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C O N T E N T S

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Short Communication

A Global Update on COVID-19 Pandemic

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Abstract: As of October 10, 2021, the entire planet has reported 219 million COVID-19 cases, with 4.55 million fatalities. Lockdowns and softening measures have been thrown into turmoil throughout the world since the outbreak. Our social life will only return to normal once an appropriate vaccine is produced and proper authorized preventive techniques are implemented. To tackle this pandemic, governments and health experts all around the globe are experimenting with a range of measures and preventative strategies. So far, 22 vaccines have been approved. They are effective against immunocompromised people, pregnant women, and multiple sclerosis patients. Certain nations are considered to be more successful than others in terms of providing safety to their inhabitants and increasing their economic activities. A plethora of vaccinations have been produced, and a research anthology has been published. However, medical personnel are still searching for a viable treatment to limit this pandemic.

Keywords: COVID-19 pandemic, Hybrid immunity, Mass vaccinations, mRNA Vaccines, DNA vaccines, FDA.

1. INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes COVID-19, a respiratory disease. Researchers from all around the world are working to create a vaccine [1, 2]. Numerous potential vaccines are in the conduit or are in their nascent phases of clinical trials, while others are clinically accessible and have been authorized. Animal studies, as well as human trials, have already revealed possible tendencies toward achieving a high level of neutralizing antibodies. As of today, there are now 151 potential vaccine candidates, currently, 41 potential vaccines are in stage 3 clinical studies. So far, 22 vaccinations have been approved in various countries. The Food and Drug Administration (FDA) has approved the Pfizer-BioNTech COVID-19 vaccination for persons aged 16 and above, and it is being considered for kids aged 5 to 15. The vaccine is presently being marketed under the trade name "Comirnaty." Dr. Janet Woodcock, the acting FDA Commissioner, stated in the organization's news release, "While millions of individuals have already successfully received COVID-19 vaccinations, we recognize that FDA approval of a vaccine may potentially inspire further confidence in being vaccinated among the masses." Today's accomplishment puts us one step closer to changing the worldwide direction of the pandemic [3, 4]. Since the start of the pandemic, vaccine acceptance has become a great conundrum all over the world. Even rich countries have often struggled to roll out vaccines but in Global South, things are much worse [5].

2. MATERIAL AND METHODS

2.1 Global Review of Pandemic

Global review of COVID-19 pandemic is shown in Table 1.

2.1.1 What we need to know about DNA-vaccines

The field of medicine continues to see radical new techniques for combating COVID-19, with the most recent development being in vaccination. India has approved the world's first DNA vaccine for use in an emergency against COVID-19, joining almost a dozen additional DNA vaccine candidates

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in clinical testing. The ZyCoV-D vaccine works by priming the immune system against the virus that causes COVID-19, SARS-CoV-2. It is also administered without the use of an injection. This vaccine differs from the messenger RNA (mRNA) technology utilized in two of the currently approved COVID-19 vaccines, Moderna and Pfizer-BioNTech, both of which have received praise for their inventiveness [6].

2.1.2 Hybrid immunity is more effective to combat the SARS-CoV-2 virus

Several other investigations have indicated that individuals who have both contracted SARS-CoV-2 and received the COVID-19 vaccine had nearly "bulletproof" immunity against the new coronavirus and its variants. People acquire a highly powerful immune response as part of this "hybrid" immunity, as some researchers refer to it, by producing a large number of antibodies as well as "flexible" antibodies capable of repelling numerous coronaviruses, including SARS-CoV-2 subtypes. The researchers conclude, "Overall, hybrid immunity against SARS-CoV-2 appears to be astoundingly high." Whatever you call it, this type of immunity is excellent news in the middle of what appears to be an endless supply of bad COVID-19 news. Several recent investigations have revealed that certain persons have a very high immune response to SARS-CoV-2, the coronavirus that causes COVID-19. Their bodies produce very high levels of antibodies, but they also produce antibodies with great flexibility — antibodies that are likely capable of combating the coronavirus variants that are currently circulating in the world but are also likely effective against variants that may emerge in the future [7].

2.1.3 Johnson & Johnson vaccine to be 80 % effective against infection and hospitalization

Credible proof from worldwide research shows that Johnson & Johnson's single-shot vaccination provides high and stable protection against COVID-19 across time — that is, from the time the Delta variant first appeared until the time it became prevalent. The firm recently revealed the findings of a major trial, which indicated that the vaccine was 79 % effective in avoiding coronavirus infections and 81 % successful in reducing hospitalizations. The study revealed no evidence of decreased efficacy between March and late July when the number of Delta variant cases increased. "Our extensive real-world data and phase 3 trials indicate that the Johnson & Johnson vaccination provides substantial and long-lasting protection against COVID-19-related hospitalizations." Furthermore, our phase 3 study findings show further protection

Table 1. Global review of COVID-19 pandemic

COVID-19 Concerns Globally	Update & Statistics	Citation
No. of COVID-19 cases globally	313 million	[1]
No. of COVID-19 mortalities globally	4.55 million	[1]
No. of approved vaccines globally	22	[1]
No. of COVID-19 vaccination administration globally	1.93 billion fully vaccinated, 5.04 billion partially vaccinated	[1]
Can Multiple Sclerosis patients get COVID-19 vaccines	Yes	[7, 8]
Can immunocompromised people get COVID-19 vaccines	Yes	[9]
Is second dose of mRNA vaccine is safer after allergic response from the first dose?	Yes	[7]
Which countries are more reluctant towards COVID-19 vaccine jabs?	High income countries	[10]

against COVID-19-related death," stated Dr. Mathai Mammen, Ph.D., worldwide head of Janssen Research & Development at Johnson & Johnson. Previously, early results indicated that when a booster dose was provided 2 months after the initial injection, the vaccine's effectiveness against moderate-to-severe COVID-19 rose to 94 percent [3, 6].

2.1.4 FDA to approve COVID-19 vaccines for senior citizens

In October 2021, a Food and Drug Administration (FDA) advisory group decided against authorizing a booster dose of the Pfizer COVID-19 vaccination for anyone aged 16 and over. The booster dosage for younger people was defeated by a vote of 16-2. However, the same advisory group voted 18 to 0 in favor of allowing the booster dosage for people over the age of 65 and those at high risk of developing severe COVID-19 after viral infection [3, 4].

2.1.5 COVID-19 vaccines effective against Multiple sclerosis patients

Anti-CD20 monoclonal antibodies, which restrict the activity of B cells, are frequently used by people with multiple sclerosis. A recent small-scale study discovered that, despite the immunosuppression, there was still a strong T-cell response to COVID-19 immunization [8].

2.1.6 Hesitation towards COVID-19 vaccine jabs among low- and middle-income countries

According to a new study published in the journal 'Nature Medicine', populations from low - and middle-income countries (LMICs) had less COVID-19 vaccination reluctance than those from high-income nations. In addition to this, seven studies were conducted in low-income countries (Sierra Leone, Burkina Faso, Rwanda, Uganda, and Mozambique) five studies were undertaken in nations with a lower-middle-income level (India, Pakistan, Nepal, and Nigeria) and one study was done in middle-income country (Colombia). According to the data, the average vaccination acceptance rate in LMICs was 80.3 %. They also demonstrated that even the LMICs with the lowest rates — Burkina Faso and Pakistan — performed better in terms of COVID-19 vaccine uptake than Russia and the United States. Vaccine uptake rates were 66.5 % in both Burkina Faso and Pakistan. Ironically, the digits were 64.6 % in the United States and 30.4 % in Russia. Dr. Alexandra Scacco, a senior research fellow at the WZB Berlin Social Science Center and co-author of the study, observed that "Across nations, we see that adoption of COVID-19 vaccines is typically somewhat diverse, it may be due to their novelty" [9].

2.1.7 Is the second dose of mRNA vaccine is safer after an allergic response from the first dose?

An estimated 2 % of individuals experienced adverse side-effects from mRNA COVID-19 vaccinations, such as the Pfizer-BioNTech and Moderna vaccines. The great majority of these responses are mild. A new study article published in the Journal of American Medical Association examined how these people responded to their second dosage. The study included information from 159 people who experienced an adverse reaction from their first dose of mRNA vaccination and then received a second dose [10].

According to the study, 47/159 individuals took an antihistamine before the second dosage. "All 159 patients, including 19 with first-dose anaphylaxis, tolerated the second dose. Thirty-two (20 %) patients had acute and possibly allergic symptoms associated with the second dosage that were self-limited, mild, and/or alleviated with antihistamines alone" [11].

2.1.8 Experimental COVID-19 vaccines to lasts at room temperature for a month

In a recent study, mice and macaques were given a single dosage of a novel adeno-associated viral vector-based vaccination. The vaccination not only elicited a robust immune response against SARS-CoV-2 variations, but it also stayed stable at room temperature for 1 month. Although the currently available COVID-19 vaccines are extremely effective, they have limited production capacity, and several — such as the Moderna and Pfizer-BioNTech vaccines — need cold-chain storage, which limits their worldwide availability [12].

3. CONCLUSION

Coronavirus is declared a pandemic by WHO the previous year. Since its inception health pantheons and several big Whig pharmaceutical companies striving best to contain this virus by implementing a plethora of preventive measures and developing COVID-19 vaccines. So far, many vaccines have been developed and their results are very promising. Hitherto, scientists have achieved marvelous results regarding these vaccines such as these vaccines are safe for younger people, pregnant and lactating women, immunocompromised people. Prior to vaccines, many allopathic drugs were used to contract COVID-19 patients such as ivermectin, dexamethasone, actemra, etc. To date, only 5 vaccines have been approved by WHO for emergency use that includes, Pfizer-BioNTech, Moderna, AstraZeneca, Sinopharm, Johnson & Johnson. Furthermore, vaccine boosters are also efficacious against the deadliest strains of COVID-19 such as Delta, Epsilon, Beta, and Alpha. Now people across the globe are trusting these vaccines and voluntarily administering them.

4. CONFLICT OF INTEREST

There is no conflict of interest.

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Research Article

Capitalizing Trees for Carbon Sequestration as a Co-Benefit of Biophilic Urbanism

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Abstract: Biophilic urbanism as an emerging paradigm in the design field has initiated various patterns of nature-based mutation. One of the most associated environmental benefits of this amalgamation of nature in urban design is carbon sequestration [CS]. The main focus of this research was to quantify the potential of trees to act as carbon reservoirs. It was investigated by assessing the roles of several tree parameters, such as diameter at breast height [DBH], height, biomass, and age in CS. A comparison of native and exotic trees was also done for this. In a field survey at Jilani Park, Lahore, 16 different families of trees (N = 115) were measured through a non-destructive method and CS was calculated. The results revealed that sample trees sequestered 588452.9 kg of carbon with an annual rate of 19998.92 kg and Combretaceae (M = 11813.65, SD = 6492.38) and Moraceae (M = 9909.93, SD = 12695.26) were the dominant families in doing so. The Pearson's correlation and linear regression analyses indicated that biomass and DBH have a significant positive relationship with *CS*, r = 0.100, R^2 = 0.99, and r = 0.943, R^2 = 0.89, respectively. The independent-sample t-test revealed a significant difference in CS capacity between native and exotic trees, with t (67.626) = 3.016, p =.004, and the greater biomass and DBH of native trees were the distinguishable factors. To conclude, trees are the most efficient source of carbon attenuation in the urban environment, and native species have an advantage in this process. This study will inspire new endeavours in research related to the benefits of biophilic cities.

Keywords: Carbon dioxide, Carbon sequestration, Biophilic, Urbanism, Biomass.

1. INTRODUCTION

Urban design in the twenty-first century necessitates a cautious approach that not only conserves nature but also finds new ways to incorporate it [1]. A new perspective on biophilic urbanism can aid in designing with nature for a healthier, more climate-friendly, and sustainable urban environment [2]. Biophilic elements, as a natural resource, can act as carbon reservoirs, which can therefore increase adaptability to climate change in cities [3]. Africa *et al*. [4] suggested the incorporation of a biophilic design to combat climate change because sequestration of carbon is reported as a common benefit of biophilic urbanism [5, 6].

Trees act as natural purifiers by captivating carbon in their biomass [7]. The active accumulation of carbon dioxide by trees, both in the form of biomass and in the soil, can act as potential carbon sinks [8]. Urban greenery utilizes atmospheric carbon dioxide in the process of photosynthesis and stores an excess of it in the form of a reservoir [9], thus playing an active role in the natural carbon cycle [10].

Unfortunately, anthropogenic activities like deforestation disturb the natural phenomena of the carbon cycle on a global scale [11]. The aftereffects of this can be seen in the rising temperature of urban areas, which is associated with a high accumulation of carbon dioxide in the atmosphere

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[12]. Consequently, devastating destruction in the environment is observed at an alarming level with an even worse future trajectory [13].

This problem has brought the whole world on the same page by passing climate treaties, including the Kyoto protocol (2005) that was superseded by the Paris agreement (2015), highlighting the need for a reduction in carbon dioxide [CO_2] emissions [14, 15]. The analysis of the reduction in CO_2 emissions in different countries showed a positive result of the Kyoto protocol [16, 17]. However, more effort is required to achieve desirable results [14, 18]. Recent flooding, wildfires, hurricanes, and dense smog in different parts of the world have opened the eyes of all stakeholders to the urgent need to work on eliminating CO_2 emissions throughout the world.

Although there has been debate about the rate of carbon dioxide off-set by urban trees in comparison to the high anthropogenic CO_2 release in cities [19], the high potential of trees in carbon uptake and assisting in the achievement of carbon neutrality cannot be overlooked [20, 21]. Urban tree cover acts as a carbon sink, bending the rising carbon curve at a point where carbon emissions can be controlled below 2 °C [21] and helping to mitigate climate change at the local level [22, 23].

Different mitigation and adaptation strategies have been suggested as a solution and for adding resilience to live with this situation better [24]. One of the most economical solutions is to increase green spaces in cities, which would have the potential to cut down on the rising level of CO_2. The biophilic design focuses on incorporating natural elements and features, including green plantations in cities, for an enhanced environment. There is much research that has addressed different benefits of biophilic urbanism, but a gap has been identified in assessing the direct role of biophilic cities in reference to the tree's capacity for CS. This research is designed to assess the urban tree capacity for carbon pooling and compare the roles of native and exotic trees in sequestering carbon dioxide. The objectives of this study are:

- To assess the role of trees in carbon sequestration, which can be a potential benefit of biophilic urbanism.
- To evaluate the relationship between carbon sequestration and DBH, height, total biomass, and age of a tree.
- To compare native and exotic trees for carbon sequestration.

2. MATERIAL AND METHODS

2.1 Site Characteristics

This study was conducted in Jilani Park, covering an area of 88 acres on Jail Road, Lahore shown in Fig 1. This city has a composite climatic condition with marked seasons of harsh summers, cold winters, and a heavy monsoon period. The Jilani Park is renowned for its beautiful flora on fertile soil and has many other recreational facilities. One of the more dominant characteristics of this park is the presence of more than 100 types of native and exotic species of trees that made it suitable for data collection in this study.

2.2 Measurement Protocol and Data Collection

This study was developed to assess the capacity of trees for CS. The field survey was conducted from January to March 2020 to collect data in the old and densely planted areas with native and exotic tree species in Jillani Park. The two detailed lists of trees having information about local names, girth measurement (DBH), and age of the trees were provided by the Pakistan Horticulture Authority [PHA] as this park is directly under their supervision. The non-destructive method of biomass estimation was used [22, 23, 25, 26]. The DBH (1.3 m above ground) and height were measured using tape and a clinometer, respectively [22, 27]. The estimated height of the trees was computed with the help of a given formula [28, 29]:

$$h = (\tan A \text{ x } d) + \text{eye height}$$

The pilot study was carried out on ten trees at the campus of Lahore College for Women University, Jail Road, Lahore, which ensured the accuracy of the measurement technique, and after that, the actual field survey was conducted in Jilani Park. The record of girth measurement (DBH) was already done by PHA staff for the selected trees, and the height was measured by using the formula. The data was recorded in spreadsheets for further

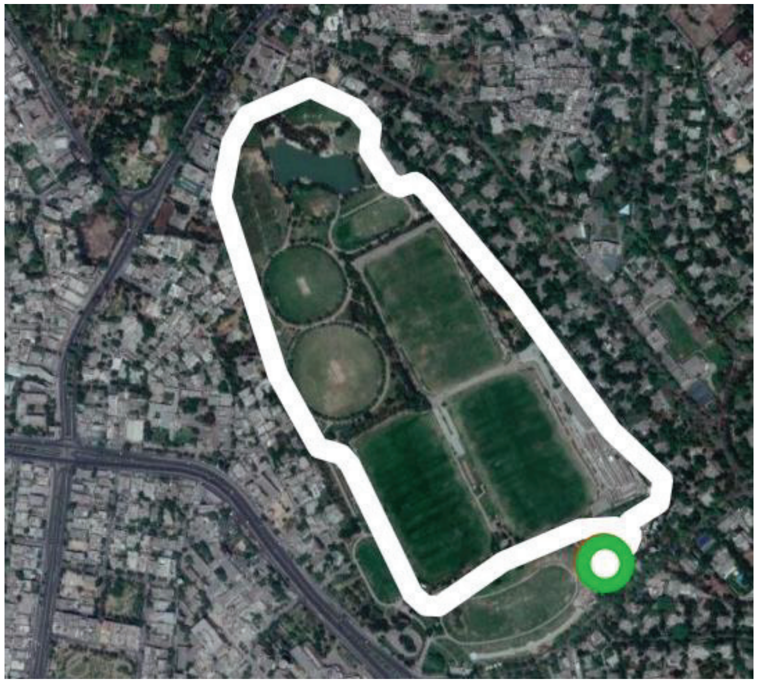

Fig.1. Google Map of Jilani Park at Jail Road, Lahore

calculation.

2.3 Data Analysis

The collected data was utilized to measure the amount of CS in a tree through a formula developed by the University of Nebraska [29 - 33].

(When D < 11inches)

$$W = \frac{0.25 \times D^2 \times H \times 120\% \times 72.5\% \times 50\% \times 3.6663}{\text{Tree age}}$$

(When D $\geq$ 11inches)

$$W = \frac{0.15 \times D^2 \times H \times 120\% \times 72.5\% \times 50\% \times 3.6663}{\text{Tree age}}$$

Here, W stands for the weight (lb., later converted into kg) of CS in a year, D denotes the diameter (inches), and H shows the height (ft.) of a tree. The tree capacity of CS was estimated in the following step.

- To calculate total green weight, the above-ground weight [AGW] was estimated by taking the product of diameter (squared) and height with 0.25 and 0.15 for trees with a diameter of more than 11 and less than or equal to 11 respectively. The below-ground weight of the root system is composed of 20 % as much as the above-ground weight that was multiplied with it to get the total green weight.
- The total dry weight of a tree was calculated by taking the product of total green weight and 72.5 % (on average, a tree has 72.5 % dry matter and 27.5 % moisture).
- The total carbon content in a tree was computed by multiplying the total dry weight by 50 % (on average, 50 % of a tree's volume is composed of carbon compounds).
- The CS by a tree was estimated by taking the product of total carbon weight and 3.67 (this value is based on the carbon ratio in CO_2 which has one molecule of carbon along with two molecules of oxygen as well).
- The final step was to calculate the annual CS of a tree that was determined by dividing the attained carbon sequestration weight by the age of the tree.

After computing the required values on Microsoft Excel, a detailed analysis of carbon sequestration by trees was done through SPSS (23.0). The descriptive statistics were used to calculate the frequencies and mean scores of the variables under study. The relationship among the variables was explored with the Pearson correlation coefficient and linear regression analysis. The independent sample t-test was applied to estimate differences between native and exotic trees in sequestrating carbon.

3. RESULTS AND DISCUSSION

3.1 Carbon Sequestration by Trees

The assessment of CS in the different families of trees in the sample was done through descriptive statistics. The results showed that a total of N=115 trees from 16 families were enumerated to acquire data, in which the distribution of native 56 (48.7 %) and exotic 59 (51.3 %) trees was almost the same. The division of trees into evergreen and deciduous domains was 57 (58.3 %) and 48 (41.7 %) respectively. The dominant families of trees in this sample were Moraceae 23 (20 %), Bignoniaceae 16 (13.9 %), Fabaceae 15 (13 %), Apocynaceae 14 (12.2 %), and Sapotaceae (Table 1).

The efficacy of trees in CS was assessed among 16 families and a total of 588452.9 kg and 19998.92 kg annually was computed for these 115 trees. Among the families of trees, the three main types, including Combretaceae *(M=11813.65, SD=6492.38),* Moraceae *(M=9909.93, SD=12695.26),* and Bombacaceae *(M=8350.60, SD=4720.94)* had a higher ability to do so (Figure 2).

Furthermore, it was found that Combretaceae (N=4) was composed of native trees, *Terminalia arjuna*, for this sample showed the highest average capacity for carbon pooling among all families. The Moraceae (N=23), with 19 native and 4 exotic trees, had the second-highest average value of CS for its native trees, including *Morus alba, Ficus religiosa, and Ficus benjamina.*

The third family, Bombacaceae (N=2), showed a prominently significant mean value for sequestrating carbon and had exotic trees like *Chorisia insignis* in it. The compatibility of these findings was found to be consistent with the results of previous research on *Terminalia arjuna* [34] (Combretaceae), *Morus alba* [35], *Ficus benjamina* [22], and *Ficus religiosa* [36] (Moraceae), in which these trees proved to be a good source of carbon

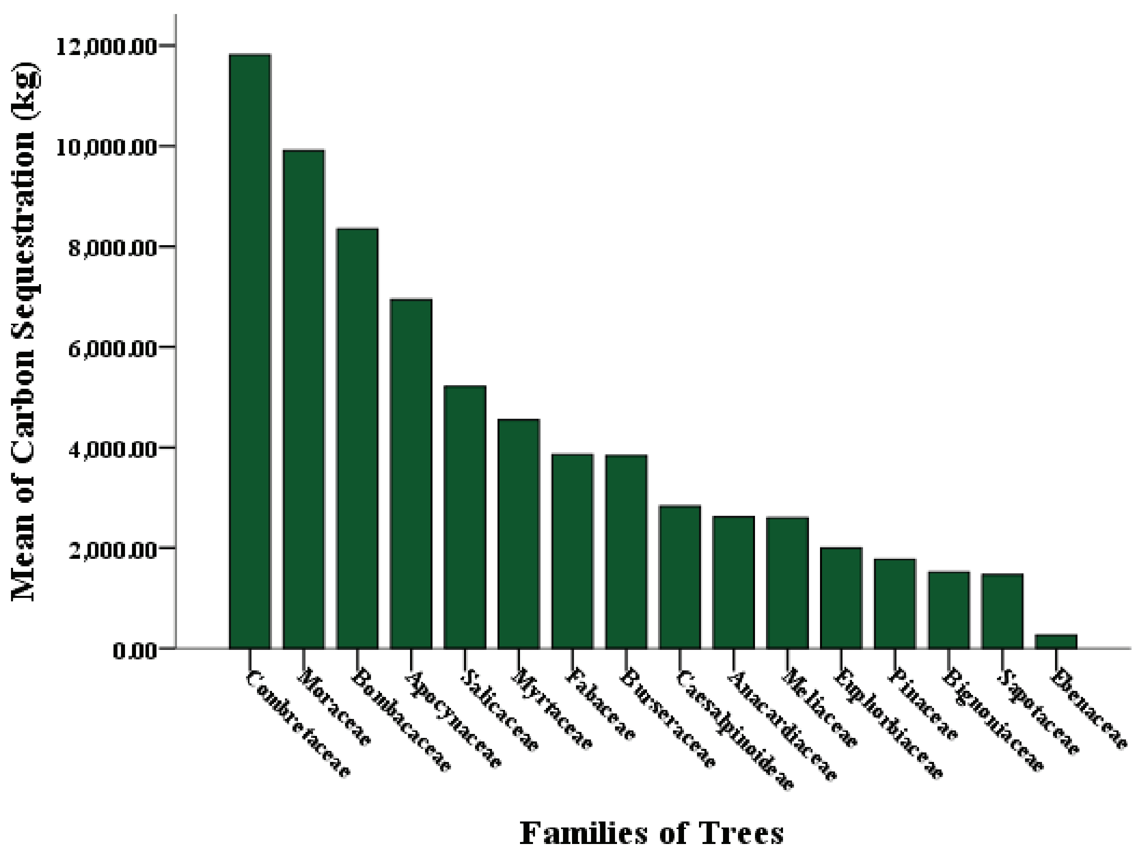

Fig. 2. Bar chart showing Carbon Sequestration by different families of trees

storage in the particular study areas.

