective) and Chlorophyll contents (photo-selective capacity in these plants).

5. Conclusion:

Remedial plant species are significant to control the various kinds of humanoid illnesses. The leaf pigments (chlorophylls and carotenoids) in these medicinal plant species are vital for the assimilatory materials and are not only responsible for variation of color from dark green to yellow as well as they play important roles in the process of photosynthesis, pollination and the production of different pharmaceuticals components in medicinal plants. Present study indicated that in the same environmental condition the different wild medicinal plant species had significant variation in their chlorophyll a, b and carotenoids contents, with different ratio. This variation in pigments contents (chlorophyll and carotenoids contents) may indicate that their shell be the variation in other physiological components, which may help to screen the medicinal plants for the preparation of drugs and may also be helpful to cure human diseases in local area. Information of this investigation suggest that, even though, all the medicinal plant species of juniper ecosystem differed with respect to the pigment contents in suitable or hostile environmental circumstances such water scarcity and lowermost temperatures, they quiet might play significant part in upholding the efficiency of this kind of rangeland ecologies. Nevertheless, investigation on leaf chemical, morphological, structural and molecular level must be focused to explain the

fundamental tools working by these wild medicinal plant species to adjust to the present ecology, through the resolve to classify important devices that enhance or decrease the color contents, and in what way they are connected to photo-chemical efficiency and photo-inhibition. These enquiries might be absorbed with together leaf and chloroplast level. Consequently, the juniper forest ecosystem in Ziarat deliver a good chance to examine the eco-physiology and photo-protective volume of the natural medicinal plant species which usually are being utilized as a fodder source for local livestock and wildlife.

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Socio-Economic Impact of Covid-19 in Pakistan: A Case Study of District Dera Ghazi Khan

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Abstract

The last couple of month of 2019 shared the news of a new virus from Wuhan (Li *et al.*, 2020) a city of China. Initially no one gave attention to this pandemic. Chinese government took it serious after the deteriorating situation. WHO warned the world about this fatal disease continuously and suggested precautionary measures. But the year 2020 is considered very important in the modern world with the human progress. It will also be remembered as a most critical time due to the dangerous pandemic of Corona which got fame as a Covid-19. This Covid-19, which wreaked havoc on the world. The most developed countries of the world looked helpless before this pandemic of Covid-19. The covid-19 devoured human demons regardless of color or religion, and the world took a new turn in the face of this dangerous and hidden enemy. During the time of most fatal pandemic of Covid-19 in modern world of 21st century the world has seriously affected due to the impacts of this Covid-19 which proved dangerous enemy of humanity. This hidden enemy changed the behavior of humanity strongly during the era of this pandemic. Covid-19 spread in the entire world, and it realized the need of global level cooperation among the world because Covid-19 influenced badly on the socio-economic life of the people of underdeveloped countries not only but also destroyed the powerful and developed western countries i.e., education, health and economy which is a serious human issue. This paper highlights the socio-economic effects of Covid-19 in Pakistan especially in Dera Ghazi Khan during this pandemic era.

Keywords: Pandemic; Covid-19; Global; Education; Economy; Pakistan; Dera Ghazi Khan

1. Introduction:

The paper primarily deals with the socio-economic impacts of Covid-19 in Pakistan with a case of Dera Ghazi Khan. Covid-19 which initially appeared from China in the end of 2019 and soon it spread globally as pandemic. Covid-19 crushed the socio-economic condition of the developed world. In Pakistan it diagnosed in February 2020. It created a fear on the society and the social distancing changed the behavior of the people. The state of Pakistan's economy was already dilapidated, but its size has shrunk further in the Corona crisis. A weak public health system, a large population, and the fact that many families live in densely populated areas due to poverty, all these indicate the devastating Corona virus epidemic in Pakistan. Dera Ghazi Khan is the most southern (Bhatti, 1987) district of

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© 2021 (Accepted for publication in August 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

Punjab and considered as a backward District also facing this crisis. Labour, health and education affected badly while school going children faced the educational loss. This paper explores the impacts of Covid-19 on the socio-economic condition of the people of Dera Ghazi Khan.