Figure 3 indicated that families with native trees had the highest mean values for CS, and at the same time, families with exotic trees also showed a good capacity for carbon storage, as demonstrated by *Chorisia insignis* of Bombacaceae in this case. Several studies have found that combining different families of native and exotic trees improves carbon sequestration performance [22, 37, 38]. Mixing different species of trees had more profound results in carbon storage [39, 40].

The findings tended to suggest that even with less frequency, a few families had shown better results for CS, and a difference was also found between native and exotic types. Therefore, there would be some major components of trees that play a vital role in absorbing and storing carbon that was further explored in detail in the next section.

3.2 Carbon Sequestration and Characteristics of Trees

It was anticipated in the previous section that the phenomenon of CS in trees would be based on certain characteristics, including DBH, average height, total biomass, and age of a tree. Therefore, to explore associations among these variables, correlation and linear regression analyses were applied. The analysis of the relationship between DBH, height, biomass, age of a tree, and CS has shown a good positive and statistically significant correlation among variables. The biomass ($r = 1.000$, $p < 0.01$) and DBH ($r = 0.943$, $p < 0.01$) of the trees showed a strong positive correlation with CS. Tree age and height both had a moderately strong positive correlation with CS, with $r = .711$, $p < 0.01$ and $r = 0.505$, $p < 0.01$, respectively (Table 2).

Following that, to determine the magnitude of

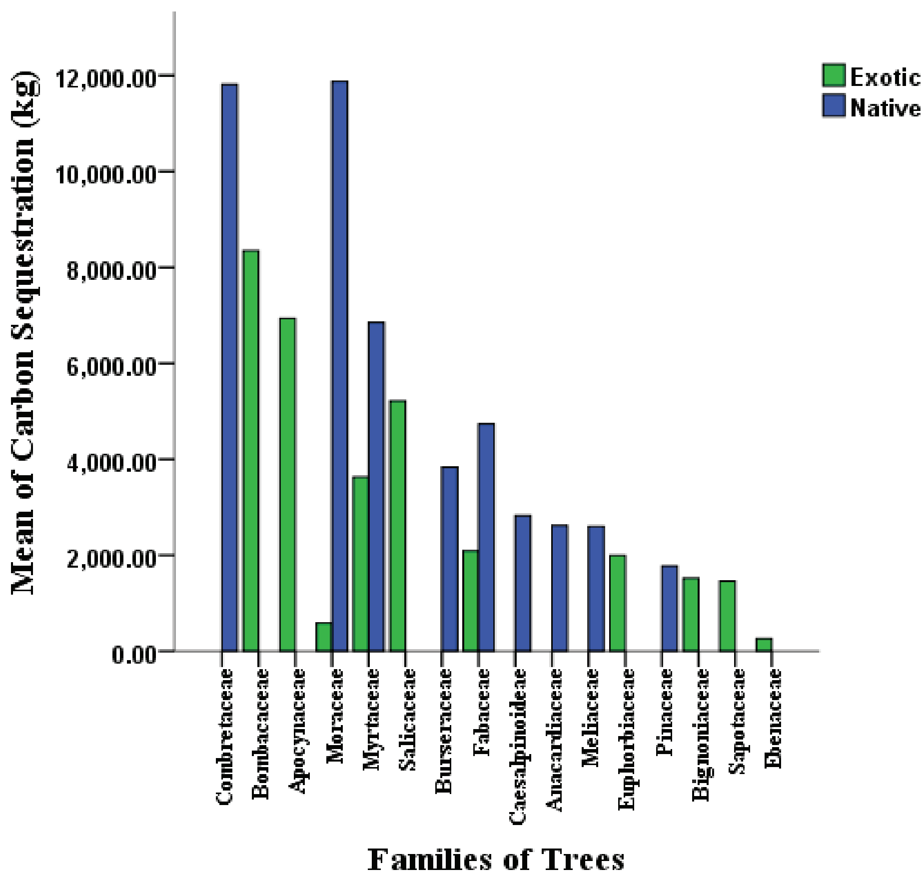

Fig. 3. Bar chart showing Carbon Sequestration by the native and exotic trees in different families of trees

the relationship between the independent variables, DBH, height, biomass, and age were regressed against CS. The regression model suggested that the biomass had shown a significantly high relationship with CS as the model explained 99 % of the variance with F (1,113) = 165441.13, p < 0.001(b=0.520, p < 0.001). The DBH also had a significant impact on CS with 89 % of variance and F (1,113) = 902.67, p < 0.001 (b = 621.68, p <0 .001). The age of the trees explained 51 % of the variance with F (1,113) = 115.705, p < 0.001 (b = 607.41, p < 0.001). The height explained 26 % of the variance, with F (1,113) = 38.58, p < 0.001 (b =337.84, p < 0.001), (Table3).

The biomass had a strong and significant positive linear relationship, which had the most notable influence on CS in this study. The findings of previous research verified that biomass was a major contributor to the carbon stock in trees [41, 42]. Wellbrock *et al*. [43] found 46 % of carbon storage in above and below biomass.

The link between DBH and CS was found to be significantly good, which confirmed its major role in developing carbon storage in trees. In a study by Mildrexler *et al*. [44], trees with a diameter of more than 21 inches accounted for only 3 % of the total sample but had a carbon content of 42 %. In another study, 93 % of carbon was stocked in stems [45]. Maren & Sharma [46] found that pine trees of large size with variation in stem size sequestered more carbon.

The age and height of a tree have shown a moderately good relationship and a considerable impact on CS. Leverett *et al*. [47] reported that large trees had dominance in accumulating carbon and aged trees had a high accumulation rate of carbon. Matured and aged trees showed high values for CS along with the R^2 0 .99 for basal area and 0.60 for height [48].

Zribi *et al*. [49] found that aged trees have the largest biomass and thus the highest capacity of CS but less potential for future CS in comparison to young trees, which have not only rapid growth but also more CS capability. The consensus found that as a tree grows older due to an increase in DBH and height, a constant increase in biomass is attained and the tree thus becomes a good carbon reservoir [50].

3.3 Carbon Sequestration in Native and Exotic Trees

In the final stage, the CS of native (n=56) and exotic trees (n=59) was assessed through descriptive analysis. The results revealed that the DBH for native trees with a mean of 24.83 (SD=12.34) is greater than exotic trees (M=17.56, SD=76.92). The average heights of 46.45 (SD=9.81) and 44.82 (SD=10.92) and age at 26.13 (SD=9.580) and 24.29 (SD=6.04) haven't shown much difference for both species.

The native trees have significantly higher biomass (M=13574.98, SD=17411.83), CS (M=7093.76, SD=9049.06) and annual carbon sequestration (M=231.57, SD= 212.81) values than exotic trees (M=6231.95, SD=6080.80; M=3240.72, SD=3162.12; M=119.17, SD=91.79) (Table 4).

Then, to compare the carbon sequestration between native and exotic trees, an independent sample t-test was conducted. A significant difference was found (t (67.626) = 3.016, p=0.004) in the scores of variables. The mean of native trees (M=7093.7610, SD=9049.06255) was higher than that of exotic trees (M=3240.72161, SD=3162.11839). The magnitude of the difference in the mean values (mean difference=3853.04491, 95 % CI: 1303.80497 to 6402.28485) was significant (Table 5).

It can be extracted from the results that the total amount of biomass is one of the key characteristics that have a vital role in the carbon sequestration of trees, along with DBH. As previously discussed, native trees with higher biomass and DBH demonstrate a greater capacity to capture and store carbon dioxide from their surroundings. So, it can be inferred from the findings that native trees are more capable of sequestering carbon.

These findings were supported by other studies as Rodríguez-Loinaz *et al*. [51] suggested plantation of native trees for better CS in the long term. Ajani and Shams [52] compared the carbon sequestration between native trees like *Azadirachta indica* and exotic trees like *Conocarpus erectus* in Karachi and reported that native species showed a significantly

Table 1. Descriptive analysis of types of family

S. No.	Family	*f*	%	Mean					
				DBH (inches)	Height (ft.)	Age	Biomass (kg)	Carbon Sequestration (kg)	Annual Carbon Sequestration (kg)
1	Anacardiaceae	4	3.5	17.55	46.50	20.50	5034.35	2617.94	128.47
2	Apocynaceae	14	12.2	25.03	56.36	33.71	13342.84	6938.50	206.05
3	Bignoniaceae	16	13.9	15.71	32.74	20.88	2923.79	1520.42	72.78
4	Bombacaceae	2	1.7	30.65	46.65	35.00	16058.34	8350.60	238.59
5	Burseraceae	6	5.2	19.68	45.50	25.00	6753.93	3835.25	135.36
6	Caesalpinoideae	9	7.8	20.43	32.99	20.00	5436.91	2827.28	140.00
7	Combretaceae	4	3.5	31.53	61.50	35.00	22638.48	11813.65	341.51
8	Ebenaceae	1	0.9	8.40	34.00	20.00	501.11	260.59	13.03
9	Euphorbiaceae	1	0.9	15.00	49.00	20.00	3838.16	1995.91	99.79
10	Fabaceae	15	13.0	17.61	48.72	21.13	7418.41	3857.69	170.72
11	Meliaceae	1	0.9	19.00	39.40	22.00	5003.88	2602.10	118.28
12	Moraceae	23	20.0	28.20	45.05	30.00	19070.81	9909.93	270.05
13	Myrtaceae	7	6.1	20.43	53.49	21.00	8750.60	4550.46	217.90
14	Pinaceae	1	0.9	14.00	50.00	22.00	3411.70	1774.14	80.64
15	Salicaceae	1	0.9	24.00	50.00	25.00	10026.21	5213.79	208.55
16	Sapotaceae	10	8.7	12.89	47.40	20.50	2809.37	1460.92	70.30
	Total	115	100	21.10	45.62	25.18	9807.69	5116.98	173.90

Note: *f* = Frequency; % = Percentage

Table 2. The correlations coefficients for DBH, height, biomass, age of a tree, and carbon sequestration

Variables	*N*	*M*	*SD*	DBH	Height	Biomass	Age	CS
DBH	115	21.10	10.54	--				
Height	115	45.62	10.38	0.489**	--			
Biomass	115	9807.69	13366.70	0.943**	.504**	--		
Age	115	25.1826	8.14	0.695**	.567**	.711**	--	
CS	115	173.90	171.23	0.943**	.505**	1.000**	.711**	--

Table 3. Linear regression analysis of carbon sequestration

Variables	R^2	*B*	*p-value*
DBH	0.89	621.68	.000
Height	0.26	337.84	.000
Biomass	0.99	0.520	.000
Age	0.51	607.41	.000

Note: ***p* < 0.01

Table 4. Descriptive analysis of native and exotic trees

	Native			Exotic		
	n	*M*	*SD*	*n*	*M*	*SD*
DBH	56	24.83	12.34	59	17.56	76.92
Height	56	46.45	9.81	59	44.82	10.92
Biomass	56	13574.98	17411.83	59	6231.95	6080.80
Age of a tree	56	26.13	9.85	59	24.29	6.04
Carbon Sequestration	56	7093.76	9049.06	59	3240.72	3162.12
Annual Carbon Sequestration	56	231.57	212.81	59	119.17	91.79

Table 5. Independent sample *t*-test to compare native and exotic trees for CS

				Levene's Test for Equality of Variances		*t*-test for Equality of Means						
		M	*SD*	*F*	Sig.	*T*	*df*	Sig. (two-tailed)	Mean difference	Std. Error difference	95% Confidence Interval of the difference	
											Lower	Upper
Carbon sequestration	Native	7093.76	9049.06	13.9	.00	3.01	67.	.004	3853.04	1277.39	1303.80	6402.28
	Exotic	3240.72	3162.12	0	0	6	626					

Note: $^{**}p < 0.01$

higher value for carbon sequestration. Omoro *et al*. [53] found that native forests sequestered more carbon in their biomass than exotic plantations.

In sum, the trait of carbon captivation and storage among different families and types of trees according to the local scenarios of anthropogenic carbon emission was reported by different researchers with the assessment that trees are one of the major contributors to producing carbon sinks for enhancing the environment [54 - 57]. Although, at the same time, arguments related to trees' ability to extract carbon on a large scale in comparison to other methods that could lower its concentration at a required level are still under discussion [58 - 60]. In general, the importance of trees in the development of carbon footprints cannot be overstated [10]. The most important point that should be highlighted here is the potential of different trees in carbon extraction from the surroundings and their ability to have a significant role in cutting off carbon dioxide rates from the environment is confirmed by the findings [61 - 63].

4. CONCLUSION

This study was initiated with the idea that biophilic urbanism can provide an additional benefit of carbon sequestration. Therefore, urban tree potential was assessed in this regard, and synthesis indicated a significantly positive effect. In line with the findings of this study, it can be concluded with statistical evidence that urban trees are the most effective source of carbon elimination from the environment. In addition to this, biomass and DBH are major components in the facilitation of more carbon absorption and storage in trees. For instance, native trees with greater total biomass and DBH are better carbon reservoirs than exotic trees in this study. To conclude, carbon sequestration by trees can be considered as a co-benefit of biophilic cities that tend to show both compatible and complementary trends in the future. Yet, more research is required to explore this in-depth with a large and diversified sample.

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6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

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Research Article

Quantification of Soil Erosion by Integrating Geospatial and Revised Universal Soil Loss Equation in District Dir Lower, Pakistan

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Abstract: This study is aimed to estimate soil erosion risk by integrating Revised Universal Soil Loss Equation (RUSLE) and geospatial tool in District Lower Dir, Eastern Hindu Kush. Soil erosion is among the biggest threats to agricultural production. Mountainous areas of Pakistan are exposed to erosion hazards due to immature geology, fragile slope, and deforestation. RUSLE factors were derived from data acquired from various sources. The Rainfall erosivity (R) factor was derived from monthly data obtained from Pakistan Meteorological Department, Peshawar. The soil erodibility (K) factor was prepared from the map of soil, Survey of Pakistan. The topography (LS) factor was calculated from 12.5 m DEM downloaded from the Alaska Satellite Facility. The cover management (C) factor was calculated from the Red and Near-Infrared band of Landsat 8 satellite image downloaded from USGS earth explorer. The Digital Elevation Model (DEM) and Landsat image were integrated to prepare the Support practice (P) factor. These factors were combined to assess soil erosion in the study area. The estimated soil erosion ranges between 0-25407 tons/hectare/year, with a mean soil loss of 230 tons/hectare/year. The erosion zonation map was then prepared and was classified as very low, low, moderate, high, and very high erosion. 22 % of the district was affected by low to moderate erosion while 67 % area is affected by very high erosion. The areas having more rainfall and steep slopes are more exposed to erosion hazards. Therefore, Erosion control activities are essential in those areas where erosion is high to assure a viable ecosystem.

Keywords: Soil Erosion, RUSLE, Deforestation, GIS, Lower Dir.

1. INTRODUCTION

Anthropogenic activities like the cutting of trees, overgrazing, construction activities, and extensive farming accelerate the process of soil erosion and degradation of the natural environment [1]. Several natural activities like extensive rainfall, running water, forest and vegetation cover, detachment of soil, and its transportation by several agents also play a major part in the process of erosion [2].

The magnitude and influence of erosion is a major issue especially in developing nations [3, 4], where most people rely on small-scale farming [5]. It is one of the major concerns of the 20th century and will also be top challenge in the 21st century [6]. Each year around 0.90–0.95 mm of the topsoil is eroded due to erosion in the world [7]. Nearly 10 million hectares of cultivable land is washed away by soil erosion annually [8]. In the previous four decades, around one-third of agricultural land has been affected by erosion, while the world's population is increasing a quarter of a million daily, which means demand for agricultural production is increasing daily while its production is decreasing [9]. In the last five decades, agricultural production has been declined from approximately 11.9 to 13.4 % due to the deterioration of cultivable land [10].

Soil degradation also causes various effects on the natural ecosystem and economy of a region [11, 12]. Consistent soil loss results in the decline of soil fertility [10] and crop production [13, 14]. It causes the blocking of river channels and raising the level of dams which poses a high flood risk [15], disturbs

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reservoirs, increases its maintenance cost, and makes it dysfunctional [16].

Though soil erosion is a global challenge however the developing nations face more threats due to the less available resources to restore and reclaim the eroded soils and lost nutrients [17]. Highland areas are more exposed to erosion hazards due to slope gradient, intense precipitation, gushing rivers, and surface overflow [18]. Pakistan has no exception, soil erosion has affected 11 million hectares of the topsoil and nearly 40 million tons of sediments are carried to the Indus River annually [19]. These sediments are filling the Tarbela and Mangla dams which have cut down the volume and life span of these dams. It also reduces the electricity generation and supply of water [20].

Soil protection and water conservation are identified by the United Nations (UN) as a crucial land-use problem and is one of the main considerations of the UN Sustainable Development Goals [21, 22]. Conventionally, assessment of the erosion hazard offers a foundation for water and soil protection [23]. Combating land degradation, desertification, and soil erosion have drawn much attention of land conservationists, decision-makers, agronomists, and politicians all-round the globe [24]. Erosion models provide soil loss estimation qualitatively and quantitatively in several physical and environmental conditions [25]. It also provides guidelines for framing and implementing strategies for soil and water conservation [26-28].

Protecting soil by applying suitable and sustainable agricultural practices and land management strategies are the most appropriate method for controlling soil erosion in the world [29]. For this purpose, monitoring and assessment of the potentially vulnerable erosion areas become very important for controlling and managing this problem [30]. Out of numerous erosion models, Revised Universal Soil Loss Equation (RUSLE) is used widely around the globe [31], for the better estimation and quantification of soil loss [32].

2. MATERIAL AND METHODS

2.1 The Study Area

District Lower Dir is situated in the north-eastern Hindu Kush region, Khyber Pakhtunkhwa province. Geographically it stretches 34° 37′ 27″ to 35° 4′ 23″ N latitude and 71° 30′ 37″ to 72° 11′ 30″ longitude (Figure 1). Relatively, it is bounded by the Upper Dir district in the north, district Swat on the east, and district Malakand on the southeast while district Bajaur is situated to the south-western side of the Lower Dir. It also shares an international border on the western side with Afghanistan. The study region occupies a 1, 585 km^2 area and has 1, 435, 917 inhabitants [33]. It is drained by the Panjkora River. The climate is mild to hot in summer mostly warm during May-July when the temperature reaches a maximum of 38 °C whereas in the winter season the temperature decreases to 0 °C. December-March are the coldest months where occasional snowfall also occurs. Mean annual rainfall ranges between 700 to 1200 mm, most of which occur from December to April. Largely the physiography of the study region is occupied by the offshoots of the Hindu Kush Mountains. The altitude of the district ranges from 524 meters to 3268 meters. The highest altitude is found in the northern region of the district whereas in the southern region the height decreases forming a slope gradient. The gradient increases the impact of rain splash and gully erosion. The degree of erosion can be determined from a large amount of silt found in the river [34].

2.2 Methodology

RUSLE model offers an excellent methodology for measuring soil erosion and its causal factors. Like USLE, RUSLE retains the same factors and formula for assessing soil loss [35]. These factors are rainfall erosivity, soil erodibility, slope length and steepness, cover management and support practice. To facilitate the process of erosion, RUSLE has been computerized [36]. Mathematically it is denoted as;

$$A = R \times K \times LS \times C \times P \tag{1}$$

Where A is the rate of average annual soil erosion ($t.ha^{-1}.yr^{-1}$), R represents rainfall erosivity ($MJ.mm.ha^{-1}.h^{-1}.yr^{-1}$), K is the soil erodibility ($t.ha.J^{-1}.mm^{-1}$), LS is slope length and steepness, C represents the cover management, while P represents the support practice factor. LS, C, and P are dimensionless factors.

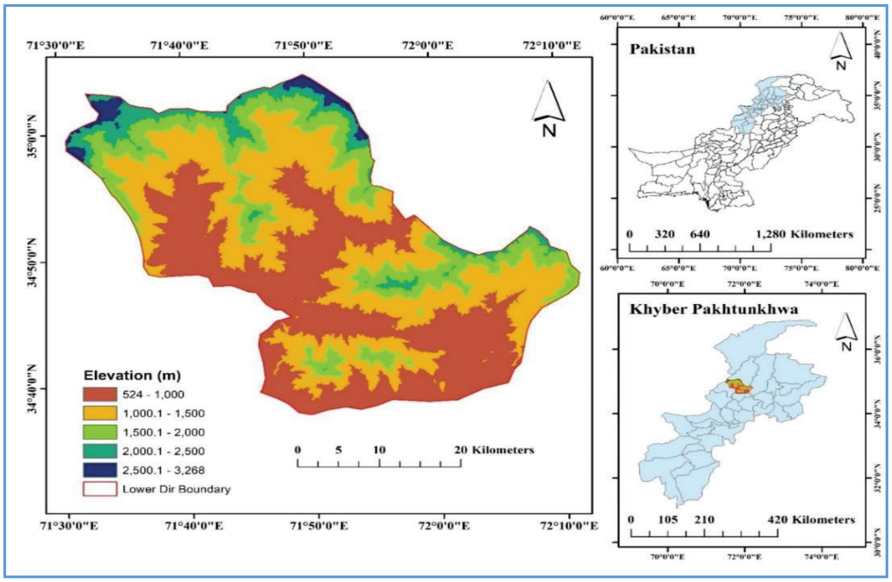

Fig. 1. Location of district Lower Dir

2.2.1 Rainfall Erosivity (R) Factor

The intensity and amount of rainfall are the most important contributor to water erosion to start [37]. The more the intensity and amount of rainfall, the more erosion will occur, related to EI_{30} (the product of kinetic energy and 30 minutes intensity of a rainfall) [38]. Calculation of R factor needs long term continuous precipitation data but in most of the countries, this EI_{30} data is not available. If sufficient data is available still it is a difficult and time-consuming process. However, some simplified methods have overcome this difficulty in numerous countries for R factor calculating from monthly rainfall data. The main advantage of these simplified procedures is that monthly rainfall data are easily available and reliable [39]. A good correlation has been identified by several researchers between rainfall erosivity and monthly data in different parts of the globe [40-43].

R factor was calculated for the study region from monthly rainfall data obtained from Pakistan Metrological Department, Peshawar. Only one met station is there in the district Lower Dir (Timergara met station), so for the accurate estimation of the R factor, the rainfall data was acquired for the seven met stations near the study area (Chitral, Dir, Darosh, Kalam, Malam Jabba, Saidu Sharif, and Timergara met stations) from Pakistan Meteorological Department, Peshawar.

A point map was generated from this data and then interpolation was applied in the ArcMap. (Table 1) shows the average annual rainfall and rainfall erosivity values calculated for each met station using equation 2 established by [41].

$$R = 79 + 0.363 * P \tag{2}$$

Where R is rainfall erosivity and P is average annual rainfall.

There is no specific equation in Pakistan for the estimation of the R factor, therefore [41] equation was used for the study area because of the similarity in the rainfall pattern of Pakistan and India (Figure 2a).

2.2.2 Soil Erodibility (K) Factor

Soil of different textures demonstrates a varying degree of vulnerability to water erosion. Erodibility is the resistance of soil to erosion from the impact of rainfall and runoff [44]. It is influenced by a variety of both chemical and physical attributes of soil. But only the physical properties of soil are considered by the RUSLE model for instance soil structure, organic matter, particle size, and permeability are the main components of soil influencing the soil erodibility [45]. District Lower Dir soil map acquired from the soil survey of Pakistan was digitized and four types of soil texture (Figure 2b) have been identified. This map was used to establish the erodibility factor assigning K values (Table 2) extracted from different literature.

2.2.3 Slope Length and Steepness (LS) Factor

The LS is a combination of two topographic factors i.e., length (L) and steepness (S) of a slope, [34], which highly influence the process of soil erosion [48]. On a steep slope, the water rushes at a higher speed which results in increased pressure on the surface consequently increasing the transport of a large number of sediments [49, 50]. Slope length also contributes to erosion, which is the area from the origin of overland flow to the place where either the slope reduces so that deposition occurs or the place where the water moves into distinct channels [49].

Nowadays, in all research studies, DEM is used to calculate the LS factor [51, 52]. In the present study, DEM with the 12.5-meter spatial resolution was used which was downloaded from the Alaska Satellite Facility (Figure 2c), using [53] established equation for the calculation LS factor in the GIS environment (eq. 3).

$$LS = (\text{Flow accumulation} * \text{cell size} /22.13)^{m}\ 0.065 + 0.045\,S + 0.0065^{2} \quad \text{Eq. 3}$$

Where the value of exponent m ranges from 0.2 to 0.5 based on the slope percentage suggested by [49] (Table 3). 0.5 was taken as the m value from (Table 3) for eq. 3 because most of the area in the study region has a steeper slope than 5º.

Table 3. Values of m for different slopes

m-value	Slope (%)
0.2	<1
0.3	1-3
0.4	3-5
0.5	>5

2.2.4 Cover Management (C) Factor

C factor reflects the combined impacts of both cover and management activities on soil loss [54, 55]. Vegetation can significantly decrease the speed of runoff and also safeguard the soil surface. This factor is mostly altered by anthropologic actions [56]. The plant cover greatly influences the process of soil erosion because it intercepts the rainwater, reduces the velocity of rainfall and runoff, and increases the infiltration rate [57]. Remotely sensed satellite image offers the latest and up-to-date information of the land surface, which can be very beneficial for earth surface dynamics and is extensively used in natural resource assessments and management [56].

The Normalized Difference Vegetation Index (NDVI) is greatly associated with the quantity of plant cover, and hence can effectively be employed to give knowledge of the plant dynamics [58, 59]. Numerous scholars and experts have calculated the C factor from NDVI using different equations [56, 59, 60]. In this study, [60] suggested equation (eq. 4) was applied to compute the C factor (Figure 2d).

$$C = (-NDVI + 1)/2 \quad \text{Eq. 4}$$

Where

$$NDVI = NIR - RED / NIR + RED \quad \text{Eq. 5}$$

2.2.5 Support Practice (P) Factor

Generally, the P factor and C factor are related to each other because both of these factors are used to reduce soil erosion [61, 62]. But the P factor is different from the C factor because it specifies the influence of management activities by controlling the runoff by changing its direction, pattern and reducing the speed [62, 63]. P factor can be estimated in various ways such as from direct investigation of the land use type at the fields and to recognizing the particular farming methods which are particularly time consuming and expensive. It can be calculated from satellite imageries classifications or previous research investigations and also from the knowledge of experts [52]. Several scientists proposed that the values of the P factor are relatively reliant on the slope gradient [49, 64, 65], whereas some others have suggested the use of farming activities to compute the P factor value [66]. In this study, Landsat classified image was used to develop land cover classes (Table 4; Figure 2e).

The land cover classes were overlaid over the

Table 1. Average annual rainfall of the selected met stations

S. No	Meteorological Station	Average annual rainfall	Time interval	Rainfall Erosivity
1	Chitral	458 mm	37 years	245.254
2	Dir	1362 mm	38 years	573.406
3	Darosh	568 mm	37 years	285.184
4	Kalam	1038 mm	16 years	455.794
5	Malam Jabba	1647 mm	16 years	676.861
6	Saidu Sharif	1050 mm	45 years	460.15
7	Timergara	796 mm	11 years	367.948

Table 2. Soil texture and K factor values of the study area

Soil Texture	Area (km^2)	% Area	K values	Erodibility	Reference
Rock outcrop loamy	765.6	48.3	0.25	Low	[46]
Mainly loamy soil	560.7	35.8	0.25	Low	[46]
Eutric cambisols	98.6	6.2	0.34	Low	[47]
Lithosols	160.1	10.1	0.2	Low	[47]

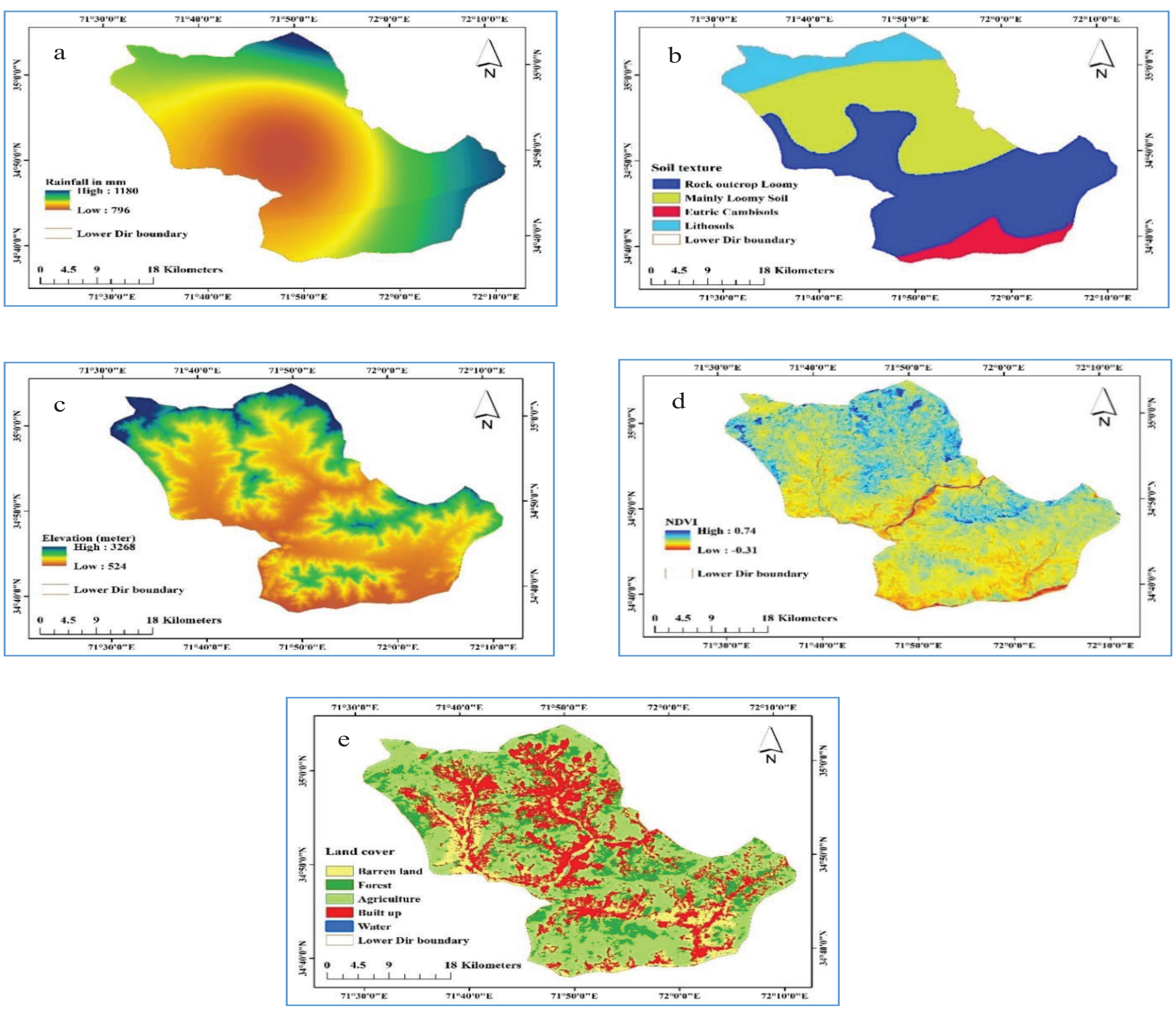

Fig. 2. District Lower Dir showing a; Mean Annual Rainfall, b; Soil texture, c; Elevation, d; NDVI, e; Land cover classes

Table 4. Landcover classes, area and percentage

Land cover	Area (km^2)	% Area
Vegetation	498.2	31.4
Forest cover	426.4	26.9
Barren land	296.4	18.7
Water bodies	39.6	2.5
Built-up area	324.4	20.5

Table 5. P values after [49]

Land cover	Slope %	P values
Agriculture	0-5	0.10
	5-10	0.12
	10-20	0.14
	20-30	0.19
	30-50	0.25
	50-100	0.33
Other lands	All	1

slope map to prepare a P factor map bored on the values based by [49] (Table 5).

2.2.6 Soil Loss Estimation

The factors of the RUSLE model were prepared in the ArcGIS environment. RUSLE formula was employed and all the layers were multiplied to calculate annual erosion for the district Lower Dir. The annual erosion map was further subdivided into erosion categories: very high, high, moderate, low and very low, less than 5 tons/hectare/year was defined as very low, while more than 75 tons/hectare/year as very high erosion. The result of the analysis shows that 12 % of the study exhibit very low and low erosion, while 67 % of the study region is affected by very high erosion (Table 6; Figure 4).

Table 6. Amount of soil erosion, categories and area

Erosion tons/hectare/year	Erosion categories	Area (km^2)	% Area
<5	Very low	63.4	4
5.1 -25	Low	126.8	8
25.1- 50	Moderate	158.5	10
50.1- 75	High	174.3	11
>75	Very high	1062	67

3. RESULTS AND DISCUSSION

The assessment and estimation of soil erosion hazards are very helpful in controlling and managing erosion in the District Lower Dir. GIS is a very effective means of assessing soil loss but the contribution of remote sensing cannot be ignored because it is a very important data source for soil loss assessment. Overall RUSLE model coupled with remote sensing and GIS were employed in this study to calculate the quantity of soil erosion and also to categorize the areas with high erosion.

3.1 R Factor

R factor is a very sensitive factor in computing soil erosion risk. Inverse Distance Weighted (IDW) interpolation method was employed to create a rainfall map and then eq. 1 was used to compute the rainfall and runoff erosivity (R) factor map. The interpolation process is essential when there is more met station data. As the study area, Lower Dir has only one meteorological station so a better estimation of the R factor data was also acquired for the surrounding meteorological station (Table 1). The mean monthly rainfall data were averaged for each met station to find out the mean annual rainfall. The areas having a high amount of rainfall have a high amount of erosivity. The northern and eastern region of the district receives more amount of rainfall while the amount of rainfall decreases going from the north to the southern and western part of the district. The same is the case with the erosivity values, it is high in the northern and eastern part of the district making it more susceptible to erosion while the risk of erosion decreases as the erosivity values decrease in the southern and western part of the district. The erosivity values ranges between 368 to 478 $Mj.mm.ha^{-1}.h^{-1}.yr^{-1}$ in the Lower Dir (Figure 3a).

3.2 K factor

In the present study, soil map acquired from soil survey of Pakistan was utilized to establish the K factor assigning K values from (Table 2) extracted from different kinds of literature. (Table 2) reveals that rock outcrop loomy is the largest soil group that approximately occupies 50 % of the study region. This type of soil is mostly found in the central part of the district. Besides this, 35 % of the district has loomy soil. The erodibility values range from 0.2 to 0.34 t.ha.h/ha/MJ/mm (Figure 3b). Though the erodibility values are low in the whole district, it increases from north to south.

3.3 LS Factor

The LS factor was calculated for the study area from the ALOS PALSAR 12.5 m DEM. Figure 2c shows the slope map and elevation of district Lower Dir. This map depicts that elevation ranges between 524 to 3268 meters. The highest altitude is found in the northern and eastern part of the district, while the slope decreases from north to south and reaches 524 meters.

The topography is divided into different slope categories based on the slope percentage (Table 7), which shows that 3.5 % of the study area has a steep slope, and has more than 40 % slope percentage. Most of the study area (45 % area) has strongly undulating.

Figure 3c shows the LS factor of the study region. The LS values range from 0 to 1303. Like the altitude, the high values are found in the northern and eastern part of the Lower Dir, while these values drop to 0, which are mostly found in the south-western valleys. The LS and slope map depict that more than 50 % of the study region is vulnerable to erosion due to its topography.

3.4 C Factor

Spectral indices like NDVI and land use land cover classified maps are preferred nowadays over the orthodox methods due to their low cost and relative accuracy. In the current study, the C factor was computed from Landsat 8 satellite imagery downloaded for the year 2020. The higher the NDVI values means high vegetation cover while low values show sparse or no vegetation. In district Lower Dir, The NDVI values decrease towards the south from 0.74 to -0.31. The higher values are found in the north of the district where the coniferous forest is found at higher altitudes with some weed species and shrubs while in the lower valleys' agriculture activities are practiced. The C factor values are inverse to the NDVI. The areas with high NDVI values have low C factor values, which means these areas have more protection against soil erosion. In the study area, the C factor value ranges from 0.12 to 0.65. The high C values are found in the southern part of the district due to low vegetation cover while it decreases towards the northern part of the district (Figure 3d).

3.5 P Factor

The P factor is computed based on farming activities on different slopes from the land use map of the study region acquired from the classification of Landsat image for the year 2020 from USGS open source. The Landsat satellite image was classified by supervised classification techniques into different land use classes i.e., Built-up area, forest, water bodies, agriculture, and barren land. The classified image reveals that agriculture and forested land have largely occupied the study region combined form 58 %, while nearly 19% area is barren land and 22 % area is occupied by built-up area (Table 4). The land use map was overlaid over the slope map and values were assigned to agricultural land on different slopes from [49] proposed values from (Table 5) while P-value 1 was assigned to all the non-agricultural land use classes (Figure 3e).

Table 7. Slope categories, percentage, and area

Slope categories	Slope %	% Area
Flat	0-2	3
Gentle undulating	2.1-5	10.9
Moderate undulating	5.1-10	11.7
Undulating	10.1-20	25.2
Strong undulating	20.1-40	45.7
Mountainous	> 40	3.5

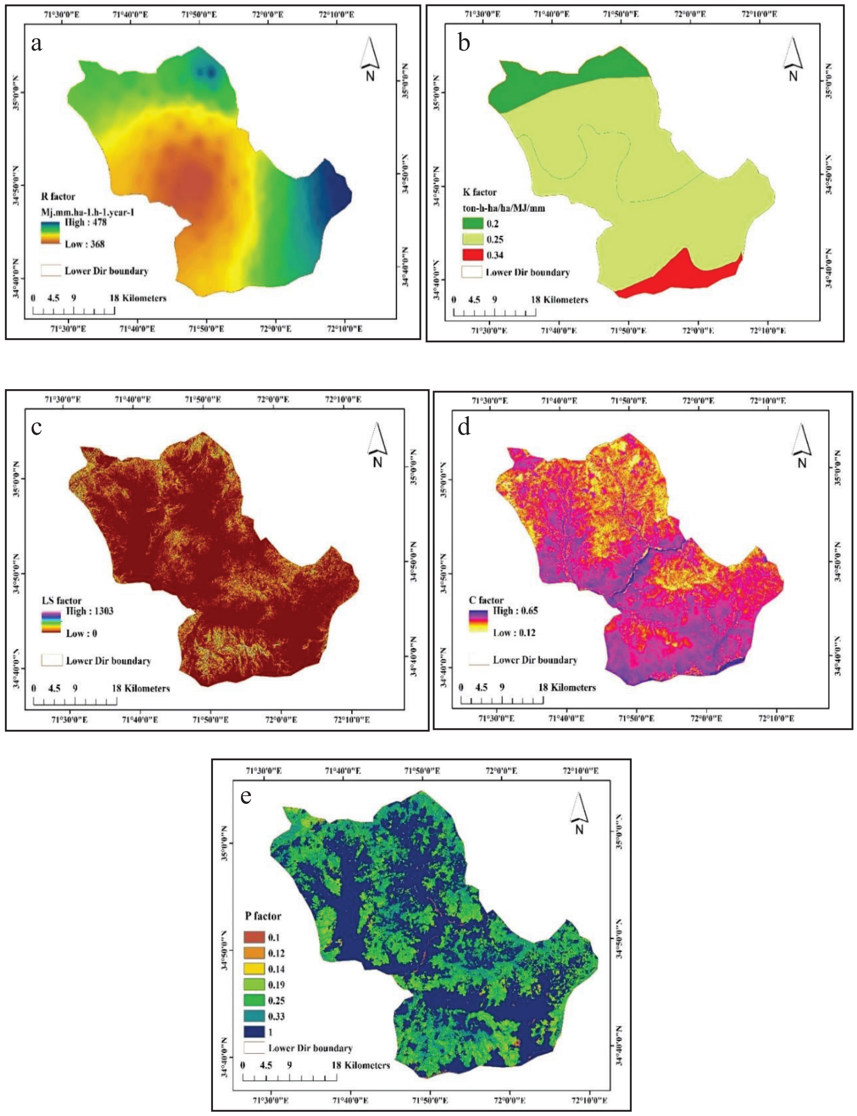

Fig. 3. District Lower Dir showing a. R factor, b. K factor, c. LS factor, d. C factor, e. P factor

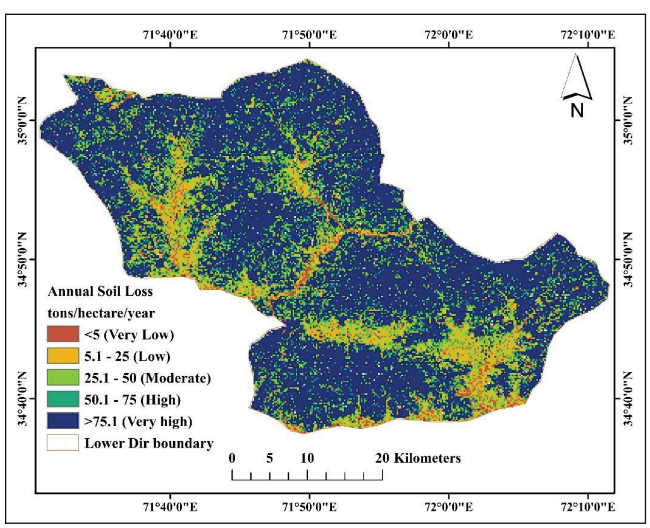

Fig. 4. District Lower Dir annual soil loss map

4. CONCLUSION

Excessive soil erosion not only reduces the productivity and fertility of land but also supplies a large amount of sediment to the reservoirs and dams which reduces its capacity and efficiency. Estimation and spatial extant of soil erosion hazards are time-consuming and difficult tasks but the combination of RUSLE and geospatial techniques are very helpful tools in quantification and mapping of erosion from an area.

This study is very important for providing firsthand information on the high erosion areas and may assist the planners and environmentalists in mitigating and monitoring the soil loss. The conservation and management of soil erosion will help increase agricultural production as well as reduction of sediments will increase the life span of dams and reservoirs. The outcome of this study would help padologist, irrigation departments, Directorate of Soil and Water Conservation as well as decision-makers for effective watershed and sediment management in the headwater region of River Panjkora.

5. CONFLICT OF INTEREST

The authors declared no conflict of interest

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Research Article

Impact of Habitat Variability on Phenotypic Traits and Seed Germination Performance of *Euphorbia helioscopia* L.; a Case Study from the Kashmir Himalaya, India

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Abstract: *Euphorbia helioscopia* L. (Euphorbiaceae), is an important medicinal plant species of the Himalayan region. The species showed wide variability in its phenotypic traits under different environmental conditions. The purpose of this study was to determine the impact of elevational gradient on the morphological traits and reproductive features of the species. The plants grown in the Kashmir University Botanical Garden were more vigorous and taller than the plants of high-altitude sites. Principal component analysis (PCA) revealed that the habitat of KUBG and Kangan (Ganderbal) were comparatively facilitated the growth of *E. helioscopia*. The regression analysis showed a positive correlation between various morphological traits. Our attempted results highlighted an elaborated account of the variation of phenotypic traits and seed germination performance in response to different environmental conditions along an elevational gradient.

Keywords: Analysis, Elevational gradient, *E. helioscopia,* Habitat, Kashmir Himalaya, Phenotypic traits, Variability.

1. INTRODUCTION

Plant species occupy different geographical areas covering contrasting habitats characterized by different environmental conditions. The adaptability of plant populations to varying environmental conditions reflects its survival and success in those particular habitats [1]. Various environmental factors may affect plants directly or indirectly by changing plant responses to other factors. To understand the response of plants to varying growth conditions over a small geographical scale, different elevational ranges are worthy model systems. Elevational gradients thus provide an appropriate scenario to study these responses and important characteristics that are required for the proper functioning of plants in varying habitats [2]. The study of seed germination methods and seed dormancy are important in understanding survival, regeneration, plant community establishment, and adaptations of plants to changing climatic conditions [3–7]. The association between plant regeneration from seeds and climate has resulted in the evolution of distinct germination needs across species [8], and this, in turn, plays a critical role in establishing vegetation type and plant distribution [9]. *Euphorbia helioscopia*, a member of the family Euphorbiaceae, is widely distributed in Europe and cooler regions of Asia, where it is a cosmopolitan weed growing in crop fields, gardens, and roadsides [10]. It is a green, fleshy annual herb growing up to 50 cm in height, with an alternate arrangement of sessile leaves; inflorescence compound umbel, cyathium subsessile; involucre campanulate (Figure 1). Male flowers many, exserted from involucre, ovary nearly reaching up to the margin of the cup. Fruit triangular capsule; seeds ovoid and dark brown. *E. helioscopia* has great medicinal importance and is a potent source of secondary metabolites such as tri-terpenoids, di-terpenoids, flavonoids, and tannins [11]. The whole plant is used to treat various diseases such as arthritis,

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edema, pulmonary tuberculosis, malaria, bacillary-dysentery, osteomyelitis, and cervical tuberculosis lymphadenitis [12, 13]. The leaves and stems are used as febrifuge and vermifuge [14], roots as anti-helminthic, and seeds mixed with roasted pepper are used to treat cholera [15]. In addition, the milky juice from the leaves and fresh stems are used to release pus [16]. *E. helioscopia* grows along an elevational gradient ranging from 1500–2450 m asl and in different habitats such as open, sloppy, etc. The present study was the first attempt to understand the degree of variation in phenotypic traits and seed germination performance in different habitats along an elevational gradient.

2. MATERIAL AND METHODS

2.1 Survey, Collection, and Documentation

Extensive field surveys were carried out across different habitats of Kashmir Himalaya to identify the specific areas. Five distinct sites including four natural sites (Kangan (1850 m asl), Drang (2230 m asl), Betab valley (2350 m asl), Gulmarg (2450 m asl), and one control site at Kashmir University Botanical Garden (KUBG) — 1595 m asl were selected for the present study. Geographical coordinates, characteristics, and distribution of these selected sites (Table 1; Figure 2). To study the phenotypic traits (microscopic and macroscopic characteristics), 10 plants were selected and tagged from each selected population site [17]. The marked individuals across different study sites were monitored on regular basis. Further, the plants were collected at the reproductive stage for analyzing the selected traits.