1.1. Statement of Problems:

The Covid-19 is a world-wide Pandemic and it spread in the entire world with all its strength. It crushed the economy, health and education system of the modern and developed power i.e USA, UK and other western countries. Pakistan is underdeveloped country of the third world, and it performed better than the developed countries. The selected region for this topic is most backward District of Pakistan and the impacts of this covid-19 could be looked during the last 14 months how this pandemic affected the society of the region is a basic problem of this research which need the answer especially the area where insufficient facilities are also big issue.

1.2. Objectives:

- To highlight the socio-economic effects of Covid-19 in Pakistan especially in Dera Ghazi Khan during this pandemic era.
- To explore the impacts of Covid-19 on the socio-economic condition of the people of Dera Ghazi Khan.

2. Research Methodology:

Socio-Economic Impact of Covid-19; A Case of Dera Ghazi Khan District is an Analytical Study. It is an attempt to analyze the impacts of Covid-19 on the socio-economic condition of Dera Ghazi Khan as a backward region. The primary aim of this study is to understand how Covid-19 affected the life of the people of the region Dera Ghazi Khan. In this Paper, the use of quantitative data has also been made to understand the point of discussions, wherever was necessary. However, the quantitative method is combined with qualitative and historical method and an attempt has been made to find the answers such as questions through sources. The sources are consisting of mostly the interviews of the Teachers, Students, Doctors, Nurses, Lawyers, Businessmen, Shopkeepers, Workers and Daily wagers. The tool of survey has also used to compile this paper. All this data is based primary Sources of research.

2.1. Literature Review:

During this pandemic, scholars and researcher are working on this serious human enemy and for this few important studies have been introduced and tried to explore the socio-economic impact with health, education, economy even religious rites of the people Abodunrin *et al.* (2020) indicated its fatal and unlimited attacks on people. While Fetzer *et al.* (2020) describes it fatal for economy. He has tried to explore the worldwide web information indicating the drastic effects of COVID-19. Sahu, (2020) is disappointed to indicate about the fatal effects of this pandemic COVID-19 in this era on education. He stated all staff, teachers and the students all are in trouble. The findings of his research explained and indicated that over 80% students have been suffering due to COVID-19 situation. The net facilities are not available everywhere especially underdeveloped areas. This condition has caused a huge stress among the educational system as well as the untrained conduct of online classes created hurdles for education. Karabag, (2020) highlighted the political and global conflict due to this pandemic. All these studies explore the impacts of Covid-19 on education, health, economics, norm, traditions and worship globally. Aarif and Yasir, (2020) explained the more rapid impact of Covid-19 on children as the survey District Sawabi of KPK. But there is no study which reflects its impacts in this selected region, which is already under the grip of poverty, illiteracy, poor sanitation system with deprivation of modern facilities of transportation and communication. This paper will be proved useful for the regional impacts of Covid-19.

2.2. Historical Perspective of Covid-19:

It is very clear that Covid-19 initially reported Wuhan from a province of China and then expands across China and beyond. First of all, people considered it a seasonal flu which was the key mistake of this global world. There is an opinion that the virus was originated in bats, but no one is aware how it jumped to the people it is unknown. After China it wreaked globally and destroyed the world. China imposed a lockdown on the city of Wuhan

on January 23, 2020, due to the Corona epidemic. After the announcement of the implementation of the lockdown in Wuhan, strict restrictions were imposed there from the end of January 2020 to the end of June 2020. It was completely cut off from China and the rest of the world. More than 125 million people worldwide have contracted the disease so far. Three million people have died. As a result, the life system of the world has become paralyzed. Covid-19 has had a devastating effect on the Pakistani economy as well as a number of social problems. In which the education and health system is very ruined. A quarantine center was set up in Pakistan to isolate covid patients, and the first quarantine center was set up at Ghazi University, the largest educational institution in Dera Ghazi Khan, bringing 800 visitors from Iran to Taftan and them first to be quarantined.