For statistical analysis, OriginPro (version 2019b) software was used. Multivariate ordination was performed to depict the impact of habitat variability on different phenotypic traits of the plant while regression analysis was performed to analyze the correlation between two different traits. Different populations were statistically analyzed for various phenotypic traits such as stem length, leaf length, number of branches per plant, plant height, and branch length. The propagules and seedlings were transferred to the Kashmir University Botanical Garden (KUBG).

2.2 *In vitro* Seed Germination

Studies on the germination of seeds were undertaken to examine the impact of various pre-sowing seed treatments using different concentrations of various chemicals to ascertain the best treatments for breaking dormancy and accelerating the germination rate. All germination tests were conducted according to the following protocol: seeds were sterilized and placed in sterilized petriplates having Whatman No.1 filter paper wetted with distilled water or given solution. Randomly collected air-dried seeds from the selected populations were used for *in vitro* seed germination studies. Each treatment consisted of 3 replicates, each with ten seeds. The seeds were sterilized for 5–7 minutes with 0.1 percent mercuric chloride ($HgCl_2$), then rinsed five times with double distilled water to eliminate any remaining $HgCl_2$. At an average temperature of 18–24 ^{0}C, the seeds were exposed to both physical (freezing for various periods) and chemical (Gibberellic acid, Thiourea, and kinetin) treatments. Seeds were kept in petriplates on moist Whatman No.1 filter papers moistened with 10 ml of the given treatment solution or distilled water (control). Radicle emergence of 1 mm was set as the germination indicator, and total germination was recorded at the end of the experiment when no new seeds germinated in respect of all the treatments. Three replicates, each with a control set for comparing mean germination time (MGT) and percentage germination of seeds were used for each treatment. Percentage seed germination was obtained by using the below formula:

$$\% \text{ Seed Germination} = \frac{\text{Number of seeds germinated}}{\text{Total no. of seeds kept for germination}} \times 100$$

3. RESULTS

The species shows significant variability in phenotypic characters under varying environmental conditions. This phenotypic variability was observed in all the selected study sites including four natural populations i.e. Kangan, Betab valley, Drang, Gulmarg, and one control site at KUBG Srinagar. Plant height (PH), leaf length (LL), branch length (BL), number of branches per plant (NBP), and stem length (SL) were among the

Table 1. The geocoordinates and characteristics of study sites.

Study sites	Altitude (masl)	Longitude (E)	Latitude (N)	Habitat	Threat factor
Gulmarg	2450	74.3642	34.05065	Open slope	Landslides
Betab-valley (Pahalgam)	2350	75.3617	34.05406	Sunny open slope	Landslides and construction of roads
Drang (Tangmarg)	2230	74.4027	34.04009	Sunny Open slope	Landslides
Kangan (Ganderbal)	1850	74.8953	34.26194	Open field across roadside	Construction of roads
KUBG*	1595	74.8343	34.12797	Sunny open field	Nil

*Kashmir University Botanical Garden, Srinagar

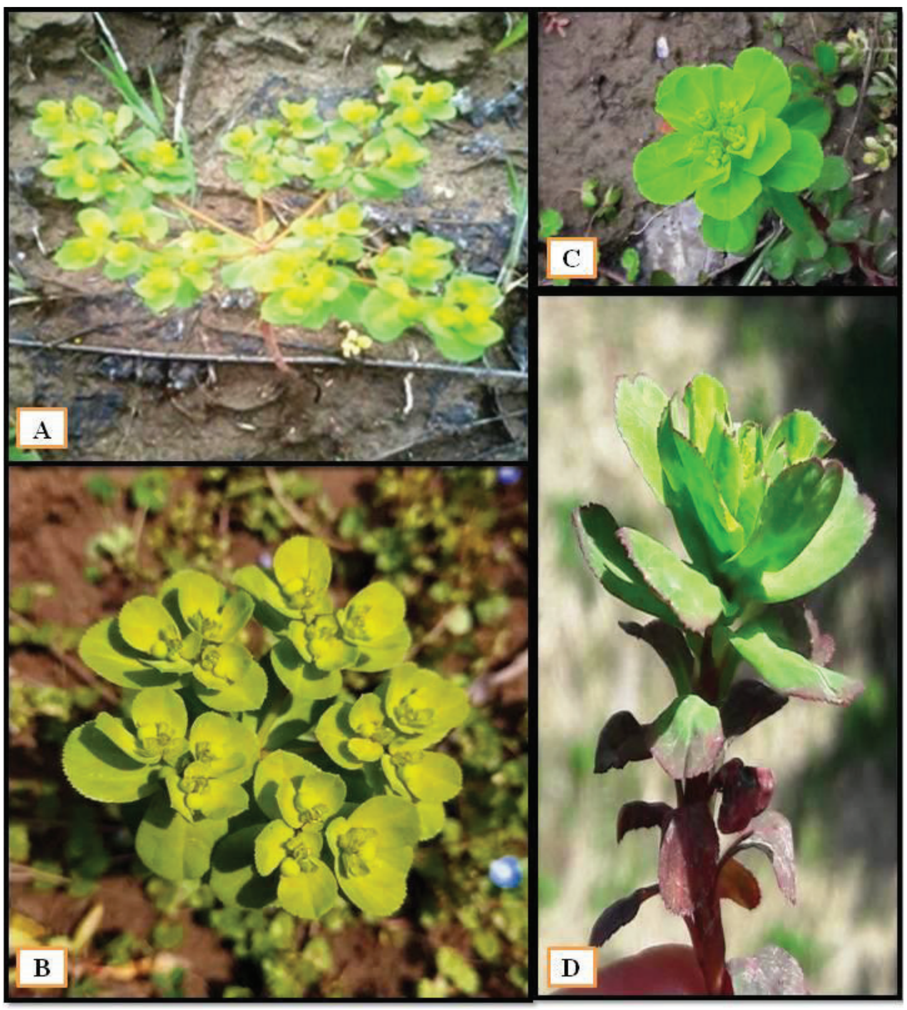


Fig. 1. *Euphorbia helioscopia* L.: A. Habit; B – C. Inflorescence; D. Leaves.

morphological parameters evaluated for phenotypic variability. Table 2 shows the differences in various phenotypic traits studied during the peak flowering period, across and within populations. The plant height ranges from 29.1–39.6 cm in all studied populations. A significant trend of a gradual decrease in plant height was observed along an elevational gradient. The present study depicted that the plant population at KUBG showed more height whereas the population at Gulmarg had less height among all populations.

The number of branches per plant also showed variation among and within studied populations. The highest and lowest number of branches per plant was recorded at KUBG and Gulmarg respectively. Thus branch number per plant showed a decreasing trend from high to low altitude. Branch length also shows a decreasing trend with an increase in altitude. Further, leaf length decreases with an increase in altitude. The population growing at high altitudes i.e., the population at Gulmarg showed smaller leaf lengths (2.4 cm long) while populations growing at lower altitudes had larger leaf lengths (3 cm long).

The impact of various pre-treatments (physical & chemical) on the germination of seeds recorded for fresh seeds is shown in Table 3. The best seed germination (60 %) was recorded in gibberellic acid (50 ppm). Though, no seed germination was observed in control.

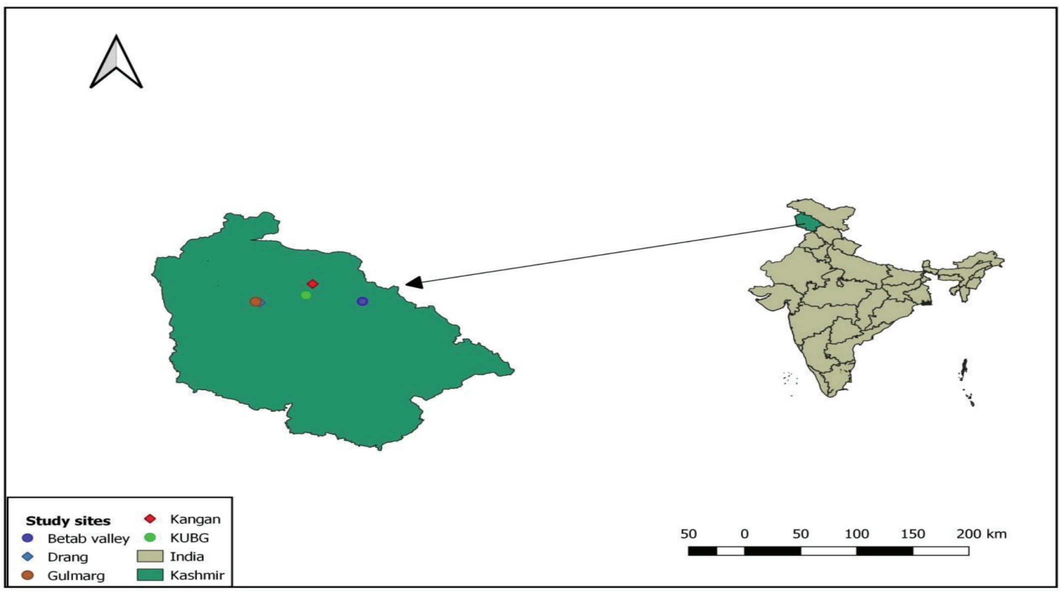


Fig. 2. Map showing the location of different study sites in Kashmir Himalaya (QGIS version 3.20).

Table 2. Phenotypic variability of *E. helioscopia* across different populations.

Phenotypic Characters	**KUBG**	**Drang**	**Betab valley**	**Gulmarg**	**Kangan**
Mean plant height (cm)	39.6	34.2	29.1	25.4	33.4
Mean length of stem (cm)	23.4	18	16.4	13.1	21.2
Mean No. of branches/plant	6	5	5	4	6
Mean branch length (cm)	10.5	10.5	8.3	7	11
Mean leaf length (cm)	3	2.6	2.5	2.4	2.8

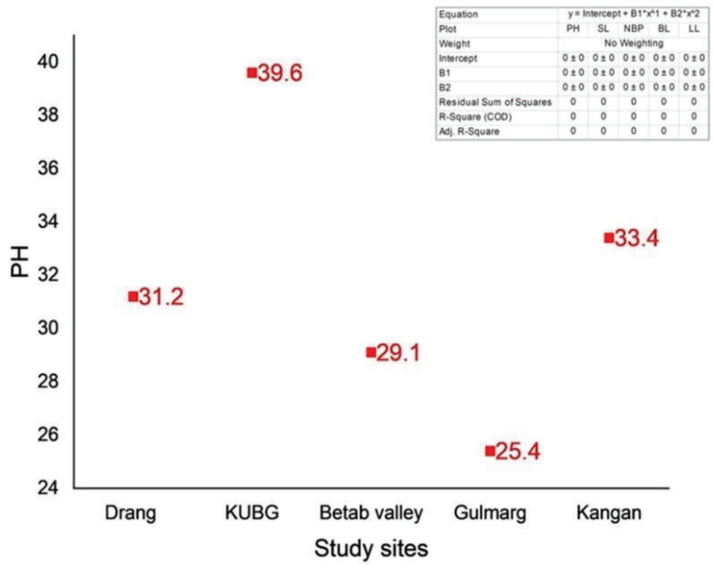
Table 3. Effect of different chemical treatments on seed germination.

Dry chilling (60 days) + different hormones	Conc. (ppm)	Seed germination	No. of days taken for 1st germination	No. of seeds kept for germination	Germination (%)
Gibberellic acid	50	6	15	10	60
	100	2		10	20
	200	3		10	30
Thiourea	50	2	15	10	20
	100	-		10	0
	200	-		10	0
Kinetin	50	-	19	10	0
	100	1		10	10
	200	1		10	10
Potassium nitrate	50	1	19	10	10
	100	1		10	10
	200	-		10	0
Control		-	-	10	0

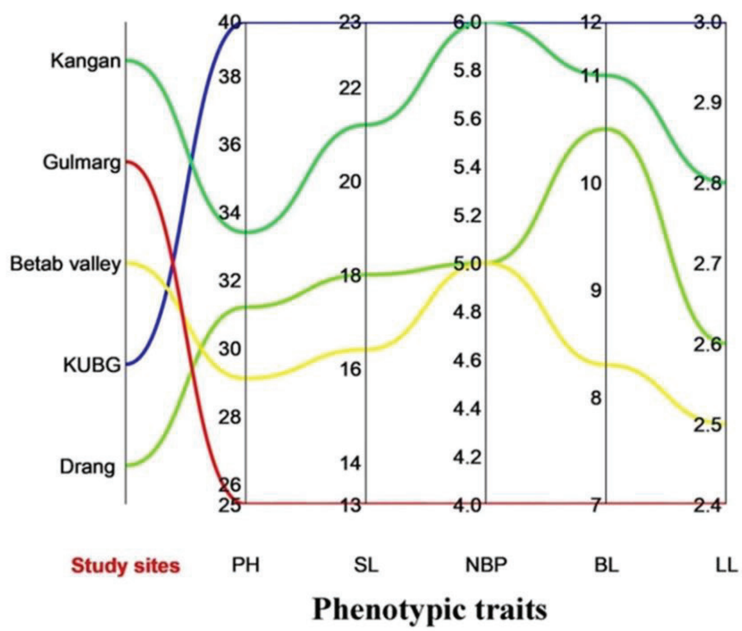
4. DISCUSSION

The present study revealed that *E. helioscopia* grows both in plains and sub-alpine regions of Kashmir Himalaya. It prefers sandy and clay loams and is often associated with light-textured soils [18]. The populations at Gulmarg mostly prefer humus moist soils. However, populations at Kangan and other sites growing across roadsides and cultivated fields prefer sandy and little moist soils. Studies on plant morphology of different populations reveal that the analyzed phenotypic characters vary considerably across different populations viz. Gulmarg, Betab valley, Drang, and Kashmir University Botanical Garden (KUBG). Figures 3A & B depict the mean value of phenotypic characters at different study sites. Both in natural and transplant populations, the plants show a high degree of variability in morphological traits, i.e plant height, length of leaves, length of branches, length of the stem, etc, along the elevational gradient. The regression analysis between different morphological characters (Figures 4A − C) revealed that there is a positive correlation (r^2 = 0.82234) between branch length (BL) and the number of branches per plant (NBP), similarly positive correlation (r^2 = 0.96528) between plant height (PH) and leaf length (LL). Further a positive correlation (r^2 = 0.94613) between stem length (SL) and plant height (PH). Scale interval (Figure 5) showed the range value and mean of different phenotypic traits. The multivariate ordination (Principal Component Analysis) (Figure 6) shows that the plant population at Kangan and KUBG encompass an environment favorable for most of the plant characteristics. This phenotypic variability allows the adaptation of species in tackling the change in environmental conditions. [19]. Similar findings were reported by several workers [20, 21]. Korner and Cochrane [22] stated that the decrease in plant height along with elevational gradient is advantageous for the species as it prevents the plant from strong winds. There is an increase in leaf dimensions in plants growing at lower altitudes as compared to those growing at higher altitudes. The results show conformity [1, 23], who reported that leaf dimensions generally decrease with an increase in altitude.

Further, chemical and physical treatments have been found to enhance seed germination in plant species. The seeds kept in the gibberellic acid (under a concentration of 50–200 ppm) show a better response towards germination, while the seeds which were kept in other chemicals (hormones) show little or negligible response towards germination. In the case of control (distilled water) treatment, the seeds show no response (Figure 7).



A



B

Fig. 3 (A & B). Parallel plots showing mean values of phenotypic traits at different study sites.

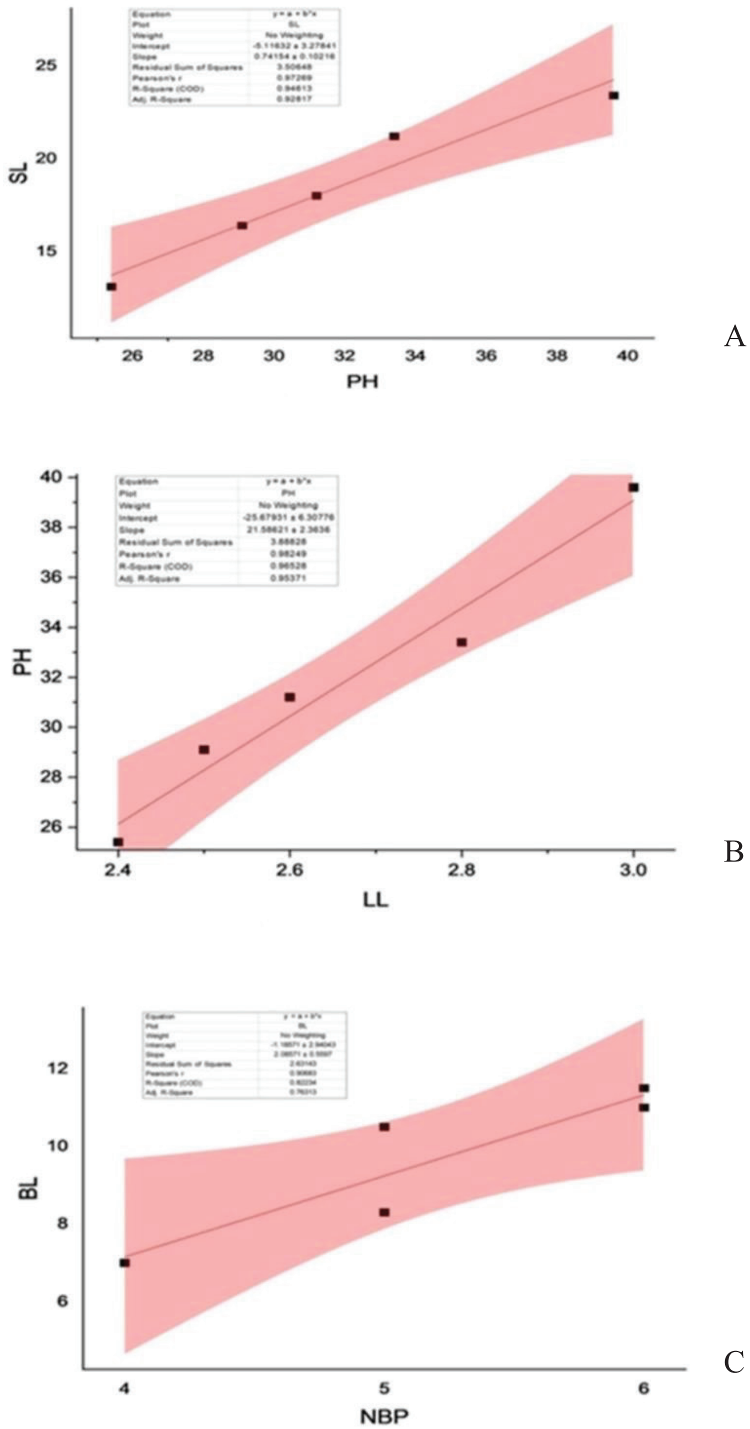


Fig. 4 (A−C). Regression analysis and scale interval between various morphological traits of *E. helioscopia*. **BL.** Branch length; **NBP.** No. of branches/plant; **PH.** Plant height; **LL.** Leaf length; **SL.** Stem length.

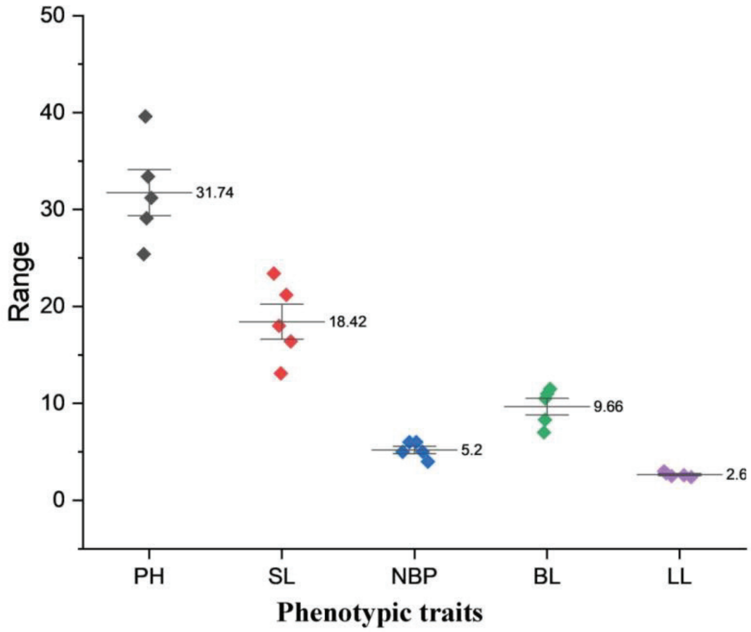


Fig. 5. Scale interval showing the mean and range of all phenotypic traits.

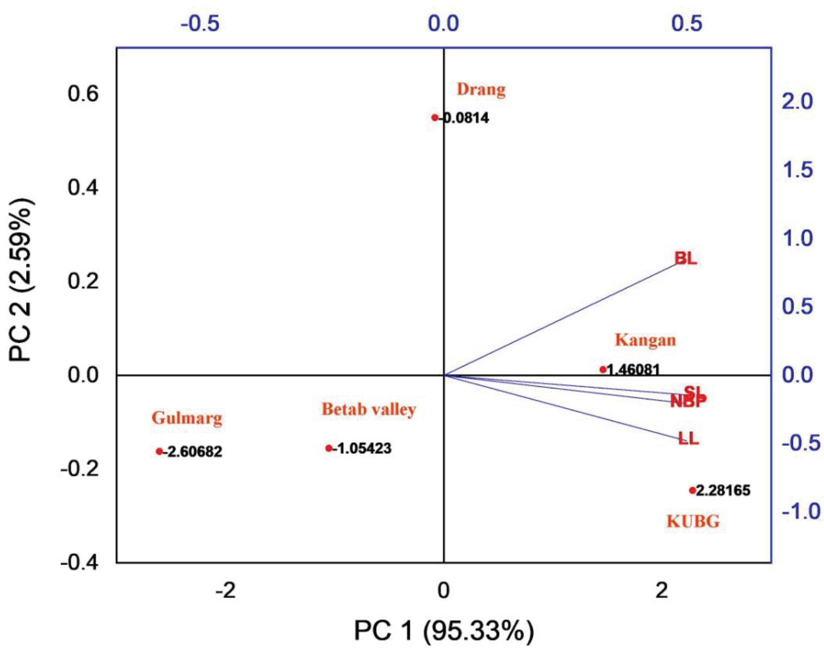


Fig. 6. Principal component analysis of morphological traits of *Euphorbia helioscopia* L. across selected study sites. **PH.** Plant height; **SL.** Stem length; **NBP.** No. of branches/plant; **BL.** Branch length; **LL.** Leaf length.

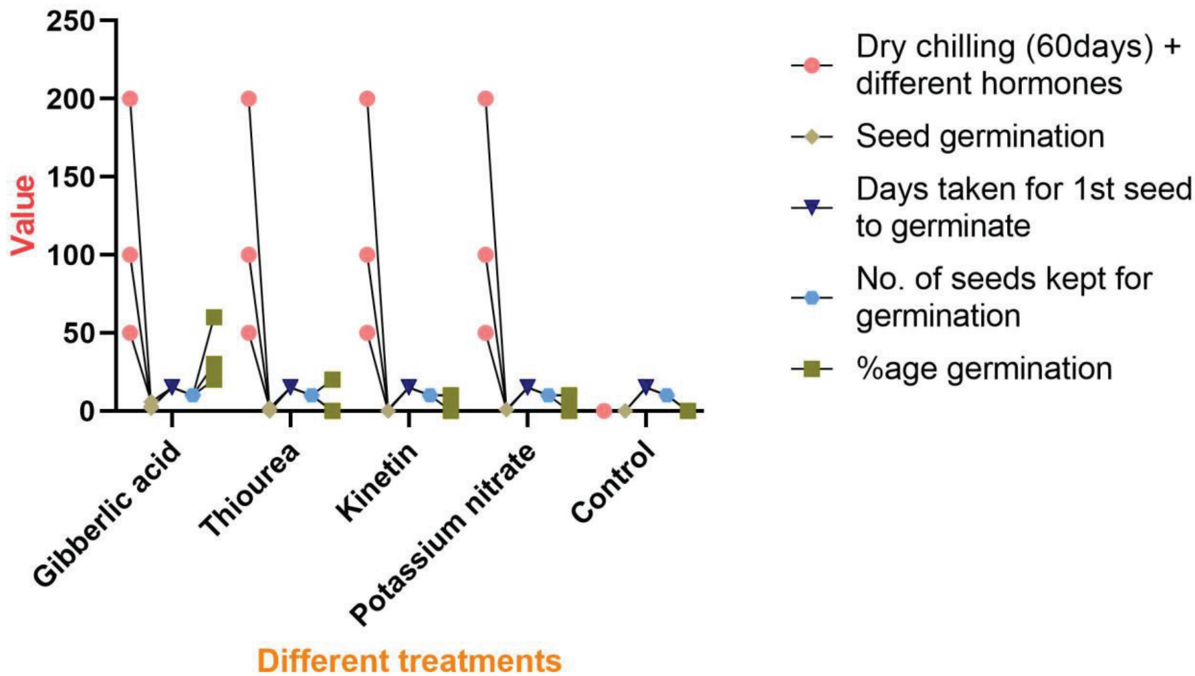


Fig. 7. Effect of hormone & chemical treatments on seed germination of *E. helioscopia.*

4. CONCLUSION

The present study revealed a high degree of variability in morphological traits i.e. plant height, length of leaves, length of branches, length of the stem, and other traits, in natural as well as transplant populations in different habitats along the elevational gradient. These variations in phenotypic traits of *E. helioscopia* may be attributed to the varying environmental conditions across habitats along elevational gradients. The negative correlation of morphological features with increasing altitude suggests that this species grows better at lower altitudes. The present results also revealed that *E. helioscopia* had a very low percentage of seed germination. Among the treatments given, gibberellic acid shows promising results in terms of the percentage germination as well as survival percentage. It can be suggested from the present study that the plant species growing at low altitudes were comparatively much vigorous in respect of the various morphological features. Further, these variations in phenotypic traits and seed formation may be due to the impact of various environmental factors, seasonal variations, and competition for resources or herbivory.

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6. CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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Research Article

Screening of Natural Dyes from Selected Fungal Species

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Abstract: Some fungi are observed as effective pigments. Its importance in the production of natural pigments has grown significantly. The pigment-producing fungi were developed and evaluated for application in dyeing cotton fabric. In the research, five fungal strains were identified as *Aspergillus terreus S10, Talaromyces atroroseus WW5A3, Penicillium oxalicum WW3A4 (DG), WW5C2* and *WW31DG*. These strains were incubated for 21 days under static and non-static conditions using MSM and PDB media. Under liquid state fermentation conditions, the production of the pigments by the fungus was improved by altering temperatures (25-35 °C) and pH (4.5-6.5). *T. atroroseus WW5A3* showed pinkish color, *A. terrreus S10* displayed yellow color, *P. oxalicum WW3A4 (DG)* presented yellow-greenish, *WW5C2* exhibited light yellow color and *WW31DG* demonstrated greenish color. The results showed the maximum percentage absorbance of *T. atroroteus WW5A3* showed 90.36 % at 600 nm, *A. terrreus S10* showed 88 % at 500 nm, *P. oxalicum WW35A4* showed 46.04 % at 550 nm, *WW5C2* showed 59.60 % at 550 nm, and *WW31DG* showed 81.9 % at 550 nm. The natural fungal pigments were tested against bacterial pathogens to check the antibacterial activity. The results indicated that *S. aureus* and *E. coli* exhibited antibacterial activity in terms of maximum zone of inhibition. In conclusion, out of five pigments producing fungi, *Aspergillus terreus S10* and *Talaromyces atroroseus* produced maximum pigment and highest percentage absorbance under liquid state fermentation conditions. Potential applications in the textile and leather industries have been discovered as a result of this research.

Keywords: Cotton fabric, ecofriendly, natural pigments, characterization, antibacterial activity, dyeing

1. INTRODUCTION

Modern times heighten the demand for prompt industrialization that has compelled the immediate formulation to use artificial colorants in the field of food, medicine leather, and other industrial areas nevertheless of their cancer-causing, immune-oppressive, and hazardous environmental effects. To attain these objectives researchers are discovering natural pigments from microbial resources as a substitute for artificial dyes [1]. Researchers revealed that environment-adapted microbial colorants from microbes are better than artificial dyes because of their fast growth, cool handling, and important roles in transcriptional and intracellular signaling. Furthermore, their applications in the food and cosmetic industry are due to their assembly and easiness of big-scale production [2]. Microbes such as fungi and bacteria deliver the availability of naturally derived pigments [3]. Synthetic dyes lead to the production of industrial effluents that are considered toxins and exhibited numerous natural issues and therapeutic problems [4]. Phycocyanins extracted from thermophilic blue-green bacteria and fungi were verbalized to use in makeup mostly an eye-shadows and lipstick shades were manufactured in the industry by using both fungi and bacteria [5]. Many filamentous fungi are used in the production of natural pigments as they are eco-friendly to the environment and less hazardous. Toxic effluents from industries utilizing different colors posed threat to the environment and caused serious health issues [6]. Natural colors in food manufacturers

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are associated with several advantages and endless therapeutic diseases [7]. Naturally, fungi produce colored pigments, secondary metabolites such as flavonoid and tannin are familiar as pigments with extraordinary pharmaceutical importance. Many filamentous fungi including *Aspergillus sp., Penicillium sp., Paecilomyces sp.,* and *Monascus purpureus* can be utilized in the extraction of colored pigments. *Thermomyces* are utilized to extract red color and found huge industrial applications [8]. Pigments produced by fungal species showed improved dyeing ability at acidic (5 pH) [9]. Shade created from *Talaromyces* under 4.5 - 5 pH to direct antacid settings (pH 4.5 and 6.0) [10]. Colors from fungal species, become steadier at extreme temperatures [11]. *A. Flavus* shade appeared 93 % to 96 % steadiness on cotton and silk fabric at 26 °C [12]. The colors extracted by *Talaromyces sp*. had plentiful benefits in the food, textile, and cosmetic industry [13]. Filamentous Fungi create a wide assortment of bio-colorants and have secondary metabolites like melanins, phenazines, flavins, carotenoids, quinones, violacein, indigo, and monascins [14]. Fungi species are curiously good for the environment and give attractive natural colors, few fungal species are good to produce consistent colorants and they have carotenoids present in them as secondary compounds [15]. These filamentous fungi have been distinguished as they have anti-bacterial activity against Gram-positive and Gram-negative microbes.

For the better growth of fungal pigments, the optimal carbon source, moderate pH, temperature, and availability of light source is very necessary. It has been detailed that fungal species can develop and create colors in the submerged state containing glucose, fructose, and dextrose [16]. Hydrogen molecules exceptionally play important role in the production of eco-friendly pigments. The development of color under 5.5 and 6.5 pH was maintained for two strains of *Pycnoporus* [17]. The calculations [18] showed the most noteworthy value of yellow color was gotten with 6.5 pH while the greatest biomass concentration was measured at pH values of 8 for *Talaromyces* sp. [19].

Temperature is the main element that influences colors and other metabolites generation by organisms. Research showed the impacts of temperature on cell development, color generation by a different number of organisms demonstrating that the ideal temperature ranges from 25 to 32 °C. Fungi also respond to light during development for metabolites generation. The impact of light on color generation is very important for the production of fungal pigments. It was observed that in production of pigment from *M. ruber* repressed color production when plates are covered and cannot coordinate with light [20]. Consequently, the extractions of pigments from these filamentous fungi are being used in many applications [21]. The main objective of this research was to replace artificial dyes with natural ones.

2. MATERIAL AND METHODS

2.1 Isolation and Collection of Fungi

Fungal strains were collected from the Biology Lab of Lahore Garrison University. The cultures were preserved at -80 °C, the fungal strains were refreshed by using PDA media. For isolation, textile and lignocellulosic waste were collected and stored in sterilized plastic bags. By using the serial dilution method samples were collected and 1 mL was dissolved in 10 mL of water in a test tube. After that (0-1 mL) will spread onto the PDA plates with the sterile spreader. All PDA plates were kept in an incubator at 28 °C for 3-4 days. Diverse fungal colonies showed up on PDA plates from which cultures of the fungi were acquired by transferring them onto new PDA plates and after that kept once more in an incubator at 28 °C for 3-4 days [22].

2.2. Qualitative Analysis of Fungal Dyes

2.2.1 Preparation of Media

PDA is a solid medium comprising the extract of 300 g skinned potato, 2.5 g glucose, 15 g agar. All PDA plates were incubated at 28 °C for 3-4 days. Fungal colonies grew on PDA plates and were placed in an incubator at 28 °C for 3-4 days [23].

2.2.2 Cultivation of Fungi on appropriate Medium for Optimum Color Production

For the cultivation of fungi, two media were used i.e. Mineral Salt Media (MSM) and Potato Dextrose Broth (PDB). MSM was prepared using the following salts i.e., dextrose, K_2HPO_4-5.0 g,

KH_2PO_4-5.0 g, $CaCl_2$-0.1 g, $MgSO_47H_2O$-0.5 g, $FeSO_47H_2$ O-0.01 g, $ZnSO_4.7H_2O$-0.01 g, $MnSO_4H_2O$ -0.03 g. All these salts were dissolved in 1000 mL of distilled water (Figure 4). Two sets of flasks were placed in an incubator, in dark, under static conditions for 4-6 weeks until the bulk of pigments appeared.

2.3 Extraction of Pigments from Fungi

The isolation of fungal supernatant was caused by utilizing a sterile muslin cloth after the incubation period. The residue was ground by mortar and pestle. Later, 2 ml water was added and incubated at 130 rpm for 60 min at 28 °C [24].

2.4 Quantitative Characterization of Natural Pigments

The soluble compounds from the fungal extract and culture filtrates of different fungal isolates were subjected to UV–Visible spectrophotometer. Compounds that were present in water extracts were subjected to a wavelength scan from 400 nm to 600 nm for the determination of absorption spectra. Spectrophotometry was used for the measurement of the optical density of pigment. This was done for the determination of a better option for obtaining more concentrated pigment. The optical density (OD) was measured at 400, 450, 500, 550, and 600 nm (a wavelength which represents the absorption maximum for yellow, red, pink, greenish, and yellow-greenish pigments respectively), thus yielding the so-called yellow, pink, reddish-brown, and red pigment production [25].

2.5 Analysis of Color on Cotton Fabric

To analyze the fabric properties, rate retention of the dyed cotton cloth, percentage absorption was calculated on UV spectrophotometer at 400 nm to 600 nm and the percentage absorption was calculated by measuring OD of concentrated pigment before dipping the cotton cloth and again OD was calculated after absorption of pigment by cotton cloth. The final percentage absorption was calculated by the given formula [26]:

$$Percentage\ absorption = \frac{OD\ before\ dyeing - OD\ after\ dyeing\ \times 100}{OD\ before\ dyeing}$$

2.6 Antibacterial Activity of Fungal Pigments

The bacterial suspension was made of *E. coli* and *S. aureus* which was arranged by taking ordinary saline sterilized test tube. Bacterial colonies were exchanged with the assistance of a wire loop into the test tube. The circle utilized was sterilized by warming. The test tube was swirled well with the assistance of a syringe. For the making of media, the L-Agar and L-broth were utilized. These media were included in distilled water containing jar and shake well with the assistance of a stirrer, the volume raised to 500 ml at that point closed the carafe with the assistance of a cotton plug and secured with an aluminum thwart, and autoclaved for 15 minutes at temperature 121 °C and weight 15 lb / inch^2. A loop full of *E. coli* and *S. aureus* bacteria were inoculated into a nutrient broth and incubated on a shaker at 28 °C for a period of 16–18 h. After incubation, 100 µL of new bacterial cultures were immunized separately onto the agar plates by the spread plate method.

3. RESULTS

3.1 Optimization of Fungal Pigments

Two different media PDB and MSM media were used for the production of pigments. In the case of submerged fermentation where the flasks were incubated at rotary conditions, the MSM showed color production for all five fungi (Fig. 1). However, *A. terreus S10, T. atroroseus WW5A3* and *WW5C2* secreted pinkish, dark, and light yellowish colors, respectively, in PDB. Moreover, the impact of incubation conditions (rotating and/or static) was observed. On the other hand, in static, and dark conditions all five fungi showed color in a liquid state under MSM media and in the case of PDB media, except *WW35A4* all fungal strains produced color. *T. atroroseus WW5A3* appeared pinkish color and *A. terreus* S10 showed yellow color. The greenish-yellow color was advertised by *WW31DG*. Beneath 25 °C to 35 °C (Fig. 3) *WW5C2* created light yellow color, whereas *WW35A4* displayed a light pale-yellow color in solid-state and fluid state maturation (Fig. 4) in 4.5 to 6.5 pH as described in (Fig. 2).

3.2. Analysis of Fungal Pigments

The different fungal isolates were subjected to UV–Visible spectrophotometer [27]. The *T. atroroseus WW5A3* showed pinkish color with a strong absorbance in the near UV region with a distinctive peak at about 600 nm as shown in (Fig. 5). However, in the case of *A. terreus* (Fig. 6) showed yellow color, *P. oxalicum* showed Pale yellow, *WW31DG* showed yellow-greenish and *WW5C2* showed light yellow bio-colorants, a strong peak at ~ 500 and ~ 550 nm, respectively, was observed. However, they may have secondary metabolites like carotenoids, melanins, azaphilones, and polyketide that showed that specific color and also possess resistance to natural variables (drying up, high temperatures, irradiations, and photo-oxidation).

3.3 Application of Cotton Dyeing using Fungal Pigments

The extracted pigments were further used for dyeing the cotton cloth. For dyeing ferrous sulphate was used as a mordant. The capacity of extracted colors was evaluated on cotton cloth which weighed around 10 g. For detecting any change in color on the cotton, control was also held as shown in Fig. 7. It was observed that the pinkish, green, and yellow colors, exhibited an undeviating shade on cotton. The % OD was taken before dyeing and after dyeing to check the absorbance of color in cotton fabric and to check colorfastness on fabric. The overall process of pigment production from fungi is shown in Fig. 8.

3.4 Study of Antibacterial Activity of Fungal Pigments Against Gram-Positive Bacteria by Agar Well Diffusion Method

The antibacterial activity of fungal pigments against two bacterial strains was assessed by the Agar Well Diffusion method. The extracts exhibited a varying degree of antibacterial activity at 100 mg/mL against gram-positive and gram-negative bacteria. The result described that *S. aureus* and *E. coli* are susceptible to fungal pigments as described in

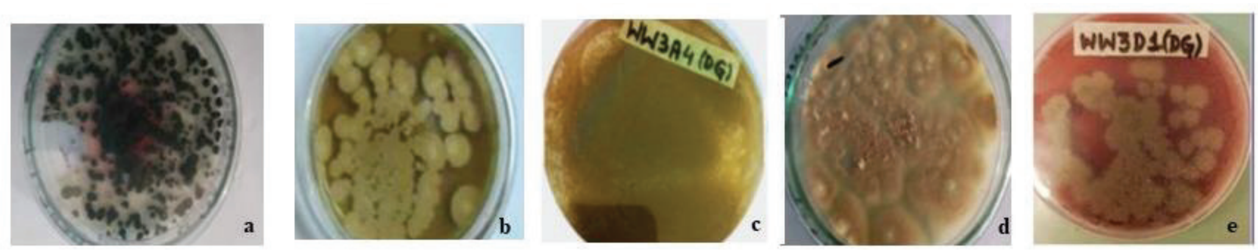

Fig. 1. Results of Solid Sate fermentation (a) *T. atroroseus WW5A3* (b) *A. terreus S10* (c) *P. oxalicum WW3A4* d) *WW5C2* (e) *WW31DG*

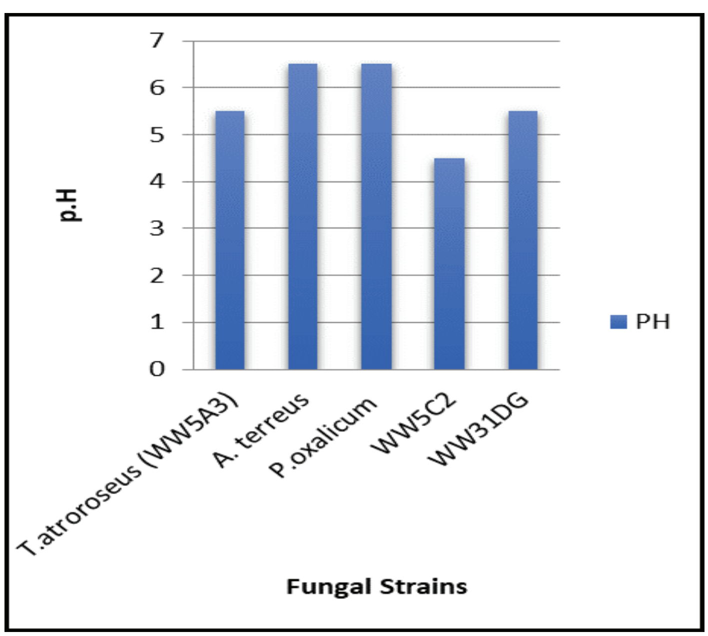

Fig. 2. Optimization of *T. atroroseus, A. terreus, P. oxalicum, WW5C2 and WW31DG* on different pH ranges

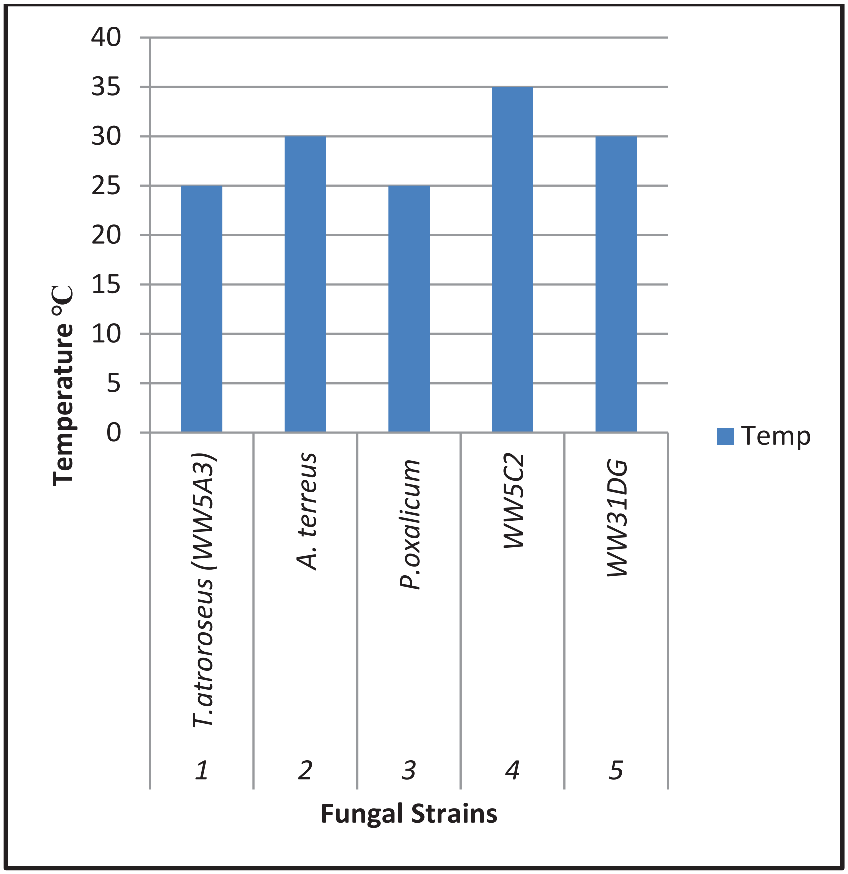

Fig. 3. Optimization of different fungal strains on different temperatures ranges

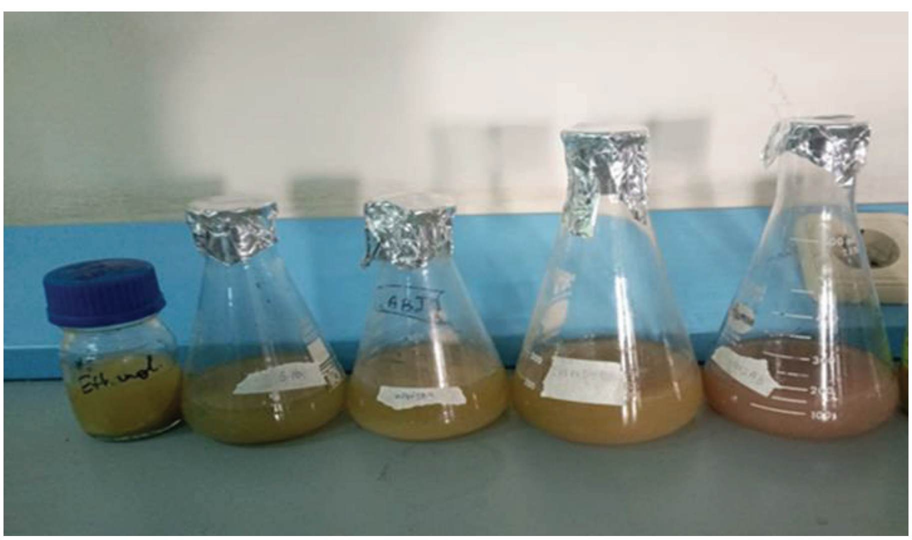

Fig. 4. Production of fungal pigments under liquid-state fermentation (a) *T. atroroseus WW5A3* (b) *A. terreus S10*, (c) *P. oxalicum WW5A4* (d) *WW5C2* and (e) *WW31DG* (left to right)

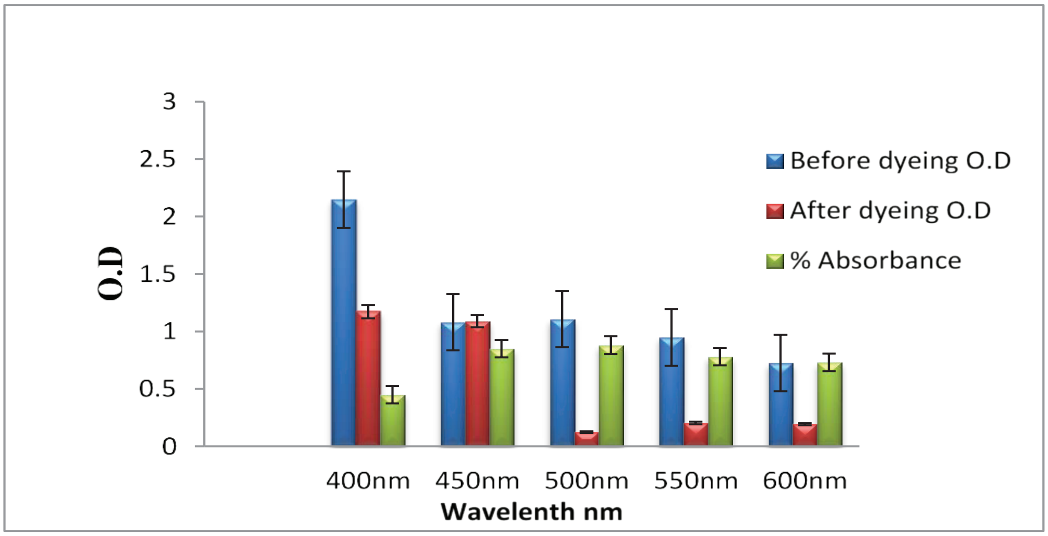

Fig. 5. Results of *T. atroroseus WW5A3* before and after OD at a different wavelength