3. The Impacts of Covid-19 in Pakistan:

The situation caused by the corona virus has become an economic and social threat and has completely gripped the entire world. Measures to ensure a reduction in social interaction are necessary for the safety of the people, but it is affecting the student's academic year the most after the economy, the routine of life. The initial steps taken by the Pakistani government to curb the spread of the corona virus included closing educational institutions until conditions improved. But the situation is still out of control, which Pakistan's economy and society are still facing. "On the one hand, the education of the students is being harmed and on the other hand, the economic condition of the people is deteriorating further, and the nation is suffering from psychological pressure which is deteriorating the social and economic conditions. The poverty-ridden and debt-ridden economy is already mired in more problems, so it is safe to say that a weak economy is making it worse. Doctors were initially skeptical that people would start treating patients at home because of false reports of patients being 'poisoned'. But the decline has been constant. According to the UNDP, about 42 million children in Pakistan are currently deprived of education. More than 17 million children in the country are not getting vaccinated. Forty million people were already facing food shortages, an increase of nearly two and a half million due to the epidemic. According to the UNDP, there is one doctor for every 963 people in the country and an average of one hospital bed for every 1,608 people. The country is facing a shortage of 200,000 doctors and 1.4 million nurses. Experts have warned that the lockdown will also affect agriculture, while disruption in transport, labor and raw material supply will affect the sector for next year.

One third of Pakistan's population lives below the poverty line. Another one-third of the population is in the lower or lower middle class. Overall, about 66% of the population lives around the poverty line. That means 145 million people need help. According to the survey, as a result of the corona virus epidemic, the unemployment rate in Pakistan could reach 28 percent. The number of unemployed is expected to reach 6.64 million in the next financial year. There are also concerns that a long-term lockdown could lead to 30 per cent manpower unemployment in the formal sector, costing the private sector Rs 190 billions. About 42 million children in Pakistan are currently deprived of education. More than 17 million children in the country are not getting vaccinated. Forty million people were already facing food shortages, an increase of nearly two and a half million due to the epidemic. In Pakistan, people are not taking the pandemic seriously and many educated people considered the pandemic as a political strategy of government to control the protest of opposition and received foreign aid. That's why the ratio of cases is going to increase rather than reduce as compared to the first and 2nd wave of this covid-19. Pakistan economy is already needing Oxygen and the pandemic affected badly as the export, trade, transport, train and even PIA suspended its service due to Covid-19 which is a big loss for the economy. After the deaths of about 13,000 people out of 230 million, it can be said that Pakistan has apparently outperformed than many Western countries in this regard. While in UK, for example, there have been more than 41,000 deaths among 67 million people. In neighboring India, the situation is even worse in cities like Delhi and Mumbai. But in Pakistan, government's efforts to improve Pakistan's weak economy, which had begun to show signs of improvement, led to the collapse of the Corona Crisis. Experts fear that the economic and financial losses of the crisis have eroded all the government's efforts and led to an increase in poverty and inflation.

The situation affected bitterly the social and economic environment of the world which created in the result of global pandemic Covid-19. The pandemic proved havoc for educational sector and the loss is not recoverable for the nations especially in Pakistan. Covid-19 destroyed the educational system of developing countries and the lock down of schools badly affected the educational career and future of young generation in Pakistan. Although educational institutions tried to compensate the students through online education, but online education is not an alternative of physical and proper education. Globally nations tried their best to continue education on virtual basis but in Pakistan this mode of education is considered unimpressive due to lack of technical facilities and poor sources of technologies. In Pakistan, where the ratio of online classes is not better and there are very low attendance position students also became lazy and useless. Due to fourth wave of pandemic institutions are again closed till 15th September 2021 and it will be again educational loss of the students. Poor internet and networking condition created

difficulties during online education not only it also created psychological issues for the students and teachers. As the teachers raised their voice many time with question is you listening to me? Or the students always claim that due to poor network the voice of teachers is not communicated to them. Even online classes created distance between teachers and students during this pandemic of covid-19. Technology in Pakistan has not advanced enough to provide online education to students in remote areas like Dera Ghazi Khan. Students in Dera Ghazi Khan also reflect the totality of Pakistanis as almost the entire country is in lockdown, businesses are closed, human lives are in imminent danger and educational institutions, especially private universities, have to pay fees to students. Being called This facility was given to poor parents because it was not possible for them to pay their children's fees in one lump sum. While economists say that in this situation, the rate of inflation in Pakistan is likely to remain at 9.6%. Growth is expected to slow further. If the lockdown and curfew are maintained, more than one million-day laborers could face temporary unemployment.