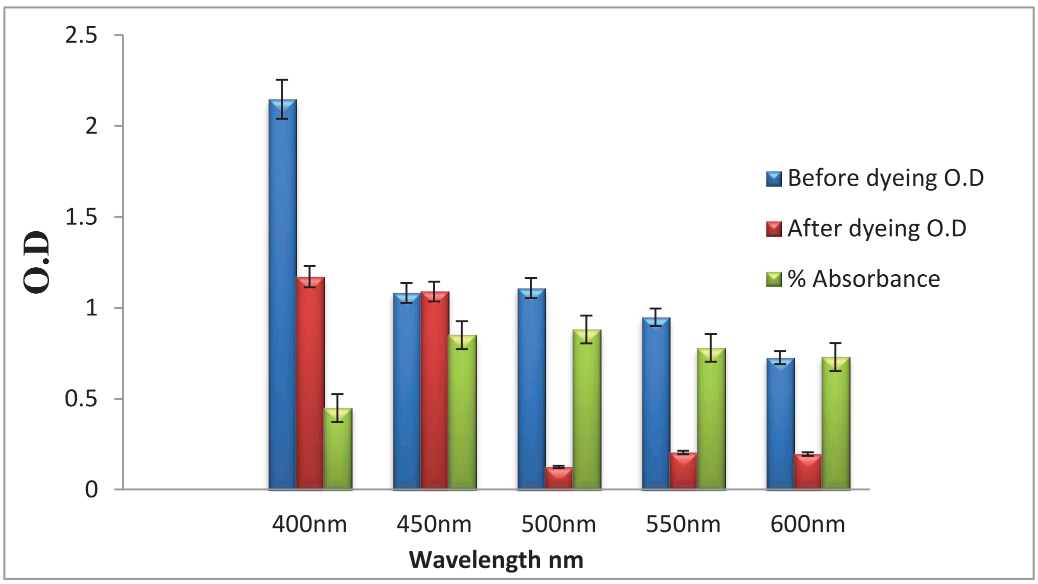

Fig. 6. Results of *Aspergillus terreus* S10 before and after OD at a different wavelength

Fig. 7. Dyed cotton cloth by fungal pigments showing dyeing of cotton cloth from fungal pigments

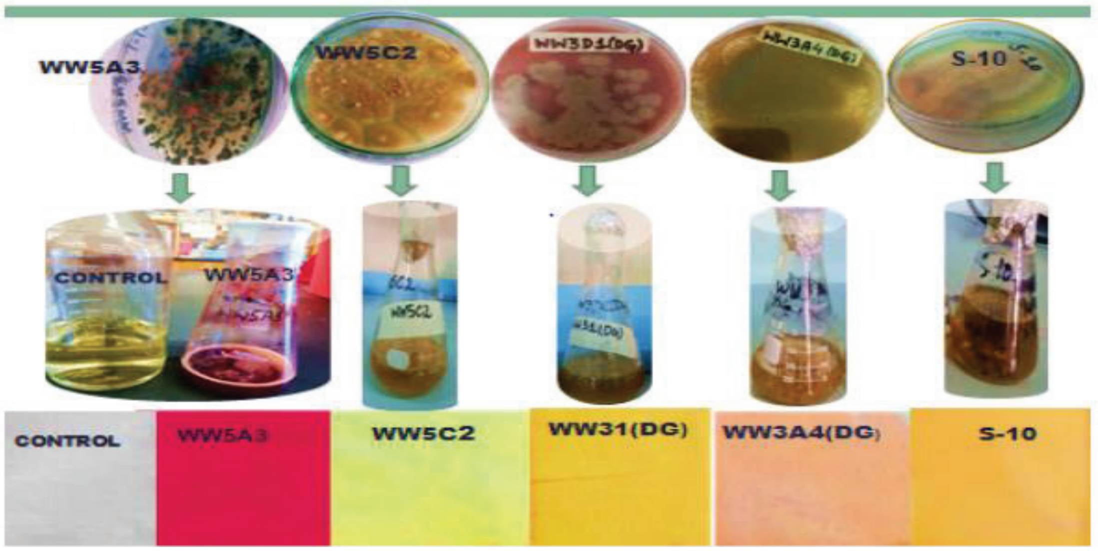

Fig. 8. The overall process of pigment production from fungi

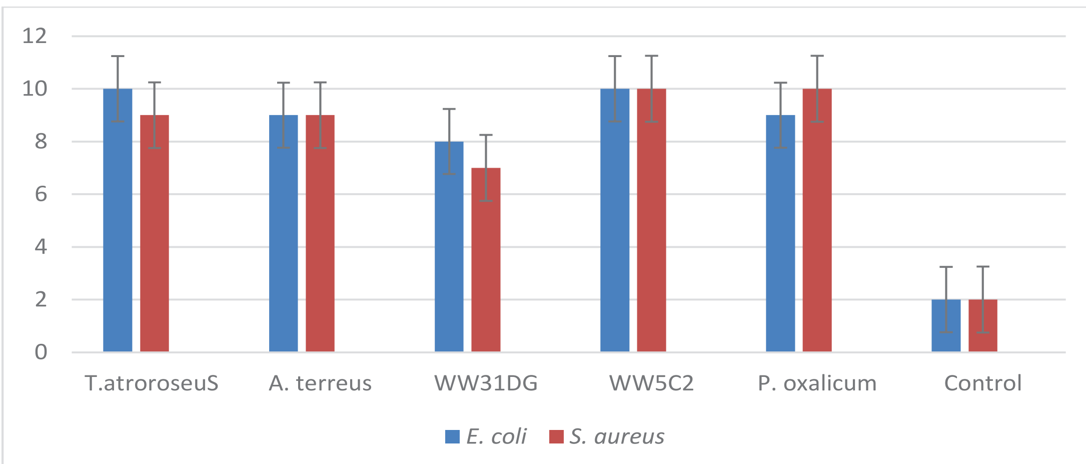

Fig. 9. Result of Zone of Inhibition for Fungal extracted pigments against strain *E. coli* and *S. aureus*

Table 1. Different fungal stains were grown on two different media that is MSM and PDA: They were grown at different temperatures and pH: on solid-state and liquid state fermentation the fungal strains showed pigments under static and non-static states.

S. No.	Fungi	Temperature	pH	Color both in solid and liquid condition	Pigments Production		Media	
					Static Condition	Non-Static condition/Dark	PDB	MSM
1.	*T. atroroseus WW5A3*	25°C	5.5	Pinkish	No	Yes	Yes	Yes
2.	*Aspergillus terreus S10*	30°C	6.5	Yellow	Yes	Yes	Yes	Yes
3.	*P. oxalicum*	25°C	6.5	Greenish Yellow	No	Yes	No	Yes
4.	*WW5C2*	35°C	4.5	Light Yellow	Yes	Yes	Yes	Yes
5.	*WW31DG*	30°C	5.5	Greenish	No	Yes	No	Yes

Table 2. Results of Zone inhibition of fungal extracted pigments against *E. coli* and *S. aureus*

Bacterial Strains	*T. atroroseus* WW5A3	*A. terreus* S10	*P. oxalicum* WW3A4 (DG)	WW5C2	WW31DG	Control
Gram Negative *E. coli*	10	9	8	10	9	2
Gram Positive *S. aureus*	9	9	7	10	10	2

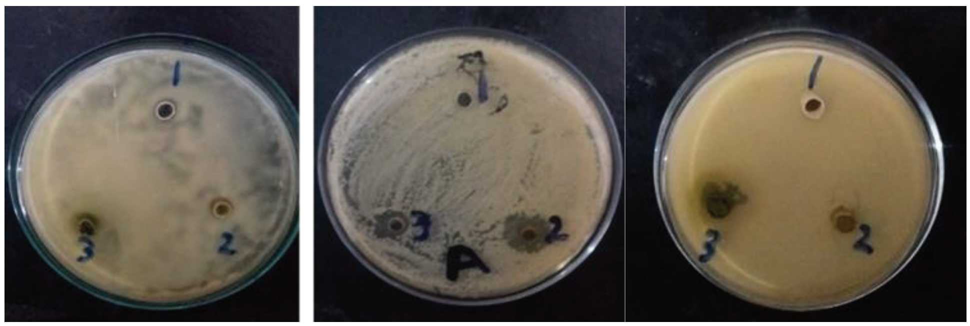

Fig. 10. Results of Antibacterial Activity against *E. coli* by Agar Well Diffusion Method

Fig. 11. Results of Antibacterial Activity against S. aureus by Agar Well Diffusion Method

(Fig. 9) and (Table 2). The antibacterial activity against *E. coli* and *S. aureus* is shown in Fig. 10 and Fig.11.

4. DISCUSSION

An effort was made to improve an eco-friendly process for the formation of pigments. This was attained by fungi and its utilization in cotton dyeing. It was observed that *T. atroroteus* and A. terreus showed a bulk of production of pinkish and yellow color pigments under MSM media while *WW31DG* showed good production of greenish-yellow pigment under both media. While *WW5C2* and *WW35A4* showed pale yellow color under MSM media. All these pigments presented a double advantage by giving the bulk of natural pigments that minimize the use of artificial dyes in several industries. Various parameters, such as the pH of the culture medium and temperature, promote the growth of fungal strains, resulting in the good synthesis of natural colors from fungus. The temperature ranges of 25 to 35 °C and the pH range of 4.5 to 5.5 were shown to be optimal for fungus growth and pigment synthesis. For both mycelial development and pigment formation, the pH of the media fluctuates. The optimum pH and temperature of the fungal strain were 4.5-5.5 and 25 ± 5 °C were observed for growth and secondary metabolite production. A pale yellow (537– 540 nm); neutral arrangement, violet (529–536 nm) and acidic arrangement, ruddy (500–507 nm) [28]. The samples were exposed to a wavelength scan from 400 nm to 600 nm to determine the maximum absorbance of chemicals present in water extraction. The greenish and pinkish color exhibited as a solid absorber inside the UV area, with a typical absorption crest observed around 550 and 600 nm. In addition, the pinkish color showed a strong absorbance in the near UV area, with a prominent peak at nearly 600 nm. However, substantial peaks at 500 and 550 nm were seen in the case of yellow-greenish and light-yellow bio-colorants, respectively. Due to the proximity of a large conjugated framework, which induces absorbance at longer wavelengths, higher absorbance values were observed. Shade broken down within the water is effectively particle pulls in the water particles and make cotton retains water well. Shades broken up within the water are effectively bound and express their tint. The absorbance of *A. terrreus* is 88 % under 500 nm of wavelength, *T. atroroteus* showed 90.36 % of absorbance under 600 nm of wavelength, *WW31DG* showed 81.9 % of absorbance under 550 nm of wavelength, *WW5C2* showed 59.60 % of absorbance under 550 nm of wavelength and *WW35A4* showed 44.04 % of absorbance under 550 nm of wavelength. The results of the study advocated that the fungal extracts were effective against tested human pathogens i.e. gram-negative *Escherichia coli*. The results indicated that fungal pigments were more effective against gram-negative bacterial strain *E. coli* showed a 10 mm zone opposite to *T. atroroteus*, 9 mm against *A. terreus,* 8 mm adverse *WW31DG,* 10 mm *WW5C2*, and 9 mm differing *WW5A4.* Streptomycin served as control which showed 2 mm ± 0 zones of inhibition respectively. Unique organisms were isolated from the samples. These fungal species are safe to use due to all of the safety precautions taken during the generation process. There are only a few ideas about how to use bio-colors from organisms for cotton coloring.

5. CONCLUSION

Fungi are rich in natural pigments hence we can extract natural dyes from them and use them in industries as an alternative to artificial dyes. Natural colors are non-toxic, non-polluting, and less health dangerous. Additionally, their antioxidant and antimicrobial nature encourage includes their positive impacts. Fungal colors have a few focal points over the plant and animal-based colors as organisms are fast in developing colors and have the potential of being standardized commercially. Efforts have been made to synthesize fungal colors to be utilized within the leather and textile industry. The extracted shades can be tried for their better utilization in industries such as cloth or leather dying, makeup, food colorant, and pharmaceuticals industries, etc. Due to its natural pigments, it is considered eco-friendly. Within the show work, efforts have been made to screen and extricate certain color-creating species of organisms and optimize their yielding conditions for the most extreme generation of colors which can be utilized productively in industries.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest

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Research Article

Role of Manganese Forms on Black Gram (*Vigna mungo*) Seedling Growth

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Abstract: The impact of concentration of manganese (Mn^{2+}) forms on early seedling growth and some physiological attributes of black gram (*Vigna mungo* L.) have been reported in the current study. An adequate amount of 22.32 µg/mL (as in control solution) of Mn^{2+} was found to be crucial for proper growth and it also greatly impacts the process of photosynthesis and the amount of chlorophyll in the growing seedling. Reduced growth was observed as the concentration of Mn^{2+} was increased from 50 ppm up to 250 ppm. Reduced growth is due to various non-enzymatic coping mechanisms invoked by the plant to ease the metal stress which has several side effects on key plant growth attributes. One such defense strategy to reduce metal overload is the production of proline that can dilute the excess metal content. Chlorophyll content with respect to the age of the seedling is also studied and it brought interesting results.

Keywords: Mn Toxicity, Growth Analysis, Black Gram

1. INTRODUCTION

Manganese (Mn^{2+}) is a micronutrient metal that is essential for many metabolic, physiological, and growth processes of plants including respiration, synthesis of enzymes and photosynthesis, etc. [1]. Essential enzymes necessary for proper plant growth such as nitrate reductase, and isocitrate dehydrogenase require Mn^{2+} for their biosynthesis [2]. Manganese is also utilized as a cofactor of the Mn^{2+} -dependent-superoxide-dismutase enzyme. It also regulates the synthesis of fatty acids, carotenoids, acyl lipids, and nitrogen metabolism [1, 3, 4]. Manganese also contributes to the functionality of photosystem II (PSII) when water molecules split into oxygen, and also in the protection of photosystem II from photodamage [5].

In comparison with aluminum, copper, and other metals, Mn^{2+} has a higher tendency of mobility for translocation from roots to the upper part of the plant, therefore, the toxicity induced by manganese shows symptoms clearly in the upper parts of plants. The effect of toxicity caused by manganese is greatly attributed to the type of species, age of the plant, environmental temperature and intensity and quality of light, etc. Several studies describe these visual features such as reduced pigmentation, reduced leaves size, and reduced height of the plant caused by manganese toxicity [6-9].

Mn^{2+} is the most stable and water-soluble form of Mn^{2+} present in soil and is responsible for the majority of Mn^{2+} -induced contamination and toxicities. Certain environmental conditions like lower pH of the soil, low quantities of organic matter, and decreased redox potential can greatly increase the risk of Mn^{2+} toxicity. Darkening of leaves, brown spots on leaves, black specks on the stem, and crinkled leaves are some of the most commonly induced Mn^{2+} toxicity. Literature information revealed high quantities of Mn^{2+} in the soil causes impaired carbon dioxide absorption in *Citrus grandis* (Pomelo) seedlings [9], reduced content of chlorophyll A in *Pisum sativum* L. (Common Pea) [10, 11], and *Glycine max* L. (Soybean) [12].

Vigna mungo is mainly grown in the Indian

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subcontinent, comprises a major part of Punjabi cuisine, where it is locally called "urad ki daal" in the local Punjabi language. It is largely being used to make daal from both wholes and split seeds. It is an erect annual herb with dense hairs and sub-erect and trailing branches. These beans were originally placed in the *Phaseolus* genus of the *Fabaceae* family but have recently been transferred to the *Vigna* genus of the legume family because of the difference of the two genera in biochemistry, the structure of pollen, style, and stipules [13].

Literature study on the subject suggests that the effect of manganese on growth parameters in early seedlings and growing plants is important in the understanding pattern of manganese distribution though out the plant body under metal overload, the underlying physiological mechanisms at molecular levels of manganese toxicity, and its tolerance. In this study, we have reported for the first time the role of manganese in two of its common forms found in biological systems (Mn^{+2} and Mn^{+7}), on the growth of early seedlings of *Vigna mungo* as a step forward towards understanding the role of micronutrient-manganese in the development of early seedlings.

2. MATERIAL AND METHODS

Vigna mungo species were used throughout the experiments. The seeds were obtained in a single lot from local seed suppliers in Karachi. The seedlings were grown in Hoagland hydroponic nutrient solution in custom-made hydroponic-styled pots facilitated with lids with some ¼ inch holes approximately 1 inch apart, as shown in Figure 1. All pots were placed in a partial shade environment and as soon as the primary leaves had unfolded, the test solutions of various oxidation states of manganese were introduced in all test pots in concentrations ranging from 50 to 250 parts per million (ppm). Seedlings were grown in a soil-less water-culture half-strength Hoagland solution [14]. For this purpose, analytical grade chemicals were dissolved in double-distilled water with a balanced composition of macro and micro-nutrients to prepare the Hoagland hydroponic culture (Table S1), while Table S2 lists the recipe to prepare the half-strength Hoagland solution.

The growth of the beans was constantly monitored and it was noted that for both test and control pots, the seedling started to grow from the 5^{th} to 7^{th} day of germination. The data was recorded from 10 to 20 days of germination that used primary leaves of the plant, root, and hypocotyl. These were measured, weighed, and dried.

The area of the leaves was simply determined by drawing the outlines of the leaves on paper with the help of a graphite pencil.

The leaves were dried afterward and stored in small glass vials with plastic lids until their chlorophyll concentration per unit area was determined spectrophotometrically. About a half gram of fresh leaves was cut into small pieces and grounded in acetone and was centrifuged at 3000 RPM (rcf = 1107 x g) for about 20 min. Then the supernatant was separated with about 20 ml acetone. The absorbance of samples was measured at wavelengths of 661.6, and 644.8, nanometers for chlorophyll A, and chlorophyll B respectively. The following equations were employed for calculating the quantities of pigments per unit area on the surface of the leaves [15].

$$\text{Chlorophyll "A"} = (11.24 \times E661.6) - (2.04 \times E\ 644.8)$$

$$\text{Chlorophyll "B"} = (20.13 \times E644.8) - (4.19 \times E\ 661.2)$$

$$E = \textit{absorbance wavelength}$$

It is a common observation that the chlorophyll concentration per square area would be greater in the full-sized leaves as compared to younger leaves. When it comes to studying the concentration of chlorophyll in the presence of higher doses of heavy metals, the necessity to study the effect of age naturally arises.

It is very important to know whether there had been a natural increase or decrease in the chlorophyll concentration during the study regardless of the concentration of heavy metal. This study was also important for the fact that etiolated leaves may not regain the chlorophyll level unless there is a soluble carbohydrate supply [1]. Another very important relationship that must be counted during the study of the chlorophyll concentration is that the presence of cotyledons, the chlorophyll concentration

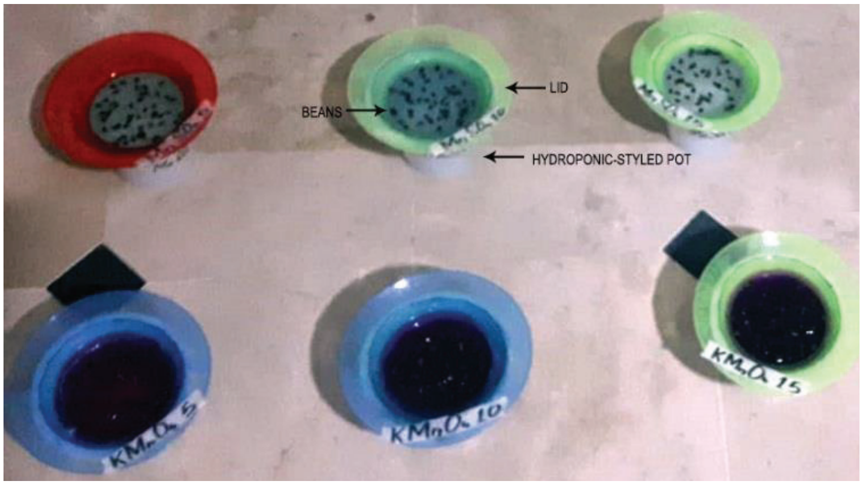

Fig. 1. Hydroponic-styled pots for germinating seedlings

before and after the shedding of the cotyledon was therefore also studied. To count for the effect of age, the leaves were reaped at four different stages of development: - (i) "Age-A" primary leaves reaped after they unfolded, (ii) "Age-B" as the cotyledons being shed, (iii) "Age-C" as the primary leaves were of considerable size and (iv) "Age-D" at the 20th day of germination.

3. RESULTS AND DISCUSSION

Results of our studies showed the overwhelming effect of manganese toxicity on seedling's morphology, germination, chlorophyll concentration, and pigmentation, especially of the manganese in its 2+ oxidation state. As the doses were increased, the effect worsened which is thought to be the side effects of plant defense mechanisms. Whenever plants employ one of these defense strategies to tolerate or detoxify the heavy metal stress, it has a direct effect on various growth parameters such as physiological malfunctioning of the vascular bundle that results in poor growth rate, limited germination, reduced length and diameters of epicotyl and roots to name a few [6]. Figure 2 shows the impact of Mn^{2+} toxicity on seedling germination of *Vigna mungo.*

Similar results were observed in the first 10 days of germination when various doses of Mn^{2+} were introduced to the pots before the emergence of the primary leaves. A direct relationship of reduced growth was observed as the doses of Mn^{2+} were increased and the test pots. Table 1 lists the morphological parameters observed in seedlings of age 10-12 days after germination after they were exposed to the Mn^{2+}.

3.1 Effect on Plant Morphology (Growth)

Generally, heavy metals can interrupt cell processes and interact with vital bio-molecules such as proteins and DNA, this is because they have a high affinity for thiol groups in these molecules. These interactions can lead to the production of reactive oxygen species (ROS) [16-18]. Severe morphological, metabolic, and physiological damages in plants can result from these toxic interactions. These damages may include chlorosis, protein degradation, reduced growth, and germination inhibition [8, 19]. Several studies reveal these damages mainly depend upon the concentration of heavy metals the plants were exposed to or the effect of the dose of heavy metals in combination with environmental conditions [8].

Nature has provided plants with some defense strategies to reduce the toxicity of heavy metals. One important defense mechanism is to remove the heavy metals by phytochelation and production of metallothioneins complexes at intracellular levels occasionally and intercellular levels more frequently [8]. These defensive detoxification processes can come into action at sites where toxicity occurs, as a result of defensive action, the produced phyto-complexes are removed from the affected sites by a process of vacuolar requisitioning. A non-enzymatic defensive mechanism to reduce heavy metal toxicity is the production of a proteogenic amino acid called proline (Pro), which acts essentially as a solute and helps greatly in the detoxification of heavy metals

[20, 21].

A combined effect of deteriorated xylem and phloem cells in the vascular bundles as a result of one of these stressful processes adopted by the plant is thought to be responsible for the reduced growth of the seedlings in this study. This combined effect may involve the slumped ability of the xylem in moving water and minerals upwards and contracted phloem cells leading to the deficient distribution of nutrients in the plant body [8, 22, 23].

The effect of Mn^{7+} was observed to be similar in results but with a mild-to-low toxic effect on plants morphology that may be due to the fact that Mn^{7+} can easily get reduced into Mn^{2+}, this conversion may not be 100 % in the context of any plant defense mechanism hence there is a mixture of both species and mild-to-low toxic effect is observed. Table 2 lists the results obtained for Mn^{7+} tests, and growth reduction under the influence of Mn^{7+} is shown in Figure 3.

3.2 Effect on Photosynthesis

For all photoautotrophs in the Kingdom Plantae, it is well known that the photosystem-II is responsible for the production of oxygen by photosynthesis. This photosystem mainly consists of a water-oxidizing complex (WOC) that has a cluster of 4 manganese atoms that catalyzes the production of oxygen from water during the photosynthetic process. The oxidation states of manganese are now known to be "3 Mn atoms in 3+ oxidation states while one atom in 2+ oxidation state" [24].

The four-manganese-atom inorganic core of the photosystem-II extracts electrons from water by a series of four consecutive redox steps denoted as S steps or states. These states are frequently labeled as S_0, S_1, S_2, and S_3 [24]. The subscript number refers to the oxidized equivalent obtained by the successive electron removals. Although it is not known so far, the individual oxidation states of manganese atoms in all four S states in the redox process however, in the presence of excess Mn^{2+} the photosynthetic ability of the plant is observed to be reduced that can be associated with the dis-balancing effect on the inorganic core resulting in interfering the redox chain reaction of electron removal [24]. Our studies confirm this hypothesis, as we introduced higher doses of Mn^{2+} to the test seedlings, the reduced growth and pale coloration of the leaves confirms the reduced photosynthesis, a clear indication of malnutrition and is caused by the malfunctioning of the vascular bundles.

3.3 Effects on Pigmentation

As the doses of the manganese were increased especially of Mn^{2+}, when the primary leaves unfolded, they noticeably appeared pale-colored. For the structure of the chlorophyll molecule, the central magnesium atom is in a 2+ oxidation state, when the seedlings were exposed to the excessive quantities of Mn^{2+}, a competition reaction presumably started between Mn^{2+} and Mg^{2+}. Due to the difference in the redox potential of these two ions, Mg^{2+} can get replaced by Mn^{2+} which can lead to the disintegration of chlorophyll molecules. Figure 4 and figure 5 show the impact of high concentrations of manganese ions on leaves.

Magnesium ion in chlorophyll molecule is bonded to four nitrogen atoms by coordinate covalent bonds. These four nitrogen atoms have a greater tendency to become covalently bonded to Mn^{2+} ions. Hence this reaction is favored and is the main reason for the seedlings to have pale pigmentation [1].

Results confirmed the hypothesis, that quantities of chlorophyll per unit area in leaves of all ages of seedlings decreased and this reduced pigmentation is proportional to the doses of Mn^{2+} the seedlings were treated with. Table 3 to table 6 list the chlorophyll concentration for all ages with respect to averaged leaf area and fresh weight of leaves per unit area for all ages sequentially. The numbers of plants for which the average data have been collected are as follows: for age-A: 16 plants, for age-B: 24 plants, for age-C and age-D: 32 plants. A closer look at the results can easily reveal a direct relationship of reduced chlorophyll concentration as the doses of the Mn^{2+} increases with one exception, a comparatively better chlorophyll concentration was detected at age-B, this is because the cotyledons were just being shed, the higher concentration of chlorophyll A and B can be attributed to the dissolved carbohydrate supplied by the cotyledons even in the presence of higher doses of Mn^{2+}.

Fig. 2. Reduced germination and growth under Mn^{2+} exposure

Table 1. Growth Analysis and Morphology on *Vigna mungo* exposed to Mn^{2+}

Mn^{2+} Dose (ppm)	**Germination** (%)	**Root Length** (cm)	**Epicotyl** **Length** (cm)	**Epicotyl** **Morphology**	**Leaf Area** (cm^2)
Control	90	6.9 + 0.2	13.5 + 0.1	ERECT	4.4 ± 0.1
50	80	5.8 ± 0.1	11.0 ± 0.1	ERECT	3.8 ± 0.2
100	65	4.3 ± 0.2	10.4 ± 0.2	CURVED	3.0 ± 0.3
150	50	3.0 ± 0.2	9.2 ± 0.4	CURVED	2.4 ± 0.2
200	25	2.2 ± 0.1	5.5 ± 0.1	DOWN	1.8 ± 0.2
250	10	1.1 ± 0.1	3.8 ± 0.3	DOWN	1.2 ± 0.1

Table 2. Growth Analysis & Morphology on *Vigna mungo* exposed to Mn^{7+}

Mn^{7+} Dose (ppm)	**Germination** (%)	**Root Length** (cm)	**Epicotyl** **Length** (cm)	**Epicotyl** **Morphology**	**Leaf Area** (cm^2)
CONTROL	95	7.0 + 0.1	13.0 + 0.1	ERECT	4.2 ± 0.1
50	82	6.1 ± 0.1	11.2 ± 0.1	ERECT	3.5 ± 0.1
100	70	5.4 ± 0.1	10.8 ± 0.1	CURVED	3.6 ± 0.2
150	65	3.7 ± 0.1	8.1 ± 0.1	CURVED	2.9 ± 0.1
200	35	2.9 ± 0.1	6.4 ± 0.1	CURVED	2.1 ± 0.1
250	25	2.0 ± 0.1	2.1 ± 0.2	DOWN	2.0 ± 0.1

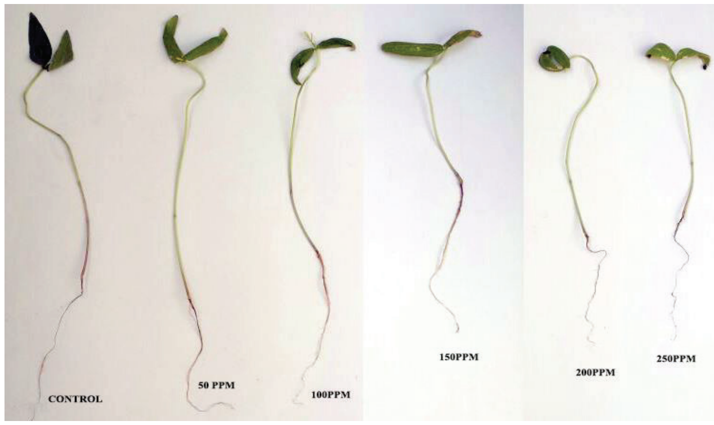

Fig. 3. Mildly reduced germination and growth under Mn^{7+}

Fig. 4. Mn^{2+} exposure, reduced chlorophyll

Fig. 5. Mn^{7+} exposure, similar but mild impact

Table 3. Chlorophyll content for "age-A" of *Vigna mungo* exposed to Mn^{2+}

Mn^{2+} Dose (ppm)	Avg. Leaf Area (sq.cm)	Avg. Fresh Weight (per sq.cm (g))	Chlorophyll A (per sq.cm (mg))	Chlorophyll B (per sq.cm (mg))
CONTROL	3.72 ± 0.1	0.0097	0.0097	0.0058
50	3.30 ± 0.1	0.0088	0.0080	0.0048
100	2.86 ± 0.1	0.0060	0.0070	0.0042
150	02.5 ± 0.1	0.0053	0.0042	0.0025
200	01.9 ± 0.1	0.0038	0.0028	0.0017
250	01.3 ± 0.1	0.0029	0.0016	0.0010

Table 4. Chlorophyll content for "age-B" of *Vigna mungo* exposed to Mn^{2+}

Mn^{2+} Dose (ppm)	Avg. Leaf Area (sq.cm)	Avg. Fresh Weight (per sq.cm (g))	Chlorophyll A (per sq.cm (mg))	Chlorophyll B (per sq.cm (mg))
CONTROL	09.8 ± 0.1	0.0257	0.0354	0.0212
50	08.7 ± 0.1	0.0233	0.0293	0.0176
100	07.5 ± 0.1	0.0160	0.0255	0.0153
150	06.8 ± 0.1	0.0140	0.0153	0.0092
200	05.0 ± 0.1	0.0102	0.0103	0.0061
250	03.6 ± 0.1	0.0077	0.0060	0.0036

Table 5. Chlorophyll content for "age-C" of *Vigna mungo* exposed to Mn^{2+}

Mn^{2+} Dose (ppm)	Avg. Leaf Area (sq.cm)	Avg. Fresh Weight (per sq.cm (g))	Chlorophyll A (per sq.cm (mg))	Chlorophyll B (per sq.cm (mg))
CONTROL	13.5 ± 0.1	0.0353	0.0257	0.0154
50	12.0 ± 0.1	0.0321	0.0213	0.0128
100	10.4 ± 0.3	0.0219	0.0185	0.0111
150	09.4 ± 0.1	0.0193	0.0111	0.0067
200	06.9 ± 0.2	0.0140	0.0075	0.0045
250	04.9 ± 0.1	0.0106	0.0043	0.0026

Table 6. Chlorophyll content for "age-D" of *Vigna mungo* exposed to Mn^{2+}

Mn^{2+} Dose (ppm)	Avg. Leaf Area (sq.cm)	Avg. Fresh Weight (per sq.cm (g))	Chlorophyll A (per sq.cm (mg))	Chlorophyll B (per sq.cm (mg))
CONTROL	18.6 ± 0.1	0.0484	0.0485	0.0291
50	16.5 ± 0.2	0.0440	0.0402	0.0241
100	14.3 ± 0.3	0.0301	0.0349	0.0209
150	12.9 ± 0.2	0.0265	0.0210	0.0126
200	09.5 ± 0.3	0.0192	0.0141	0.0084
250	06.8 ± 0.2	0.0146	0.0082	0.0049

4. CONCLUSION

Our results showed that the quantity of Mn^{+2} in the control pot (22.32 ug/ml) was necessary for proper seedling development, no visible sign of growth malfunction was observed, even at a moderately higher level of Mn^{+2} (50 ug/ml), but as the concentration of Mn^{+2} was further increased, deteriorations were observed and found to be concentration-dependent i.e., as the concentration was increased, the more prominent effect was observed. Beyond 50 ug/ml of Mn^{+2}, the epicotyl started curving down, chlorophyll concentration and percent germination were also reduced. For Mn^{+7}, similar but mild effects were observed. The impacts of manganese toxicity were seen as the result of the plant's non-enzymatic defensive measures to reduce the metal overload. The high concentration of Mn^{+2} in the system can cause the disintegration of the chlorophyll molecule, primarily affecting the pigmentation and ultimately contributing to the malfunctioning of chloroplast, and causing the reduced chlorophyll concentrations. The mild impact on the overall growth observed in the case of Mn^{+7} was attributed to its ability to get reduced into Mn^{+2} in the biological system. The experiments perfumed to observe chlorophyll in the aging seedling showed that at the age where the cotyledons were just being shed (age-B), the chlorophyll concentration was found comparatively better than expected and it was attributed to the carbohydrates supplied by the cotyledons.

5. CONFLICT OF INTEREST

There is no conflict of interest.

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Supplementary Data

Table S1. Composition of macro and micro-nutrient in Hoagland culture

S. No.	**Macronutrients**	**Quantity** (mg/100ml)
1	$Ca(NO_3)_2$	22.48
2	KCl	7.22
3	KH_2PO_4	13.22
4	$MgSO_4$	23.98
S. No.	**Micronutrients**	**Quantity** (g/L)
1	H_3BO_4	2.4
2	$MnCl_2/MnSO_4.H_2O$	1.48/1.31
3	$ZnSO_4.4H_2O$	0.11
4	$H_2MoO_3/NaMoO_4.H_2O$	0.5/0.083
5	CuSO4	0.78
6	$Fe(EDTA)^{-2}$	24.8 g $FeSO_4$ $.7H_2O$+25 mg EDTA in 500 ml distilled water with few drops of KOH

Table S2. Half-Strength Hoagland Recipe

S. No.	**Chemicals**	**Volume of Stock Solution** (mL)
1	$Ca(NO_3)_2$	1.5
2	KCl	1.0
3	KH_2PO_4	1.2
4	$MgSO_4$	0.8
5	Micronutrients	0.5
6	$Fe(EDTA)^{-2}$	0.6

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Research Article

Efficacy of Indigenous Crude Plant Extracts against Rice Weevil, *Sitophilus oryzae* L. 1763 (Coleoptera: Curculionidae)

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Abstract: Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is a widely distributed and major insect pest of rice that causes considerable losses to both husked and unhusked rice grains. Plant-based pesticides are alternative to synthetic pesticides in managing stored product pests owing to their severe hazardous effects. Thus, locally available plant leaf crude extracts i.e., neem (*Azadirachta indica* A. Juss 1830), turmeric (*Curcuma longa* L. 1753), and mint (*Mentha longifolia* L. Huds. 1762) were investigated at 2 % (w/w) concentration against *S. oryzae* in unhusked and husked rice. Adult mortality was recorded at 24, 48 hours, and seven days after the application whereas, population build-up was recorded at 1-, 2-, and 3-months intervals. Overall and grain weight loss was also recorded at the end of the experiment. In husked and unhusked rice, the highest mortality percentage was observed in turmeric powder (93.30 %) and neem (80.00 %), respectively. In comparison to plant extracts, relatively higher population growth of *S. oryzae* was recorded in control husked and unhusked rice. At the end of the 3rd month, the highest and lowest population in husked rice grains was recorded in control (70.67 ± 3.18) and turmeric treatments (18.00 ± 2.65), respectively, whereas control and neem treatments exhibited the highest (83.67 ± 3.28) and lowest (39.67 ± 2.33) populations in unhusked rice. The lowest grain weight loss in husked and unhusked rice was recorded in turmeric (9.33 %) and neem (16.67 %), respectively. Thus, turmeric and neem extracts should be investigated on large scale under commercial warehouses for the management of *S. oryzae* in husked and unhusked rice.

Keywords: Biopesticides, Phytochemicals, Plant Extracts, Rice Weevil, Mortality

1. INTRODUCTION

Rice grains are the richest source of energy and account for one-fifth of the global calorie supply. It is also a major source of nourishment for about 2.5 billion people around the world [1]. More than 1,200 species of pests have been previously reported to destroy stored products [2]. The post-harvest losses due to the stored grain pests are estimated at 9 and 30 % in developed and developing countries, respectively [3]. Major insect pests of stored rice grains include rice weevil (*Sitophilus oryzae* L.), maize weevil (*Sitophilus zeamais* Motsch.), granary weevil (*Sitophilus granaries* L.), lesser grain borer (*Rhyzopertha dominica* Fabr.), and sawtoothed beetle (*Oryzaephilus surinamensis* L.), which caused heavy grain losses [4]. Among them, *S. oryzae* is the major insect pest of rice and causes a considerable loss to both husked and unhusked rice [5]. Under favourable prolonged storage conditions, it can cause losses up to 80 %, whereas average losses between 10–65 % are reported under moderate storage conditions [6].

Management of stored grain pests is generally based on the use of synthetic chemicals throughout

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the world [7]. These chemicals create severe problems such as resistance in pest species, health hazards, and chemical residues in food products [8]. Accordingly, rice weevil has also developed resistance against many widely used insecticides and fumigants in the stored grains [9, 10].

Thus, there is an urgent need to search for alternate tactics that are readily available, affordable, cost-effective, relatively less toxic, and less harmful to the environment [11]. Plant materials and their derivatives are usually less expensive, easily available, target-specific and safe for the non-targeted species and environment [12, 13]. Accordingly, these naturally occurring plant products and bio-pesticides are potential alternatives to manage agricultural insect pests [14]. Therefore, a broader range of plant derivatives and their products has been studied against several insect pests of stored products as most of them are less toxic, increased food safety, non-pollutant, and biodegradable, improved the production profitability, and reduced pesticide resistance [15]. Among the botanicals evaluated against rice weevil includes neem, eucalyptus, black pepper, Chinese cinnamon, garlic, yellow oleander, mint, basil, black seeds, and lavender in various regions of the world with varying degrees of effectiveness [16-19]. However, in Pakistan, there is little or no studies conducted to exploit locally available crude plant extracts to manage the pest population of stored grains. Therefore, the present study was designed to evaluate the potential of *Azadirachta indica* A. Juss (1830), *Mentha longifolia* L. Huds. (1762) and *Curcuma longa* L. (1753) in managing rice weevil in both husked and unhusked rice grains. The outcome of this research will be useful in the development of bio-rational insecticides.

2. MATERIAL AND METHODS

2.1 Test Insect

Sitophilus oryzae was obtained from the previously maintained culture at the laboratory of the Department of Entomology, Sind Agriculture University Tandojam, Pakistan. Adult weevils were reared on brown rice at 25 ± 2 °C and 65 ± 5 % relative humidity adopted from Mehta *et al.* [20].

2.2 Rice Grains

Husked and unhusked rice variety IRRI-9 used in the study was purchased from the local market. Prior to experiments, the grains were disinfested in the oven at 40 °C for 4 h [21].

2.3 Preparation of Plant Materials

Matured fresh leaves of *A. indica* and *M. longifolia* were obtained from the surroundings of the Sindh Agriculture University, Tandojam, whereas *C. longa* L. powder was purchased from the local market. The plant materials were air-dried for two weeks in a well-ventilated place, ground using an electric blender (GEEPAS China GCG289) and sieved through muslin cloth to obtain a fine powder.

2.4 Experimental Outline

Two experiments (mortality and population build-up) were conducted in the study. In the mortality experiment, the powder of each plant was applied individually at the rate of 2 % into 20 g of both husked and unhusked rice grains in individual petri dishes. The powder and the grains were shacked thoroughly for 3 min to create a homogenous mixture. Thereafter, in each petri dish, five pairs of freshly emerged one-day-old *S. oryzae* were transferred. Moreover, to ascertain the effect of plant powders on the population development of rice weevil in both husked and unhusked rice grains, 2 % concentration of each material was thoroughly mixed with individual grains in plastic jars (Width: 21.855” x Height: 13.875”). Ten pairs of *S. oryzae* adults were introduced into individual jars. A control treatment (husked and unhusked rice grains) without plant powders was included in the experiments. The mouth of the jars was covered with a muslin cloth to ensure aeration and restrict insects inside the jars. Both experiments were arranged in Completely Randomized Design with each treatment replicated thrice.

2.5 Parameters Measured

The mortality of *S. oryzae* was recorded at 24 and 48 hours, and 7 days after the application of treatments, where numbers of live and dead *S. oryzae* were counted. Moreover, monthly observations were done for three months to count live *S. oryzae* to record its population build-up in both husked and unhusked rice. At the end of the third month, weight loss in the individual treatments was also recorded.

2.6 Data Analysis

The collected data was analyzed using ANOVA by STATISTIX 8.1 statistical software, whereas Least Significant Difference (LSD) at 0.05 significant level was used for mean separation [22].

3. RESULTS

Results of the study confirmed a profound impact of plant extracts on the mortality and population development of *S. oryzae* in rice grains. Among plant extracts, neem and turmeric were found more effective against *S. oryzae* in unhusked and husked rice, respectively as comparatively higher mortality of weevils was recorded in husked rice.

3.1 Effect of Crude Plant Extracts on Mortality of *S. oryzae* at Different Intervals

Mortality experiment results (Table 1) revealed that at 24 h after treatment, the highest (2.33 ± 0.33 weevils) mortality was observed in husked rice treated with turmeric powder whereas, in unhusked rice, neem powder caused the highest (1.33 ± 0.33 weevils) mortality. A similar trend was observed at 48 h after exposure as the mortality percentage of weevils showed a gradual rise. After one week, the highest mortality percentage of *S. oryzae* in husked and unhusked rice was recorded in turmeric powder (93.30 %) and neem powder (53.30 %) respectively. Moreover, in husked rice grains, neem and mint powders caused 80.00 % and 63.30 % mortality of *S. oryzae*, respectively at the end of the week. In unhusked rice, 46.70 % and 40.00 % mortality of *S. oryzae* was recorded in mint and turmeric, respectively. No dead weevil was found in the control treatment of both husked and unhusked rice grains at the end of the experiment. Results also indicated that in all the observation intervals (24 h, 48 h, and one week), significantly ($P < 0.05$) higher mortality of *S. oryzae* was recorded in husked rice grains compared to unhusked rice grains.

3.2 Effect of Crude Plant Extracts on the Population Fluctuation of S. oryzae

The application of plant materials also influenced the population build-up of *S. oryzae* in husked and unhusked rice (Figure 1) as relatively higher population build-up was recorded in control husked and unhusked rice treatments. However, significantly less population build-up of *S. oryzae* was observed in both rice grains treated with turmeric and neem powders. After one month, the highest (26.33 ± 1.45 weevils) population was observed in the unhusked rice control treatment, whereas the lowest (7.33±0.33 weevils) population was recorded in husked rice treated with turmeric powder. At two and three months after treatment, steady growth in the *S. oryzae* population was observed in the neem, mint, and turmeric treatments; however, rapid growth was recorded in the control treatment of both rice conditions. At the end of the third month, the highest mean population of *S. oryzae* in husked rice grains was recorded in control (70.67 ± 3.18 weevils), followed by mint treatment (31.33 ± 2.03 weevils). The lowest mean population of *S. oryzae* was recorded in turmeric treatment (18.00 ± 2.65 weevils), followed by neem treatment (28.67 ± 2.03 weevils). Like husked rice, the highest population of weevils was recorded in unhusked control (83.67 ± 3.28 weevils), whereas the population observed in turmeric, mint, and neem treatments was 57.33 ± 2.03 weevils, 47.00 ± 4.16 weevils, and 39.67 ± 2.33 weevils, respectively.

3.3 Weight loss of Unhusked and Husked Rice Grains Mixed with Different Crude Plant Extracts by *S. oryzae*

Similar to the population build-up results, the mean weight loss recorded in husked and unhusked rice grains treated with turmeric and neem powders was significantly lower than the remaining treatments (Table 2). At the end of the experiment, in unhusked rice grains, significantly ($p > 0.05$) highest weight loss was observed in the control treatment (77.33 g or 38.67 %), followed by turmeric treatment (39.67 g or 19.83 %). In husked rice, the highest overall weight loss was recorded in control (65.33 g or 32.67 %), followed by mint (25.33 g or 12.67 %) and neem treatments (18.33 g or 9.17 %).

Table 2. Weight loss of rice grains mixed with plant materials by *S. oryzae* under laboratory conditions

Rice	Plant	Initial weight	Final weight	Grain weight	Overall weight loss	Grain weight loss
Unhusked	Neem	200 g	173.33±2.33c	166.67±2.40b	26.67(13.33 %)	33.33(16.67 %)
	Mint	200 g	168.33±1.76c	162.33±2.03bc	31.67(15.83 %)	37.67(18.83 %)
	Turmeric	200 g	160.33±2.03d	154.33±2.03c	39.67(19.83 %)	45.67(22.83 %)
	Control	200 g	122.67±3.28f	115.67±3.28e	77.33(38.67 %)	84.33(42.17 %)
Husked	Neem	200 g	181.67±1.86ab	175.33±2.19a	18.33(9.17 %)	24.67(12.33 %)
	Mint	200 g	174.67±2.03bc	167.00±2.65b	25.33(12.67 %)	33.00(16.50 %)
	Turmeric	200 g	187.00±0.58a	181.33±0.88a	13.00(6.50 %)	18.67(9.33 %)
	Control	200 g	134.67±4.10e	127.00±4.62d	65.33(32.67 %)	73.00(36.50 %)

*Means followed by the same letters in columns are not significantly different at 0.05 % significance level

Moreover, in unhusked and husked rice grains, the lowest overall weight loss was recorded in neem (26.67 g or 13.33 %) and turmeric (13.00 g or 6.50 %) treatments, respectively.

In continuation to an overall weight loss of grains, the highest grain weight loss of unhusked and husked rice was recorded in their control treatments (84.33 g or 42.17 %) and (73.00 g or 36.50 %), respectively, whereas the lowest grain weight loss was observed in neem treated unhusked rice (33.33 g or 16.67 %) and turmeric treated husked rice (18.67 g or 9.33 %).