According to him, 9 million to 1.5 million people could go below the poverty line in this way. Government expenditure is expected to increase by 8-12% while development expenditure is expected to increase by 10-15%. Experts say economic losses can be reduced by providing facilities to the industrial sector, reducing energy tariffs and protecting small industries. According to economists, the amount of aid per family should be fixed at Rs 7,000. According to a report by the Asian Development Bank, Pakistan's economy could face losses of up to 5 billion in the event of a coronavirus outbreak. If we look Dera Ghazi which was the first quarantine center for Covid-19 in Pakistan also affected from this pandemic. Dr. Khalid Tehseen is the focal person of Teaching Hospital DG Khan. He said that hundreds of patients have been treated in Covid-19 ward. This is a dangerous epidemic that can be prevented by following government SOPs. According to him, citizens should ensure implementation of government SOPs. Protect yourself and others. The first quarantine centers for Covid-19 victims were also set up in Dera Ghazi Khan, which brought from Tuftan to Dera Ghazi Khan. From Dera Ghazi Khan Quarantine, out of 829, 755 visitors with negative results have already been sent home. Dera Ghazi Khan is one of the old city of Punjab established by a Baloch Sardar Nawab Haji Khan in the west of Indus 500 centuries ago (Chand, 2005). After many dynasties the colonial forces occupied it and formed a Tumandari System to intervene in Balochistan (Sandeman, 1877). The Tumandari system brought havoc for the region, and it remained backward and under the hold of Tumadars (Hashim, 1999). After the emergence of Pakistan, the socio-economic condition did not change and that's why it considered still backward and facilities less area. Covid-19 badly affected the region it all walks of life.

3.1. Covid-19 impact in Dera Ghazi Khan:

This paper primarily deals to explore and identify that why large number of people globally affected to a big disaster after every hundred years. This mighty pandemic globally changed the people and their lifestyle and all social matters. Dera Ghazi Khan District is considered a backward region of Pakistan and there are lacks facilities in every walk of life i.e., education, health and small level business. Although government adopted only a smart lockdown policy and tried to accommodate the daily wagers with Ihsas Income Support Program, but this is not a permanent solution for the people during this pandemic which affected the life badly. The pandemic destroyed the school education and still educational activities are completely dis-functional which a national loss is for the entire people. Dera Ghazi Khan is the district which faced the more psychological pressure of this pandemic when the visitors from Iran brought in the Quarantine Center at Dera Ghazi Khan. The fear of pandemic put psychological fear on people who largely affected education, health and the trade of Dera Ghazi Khan.

3.2. Health:

If we look health system, it was bad even before the epidemic. Poor sanitation condition and the hygienic situation is also considered miserable before this pandemic, while Corona pandemic created a fear of death among the people initially and they changed their lifestyle. There is over all health crisis in Pakistan and the system has no capacity to allow such heavy burden of this pandemic as this region Dera Ghazi Khan is facing health issues where lack of hospitals and Doctors are the key factor and due to this pandemic mostly patients were referred to Rajab Tayyab Urdgan Hospital Muzaffargarh or Nishtar Hospital Multan. In the beginning people did not take the Corona seriously and frontline doctors have failed to save their own life. This situation was also alarming for the common public who were not following the safety measures directed by the health agencies. They thought it a political strategy of the government against opposition parties and that why the public gathering also continued and these activities provided a chance to pandemic in its fatal impacts. According to UNO women, children, the disabled, the elderly and minorities will be the hardest hit. Pakistani scholars emphasized that care is the better cure of this disease and they proposed social distancing and mask as compulsory tool of safety. Even now the third wave is more

dangerous, and it affected the hospital and doctors which are now overcrowded and over burden. Covid destroyed the health system and all budget consumed in this pandemic and the struggle against other fatal diseases affected.

3.3. Education:

While the Corona virus pandemic has affected Dera Ghazi Khan socially and economically, the devastation seen in the education sector does not seem to be reversible. Over the past year, the city's educational institutions, from universities to primary schools, have been hit hard. Everyday announcements of school closures have hit education hard. While children need to be protected from the virus, there is an urgent need to revive educational activities. An online education system has also been adopted to revive educational activities at the higher education level, but this is not a substitute for face-to-face education and training. Nor is it possible to promote quality education through it (Interview Shafiq, 2021).

The re-emergence of educational activities and the rise of cases in the third wave of Corona is also a matter of great concern which has left the education system in Dera Ghazi Khan in a state of uncertainty. While students are tired of sitting at home, the administration is not yet ready to take any risks. According to teachers, online education for primary level students is not effective. While the small-scale schools also disturbed badly which were providing earning to educated youth. They also became jobless which may create serious economic crisis for the person who is a custodian of family (Interview Ahmad, 2021).

3.4. Economy:

The district is a junction of four provinces and covid-19 disturbed it completely the intra-province transport has closed due to lockdown which affected the poor worker i.e., driver, conductor and cleaner and also the labor which work in different cities. The market especially small business and daily workers affected as I arranged a survey at Pakistani Chock and 100 daily wagers stated their hopeless position that they are free for many days due to the unavailability of labor (Survey, 2021). Some workers who had servant on shops, factories and mills all indicated that the owners have no business, and they are not ready to call them for duty. Similarly, shops, small hotel as tea-hotel all have become closed and the lower class have facing hand to mouth for one time meal for their children (Interview Ahmad, 2021).

3.5. Fear:

A condition of fear also created uncertainty for the people and fear of disease forced the people to stay at home and even in routine or seasonal flu or cough people became isolated in the homes from the relatives and some time they did not inform about their illness to save their selves from isolation or hospitalization. The fear seriously proved and nervous the people and upset the entire society. Due to disease many people adopted social distancing and they cut their relations from society and created new attitude in society. Nobody is ready to talk any covid-patient which is a new development in Muslim society and with the increasing time this attitude is alarming (Interview Ubaidullah, 2021).

4. Conclusion:

The globally originated pandemic Corona virus started from Wuhan and it is a most dangerous event of the last two centuries for humanity. The entire world is facing this pandemic and a living of life under isolation with social distancing. COVID-19 has brought serious challenges in Pakistan, and it has changed socio-economic condition of society. There is a much need to become careful through precaution which advised by the medical experts and Reliance on the Allah Almighty. This paper basically deals with the impacts of Covid-19 which is a world-wide pandemic at regional level especially an underdeveloped area Dera Ghazi Khan. The regional already considered a backward and the majority of the people is living a life of poverty due to the limited facilities of life with small income as the unavailability of sources of income. The region is already behind in education, health and economy and the per capita income is very small as compared to the other developed areas of Pakistan. With this alarming situation the Covid-19 proved and brought havoc for the socio-economic condition of the society, and it badly affected the economic position of the people and their life where majority is living a life of daily earn wages as labor, shopkeeper. Education system suspended from the last 14 months. Students promoted without examinations, and it is the destruction off quality education which is serious fatal for the progress of the country. Online education is not an alternative of physical education while it is only a facilitation to engage the students.

The complete lockdown and smart lockdown also affected the private schools' employees especially the teachers who were already living hand to mouth and large numbers of employees of private education sector have become jobless. Similarly, this pandemic also brought the shortage of private jobs which is very harmful for the poor people of Dera Ghazi Khan. Health system which was already very weak and there were insufficient facilities of medical with the deficiency of Doctors, Nurses and other Para-medical staff has become overloaded now. So, the discussion is concluded with this that the outbreaks of the corona virus not only spread as the deadly disease to the people of this region but also had far-reaching devastating effects in all disciplines of human life. As a whole, the situation in Dera Ghazi Khan is under control. But no reference can be made to negligence. Similarly, in Dera Ghazi Khan, the Covid-19 epidemic has badly affected small-scale businesses and educational institutions, destroying not only education but also the quality of education. At the same time, poverty has given rise to inflation, unemployment, social and economic problems which have increased the hardships of the people.

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 1- The basic question of this survey was about the current position of private job.
 2- Position of daily labour and daily wages work. 3- Position of small Business.
 Interview, Teacher, M. Shafiq, Al-Muneer Academy, Dera Ghazi Khan, 25 March 2021.
 Interview, Irfan Ahmad Private School Teacher, Al-Qasim Public School Dera Ghazi Khan, 25 March 2021.
 Interview, Saiddique Ahmad, Private Security Guard, 27 March, 2021, Saturday, 12:00 PM
 Interview, Ubaidullah, M.Phil Scholar Pak. Studies, March 26, 2021 on Friday 6:00 PM.

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