4. DISCUSSION

AIt was observed in the study that all the applied plant materials caused significant mortality of *S. oryzae* in both husked and unhusked rice in comparison to control. It has been observed in many previous studies that plant materials have the potential not only to reduce the damage potential of stored grains pests, but many of them have also caused substantial mortality of these pests. The extracts of leaves and seeds of neem, dharek, Melia azedarach, and castor have demonstrated a significant reduction in the feeding and population development of *S. oryzae*, with neem powders exhibiting the lowest weight loss, grain damage, and emergence of adults [23]. Jayakumar *et al.* (2017) also confirmed that variable fumigant and repellent properties of wintergreen, rosemary, lemon, lavender, geranium, eucalyptus, citronella, aniseed, camphor, and vetiver against *S. oryzae* [24]. Another study has also identified that *Annona squamosa* powder has the potential against the populations of *S. oryzae* with 100% death of the targeted individuals. Moreover, leaf powders of *Justicia adhatda*, *A. indica, Carica papaya* and *Ocimum tenuiflorum* also showed potential against *S. oryzae* [25]. Buatone and Indraprichate (2011) also examined the efficacy of the extracts of *Citrus hystrix*, Mentha cordifolia and Hyptis suaveolens against *S. oryzae* in milled rice regarding their insecticidal and repellent properties along with growth prevention and weight loss to grains [26]. Ethanolic extracts of *C. hystrix* showed the highest repellency after 24 h at the concentration of 13.23 mg/ml while, among water extracts, exhibited the highest repellency at 19.04 mg/ml concentration. The suppression of growth ranged from 55 % to 89 %, whereas only 16 % weight loss was recorded after 49 days of the application. The extracts of Myrtus communis, Cymbopogon citrates, *Melia azdarach, Pegnum harmala, Mentha longifolia*, *Diospyros lancifolia,* and *aquilionum* have also shown significant results in reducing the damage of *O. sativa* along with lowering their population development [27, 28].

The results also indicated that *S. oryzae* significantly caused more damage to unhusked rice grains in comparison to husked rice grains, because husk contains a high concentration of silica which may disturb the feeding of *S. oryzae* [29]. Previous studies also reported that plant powders not only affect the feeding of insects but are also capable of blocking the spiracle of insects [30], thus, can lead to suffocation and death of the targeted insects. Rani (2017) also found insecticidal properties of turmeric powder against *S. oryzae* [31] whereas biologically active constituent of Curcuma rhizome was characterized as the sesquiterpene ketone ar-turmerone by spectroscopic analysis that also showed significant insecticidal properties [32]. In a recent study in Indonesia, the application of neem leaf powder at 10 g in rice cause significant mortality of weevils and less weight loss of rice grains [33].

Therefore, in relation to many previous research works, this study also confirmed the potential of turmeric, neem, and mint to protect rice grains either husked or unhusked against *S. oryzae*. However, turmeric was proved more effective against *S. oryzae* in husked rice grains, whereas neem showed the maximum protection of unhusked rice grains. The reasons for such difference in the efficiency of the two tested materials may be that turmeric may have more retention capability in husked rice, thus, providing effective and longer protection in comparison to unhusked rice grains. The feeding behaviour of *S. oryzae* in husked and unhusked rice grains may also have played a significant role in the effectiveness of the various plants. Therefore, turmeric and neem plant powders can be added with rice during storage to lower the population development and losses of *S. oryzae*.

5. CONCLUSION

Although all the tested plant materials showed insecticidal properties against *S. oryzae*, however, turmeric and neem caused relatively higher mortality of weevils in husked and unhusked rice, respectively. The lowest population growth at the end of third month was recorded in turmeric treated husked rice and neem treated unhusked rice grains. Turmeric treated husked rice and neem treated unhusked rice grains exhibited the lowest overall and grain weight loss.

6. CONFLICT OF INTEREST

There is no conflict of interest among authors regarding the publication of this article in this journal.

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Research Article

Assessment of Physicochemical and Bacteriological Parameters of Bottled Drinking Water Marketed in Gilgit City and its Vicinity

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Abstract: This study analyzed physicochemical and bacteriological parameters of packaged water sold in Gilgit city and its vicinity. Samples from source, market, and after the process were collected randomly from three different companies and analyzed from April 2017 to May 2017. Bacteriological parameters include *Escherichia coli* and *Enterococci;* it was further examined with conformity tests. The investigated physical parameters included (pH, Turbidity, Conductivity, Total Dissolved Solids). Some important chemical parameters like Total phosphorous and Total Nitrogen were assessed through a spectrophotometer. The results revealed that the Physicochemical parameters fell within WHO tolerable limits, pH ranged from 7.0 to 8.4, TDS were observed from 260 ppm to 40 ppm, Electrical conductivity was found between 91 µs-510 µs, Turbidity varied from 0.03 NTU to 0.52 NTU. The Total Nitrogen and Total Phosphorus range from 0.09 mg/L to 2.09 mg/L and 95 mg/L to 540 mg/L. The bacteriological parameters were unsatisfactory as some of the samples were contaminated with the *E. coli* and *Enterococci*. The maximum value for *E. coli* was 288 CFU/100 ml, and that of the *Enterococci* was 267 CFU/100 ml, which strongly violates the WHO specifications for bottled drinking water quality. Based on our findings, the Bacteriological examination of some samples is classified under the high-risk category since they are found to be unsafe for drinking. It is recommended there should be strict monitoring and surveillance of bottled water quality; sources should be protected, and awareness should be given to the public regarding its quality.

Keywords: Packaged Water, Water Quality, Contamination, Filtered water, Mineral water.

1. INTRODUCTION

Water is an important nutrient-free from any harmful calories but still a vital part of the diet [1, 2]. It is essential to carry out all the metabolic processes taking place in the living body. A person with a weight of 60 kg must intake 2 liters of water a day [3]. According to WHO valuation, about 1.1 billion people globally drink contaminated water, and the vast case (88 %) of the diarrheal disease reported across the world is credited to unsafe water, sanitation and hygiene.

Furthermore, around 250 million infections each year result in 10–20 million deaths worldwide due to water-borne diseases. These widespread diseases such as cholera, dysentery, and salmonellosis are mainly due to the lack of safe drinking water and inadequate sanitation, resulting in the deaths of millions of people in developing countries every year. Diarrhea is the primary cause of death of more than 2 million people globally, most of whom are children [4].

The principal sources of water in Gilgit and Baltistan are glacier and snow deposits. The water from these glaciers enters streams after melting, which is further used for many purposes such as agriculture, domestic purposes, and livestock. For drinking and cooking purposes, water is generally stored in the pits and wells. The water supply reduces during the winter season due to a decrease in snow and glacier melting rate. This water again replenishes during the summer season [5].

Because of the growing need for safe drinking water, the world's population has begun to use bottled water. Worldwide, it is estimated that about 89 billion liters of bottled water are used each year [6]. Several scientific procedures and tools have

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been developed to analyze water contaminants. pH, turbidity, conductivity, total suspended solids (TSS), total dissolved solids (TDS), total organic carbon (TOC), and heavy metals are among the tests that are performed. These characteristics can influence the quality of drinking water if their levels exceed the acceptable limits set by the World Health Organization (WHO) and other regulatory authorities [7].

In Gilgit city, the production and consumption of bottled drinking water are increasing gradually. However, continuous surveillance or examination of its quality at retail premises is not being carried out. This may lead to the consumption of low-quality packaged water. There has also been a growing concern about the microbiological quality of the products. The current study's main objective is to analyze the physicochemical and bacterial contamination of bottled drinking water marketed in Gilgit city and its vicinity and to check their compliance with the standard.

2. MATERIAL AND METHODS

2.1 Study Sites

There are three bottled water companies named Sujo, Seven Spring, and Vividle, which supply bottled drinking water and currently operate in the Gilgit city Metropolitan area shown in Figure 1.

2.2 Population and Sampling Methods

A random sampling technique was used to collect samples from the respective companies. 4 samples were taken from each company. 1000 ml water sample was taken from the source, after the process, and from the market to evaluate bottled drinking water's physicochemical and bacteriological parameters.

Before sampling, the bottles were autoclaved at 121 °C for 15 min, and caps were appropriately sealed to form no air bubbles inside. The caps were tightened and labeled for identification. These bottles were covered with an aluminum sheet and transferred to plastic bags so that bare hands may not contaminate the water bottles. Samples were transported to the water quality lab within a few hours to investigate the microbiological parameters through membrane filtration technique and chemical contents using a spectrophotometer [7].

2.3 Physicochemical Analysis

The physical parameters included pH, Turbidity, Total dissolved solids, and electrical conductivity were determined through pH meter (AD 1020, ADWA), Turbidimeter (TB1, VELF SCIENTIFICA), Conductivity meter (AD3000, ADWA), respectively. Chemical parameters, such as Total Nitrogen and Total Phosphorus, were

Fig. 1. Map of the study area representing water samples for examination

determined through a spectrophotometer [8].

2.4 Bacteriological Analysis

The microbiological parameters included *E. coli* and *Enterococci*. The Chromogenic (EC X-GLUC Agar) selective media was used to detect Escherichia coli. At the same time, the *Enterococci* were determined by Filtering 100 ml of water from respective samples and culturing it on Slanetz and Bartley agar. The confirmation test for *Enterococci* was done by using Bile Aesculin Azide agar (BEA). The method for bacteriological examination was followed as per the standard procedure for the investigation of water and wastewater [9].

2.5 Statistical Analysis

MS Excel 2010 and Statistix 8.1 analyzed the data acquired from the laboratory.

3. RESULTS

Results of pH were observed in a range of 8.3 to 7.5. The highest pH was observed in the large market sample 8.3. However, the lowest was observed in the source sample, 7.7. The results of EC in assessed samples showed some differences. The value ranged from 496.67 µs to 165 µs. The maximum value of EC in vividle samples was observed in market large 496.76 µs, whereas the minimum value was recorded in samples after process 165 µs. TDS results in the collected samples showed significant fluctuations at different samples that ranged from 250 ppm to 83 ppm. The value was maximum at source sample 250 ppm, and the minimum value lay at market small 83 ppm. The analyzed results of Turbidity ranged between 0.5100 NTU and 0.040 NTU. It is maximum at market small 0.5100 NTU, while the minimum values were recorded 0.0400 at market large. The total phosphorus ranged between 226 µg/L and 120 µg/L. The highest value was observed in market large 226 mg/L, and the minimum value was observed in after process 95 mg/L. Total nitrogen ranged between 1.5300 mg/L to 0.740 mg/L. The highest value of total nitrogen was observed in the source 1.5300 mg/L, while the minimum values were observed in the samples after the process 0.7400 mg/L. There was no *Enterococci* contamination in the samples after the process, market small and market large, but the value was highest in source 255.00 CFU/100 ml. The highest value of *Enterococci* was observed in the source 8 CFU/100 ml, and there was no contamination in samples after the process, market small and market large. Statistics for the water quality parameters are concise in table 1.

Analyzed results of physicochemical and bacteriological parameters of four samples collected from the Seven Spring bottled Water companies and their comparison were shown in table 2. The pH recorded ranged between 7.8 and 8.00. An overall minor amount of differences were recorded in pH results. The two samples of after process and market small showed the same maximum pH values that are 8.00; likewise, another two samples of source and market largely showed the same minimum values of 7.800, respectively.

EC results in assessed samples also showed

Table 1. Physicochemical and Bacteriological results of water analysis from vividle.

	Vividle							
	pH	EC µs	TDS ppm	Turbidity NTU	Total Phosphorus µg/L	Total Nitrogen mg/L	*E. coli* CFU/ 100 ml	*Enterococci* CFU/100 ml
Source	7.7000^{B}	226.00^{B}	250.00^{A}	0.1200^{B}	170.00^{B}	1.5300^{A}	255.00^{A}	8.0000^{A}
After Process	8.2000^{A}	165.00^{C}	132.00^{B}	0.1300^{B}	95.000^{C}	0.7400^{B}	0.0000^{B}	0.0000^{B}
Market Small	7.5000^{C}	206.00^{B}	83.000^{C}	0.5100^{A}	101.00^{C}	0.7400^{B}	0.0000^{B}	0.0000^{B}
Market Large	8.3000^{A}	496.67^{A}	101.00^{C}	0.0400^{C}	226.00^{A}	0.7500^{B}	0.0000^{B}	0.0000^{B}

Note: Source (Point before filter plant), After Process (point after filtration process), Market small (1.5 liters), and Market large (19 liters). Means followed by a different letter(s) in the same column are significantly different from one another at $p \leq 0.05$.

Table 2. Physicochemical and Bacteriological results of water analysis from Seven Spring.

	Seven Spring							
	pH	EC µs	TDS ppm	Turbidity NTU	Total Phosphorus µg/L	Total Nitrogen mg/L	*E. coli* CFU/ 100 ml	*Enterococci* CFU/100 ml
Source	7.8^{A}	101.00^{C}	50.000^{C}	0.3267^{B}	530.00^{A}	1.8300^{A}	20.00^{A}	65.000^{A}
After Process	8.0^{A}	143.00^{B}	111.00 ^{A}	0.1300^{C}	114.00^{D}	0.8800^{C}	0.0000^{B}	0.0000^{B}
Market Small	8.0^{A}	127.40^{B}	70.000^{B}	0.4500^{A}	166.00^{B}	0.9900^{B}	0.0000^{B}	0.0000^{B}
Market Large	7.8^{A}	218.00^{A}	63.800^{BC}	0.2100^{C}	135.00^{C}	0.7900^{D}	0.0000^{B}	0.0000^{B}

Note: Source (Point before filter plant), After Process (point after filtration process), Market small (1.5 liters), and Market large (19 liters). Means followed by a different letter(s) in the same column are significantly different from one another at LSD ≤ 0.05.

big fluctuations at various samples ranging from 218 µs to 101 µs. EC recorded was maximum at market large 218 µs; however, minimum values were recorded in source 101µs. The TDS values also showed fluctuations where the maximum value was 111 ppm in after-process samples, while the minimum value was of source 50 ppm. There were no big fluctuations recorded in the Turbidity. Its value ranged from 0.4500 NTU to 0.1300 NTU. The measured results for Turbidity were maximum at market small 0.4500 NTU. Likewise, the minimum value was 0.1300 NTU after the process.

The total phosphorus of all assessed samples ranged between 530 µg/L to 114 µg/L. The maximum value was 530 µg/L in the source, while the other hand minimum value was 114 µg/L. The values of Total nitrogen fluctuated from point to point and ranged between 1.8300 mg/L to 0.7900 mg/L. It was maximum in source 1.8300 mg/L, and the minimum value was 0.7900 mg/L in market large respectively after the process. *Enterococci* showed a large number of fluctuations in different samples ranging from 0 to 20.00 CFU/100 ml. The *Enterococci* contamination was highest in the source of Seven Spring 20.00 CFU/100 ml while there was no contamination in the after process, market large, and market small samples

In this study, the water samples from Sujo bottled water were assessed for drinking water quality. Results of physicochemical and bacteriological parameters of different samples taken from the Sujo bottled Water Company. There were no big fluctuations recorded in different samples' pH values as it ranged between 8.2 to 7.8. The pH of the assessed samples was maximum at source 8.2, while the three remaining samples showed the same minimum results, that is 7.8. EC values of all the assessed samples ranged from 175 µs to 120 µs. EC was maximum at source 175 µs, and the minimum value was recorded 120 µs in market small. The TDS fluctuations recorded were ranged from 91 ppm to 60 ppm. The maximum value was 91 ppm from the source. However, the minimum value was 60 ppm after the process. Values of Turbidity showed minor variations at different sampling points, ranging from 0.69 NTU to 0.2400 NTU. Turbidity's elevated results were maximum at source 0.69 NTU, and the minimum value recorded was 0.2400 NTU in the market large. Total phosphorus ranged between 422 mg/L to 138 mg/L. The minimum value was 138 mg/L, while the maximum one was 422 mg/L at the source. Total nitrogen also varied at different points. Its value ranged from 1.0900 mg/Lto 0.6400 mg/L. The elevated results for total nitrogen were maximum at the source of 1.0900 mg/L. Likewise; the minimum value was 0.6400 mg/L in the after-process samples. The *E .coli* contamination was highest in the Sujo bottled water company, which ranged from 278 CFU/100 ml to 252 CFU/100 ml. The source was highly contaminated with 278.00 CFU/100 ml. The elevated results for *Enterococci* were maximum at the source 257 CFU/100 ml, but there was no contamination in market small and market large. All the average values of physicochemical and microbiological parameters of Sujo were shown in table 3.

Table 3. Physicochemical and Bacteriological parameters of water analysis in Sujo

Sujo								
	pH	EC µs	TDS ppm	Turbidity NTU	Total Phosphorus µg/L	Total Nitrogen mg/ L	*E. coli* CFU/ 100ml	*Enterococci* CFU/100ml
Source	8.2^{A}	175.00^{A}	91.000^{A}	0.6900^{A}	422.00^{A}	1.0900^{A}	278.00^{A}	257.00^{A}
After Process	7.8^{B}	120.00^{C}	60.000^{B}	0.2800^{C}	138.00^{D}	0.6400^{A}	252.00^{B}	61.000^{B}
Market Small	7.8^{B}	153.00^{B}	76.000^{AB}	0.5300^{B}	200.00^{B}	0.9900^{A}	251.00^{B}	0.0000^{C}
Market Large	7.8^{B}	174.00^{A}	87.000^{A}	0.2400^{D}	178.00^{C}	0.8500^{A}	0.0000^{C}	0.0000^{C}

Note: Source (Point before filter plant), After Process (point after filtration process), Market small (1.5 liters), and Market large (19 liters). Means followed by a different letter(s) in the same column are significantly different from one another at LSD ≤ 0.05.

4. DISCUSSION

Water contamination is influencing the lives of many people all over the world as organic and inorganic contamination, as well as a load of fecal matter in natural water, increases. Drinking water poisoning has been identified as one of Pakistan's most serious public health issues. Our study results obtained by the physicochemical and microbiological analysis were different for different water samples taken from three other bottled water companies. The physicochemical parameters were within the WHO standard guidelines of drinking water quality. Still, one company's bacteriological parameters exceeded the WHO limits despite multiple sample drawl and subsequent assessments.

The pH of water is affected by the presence of CO_2, organic and inorganic solutes in it. In this study, the pH of all companies such as Vividle, Seven Spring, and Sujo showed small fluctuations. The elevated results for pH ranged between 7 to 8.40. pH recorded at Seven Spring market small sample was minimum 7.0 while the maximum value was recorded as 8.40 in the source of Sujo. The pH range was within the limits prescribed by the World Health Organization. This result is partially in agreement with the finding of Biadglegne [10]. in the Amhara region and further supported by Shittu *et al*. [11] in Abeokuta, Nigeria. Similar results were observed by Budhathoki [12] in Nepal and Allam *et al*. [13] in Dhaka. EC (Electrical Conductivity) ranged between 91 µs/cm to 510 µs/cm. None of the samples cross-permissible limit of WHO standards of drinking water. The maximum value was recorded in the source of vividle, which was 510 µs/cm, and the minimum value was 91 µs/cm in the source of seven spring. This result was in-agreement with Sheikh *et al.* [14] in Kashmir Himalaya, Budhathoki [12] in Nepal.

TDS (total dissolved solids) also showed differences and ranged from 40 ppm to 260 ppm, which falls under the WHO permissible limit prescribed for drinking water. The minimum was 40 ppm in the source of Seven Spring, while the maximum was 260 ppm in Vividle. The current result correlates with the research of bottle drinking of Salehi *et al*. [15] in Iran and Sasikaran *et al*. [16] in the Jaffna peninsula. Water that contains TDS more than 100 ppm. High values of TDS affect the hardness, taste, and corrosive property of water [17].

Turbidity elevated from 0.03 NTU to 0.7 NTU. It was maximum in the source of Sujo 0.7 NTU and minimum in the market large of Vividle that was 0.03 NTU scale. None of the values exceeded permissible limits <5 NTU of WHO standards. Our findings are similar to the findings of Bikram [18]. In Tamdalge tank Kolhapur district Maharashtra India where the value of Turbidity increased <5 NTU. While in our study, no sample crossed the permissible limit set by WHO. The results correlate with the findings of Werkneh *et al*. [19] in Ethiopia. Total phosphate was maximum in the source of Seven Spring 540 mg/L, and the minimum was 85 mg/L in the after process sample of Vividle which falls under the category of WHO. Total nitrogen ranged from 0.09 mg/L to 2.0 mg/L, and the value lies within WHO's limits. Our study contradicts the results of Allam *et al*. [13] in Bangladesh. The bacteriological assessment of water establishes its potability of water. The permissible limit of bacteria set by WHO for drinking water quality is 0 CFU/100 ml. The value of *E. coli* ranged from 0 CFU/100 ml to 288 CFU/100 ml. This value exceeds the limits of WHO. The contamination was maximum in the source of Sujo 288 CFU/100 ml and no contamination in the Seven Spring and Vividle after the process and market samples. The current study agrees with Biadglegne *et al*. [20] and Ali *et al*. [21]. Our study contradicts the study of Warburton *et al*. [22] in Canada. The value of *Enterococci* ranged from 0 CFU/100 ml to 267 CFU/100 ml. The maximum value was calculated in the source of Sujo, while some samples were free from contamination. This range exceeds the standards of WHO. This study is in compliance with the study of Budhathoki [12].

5. CONCLUSION AND RECOMMENDATIONS

It is concluded that bottled water is thought to be pure but cannot be relied on blindly. The quality of bottled water was good from a physicochemical aspect. From a bacteriological point of view, 5 samples were contaminated with coliforms, and 7 samples were free from bacterial contamination. It can be concluded that the samples contaminated with coliforms are not fit for human consumption. One reason for contamination may be the inappropriate standard operating procedures peculiar to the container's decontamination and the source water. During the sampling process at the plant, the personnel handling water bottles were devoid of any proper personal protective equipment, especially gloves and masks. Strict rules should be made by the responsible authorities to monitor the bottled water quality regularly. Awareness should be given to the public for either using a disinfectant or boiling water instead of solely relying on bottled water. There should be small microbiological investigative units associated with the bottled water companies.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest.

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Research Article

Promoting Adoption of Water Conservation; Soil Fertility and Health Improving Technologies through Agricultural Service Provision in Pakistan

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Abstract: Agricultural service providers (Agric. SPs) play an essential role in the adoption of promising agricultural technologies by small and medium-sized farms. Similarly, agricultural service provision also generates substantial income for them. The study highlights the role of Agric. SPs in promotion of moisture conservation; soil health and fertility improving technologies at specific sites in Pakistan for three years i.e. from 2015 to 2017. It is based on primary data collected in the year 2018-19 from eighty sampled farmers, which were purposively selected to cover a range of selected technologies. It is found that Agric. SPs-induced adoption of these technologies has increased over time. They achieved considerable success in the promotion of the use of gypsum for moisture conservation and fertilizer placement drill in Pothwar-Punjab, ridge planting of crops in irrigated province Punjab, laser land leveling in irrigated areas of Sindh province, and use of biozote for improving soil fertility in both irrigated and rain-fed areas of Punjab province. These technologies have good income generation potential for Agric. SPs. The article also highlights factors hindering the large-scale adoption of the technologies in the country. Technical and entrepreneurship capacity building of the Agric. SPs in the provision of services to farmers in multiple technologies, and technical feasibility assessment of few technologies to use these for more than one crop are suggested for large-scale adoption of these technologies.

Keywords: Adoption, Agricultural service provision, Moisture conservation, Soil fertility, Soil health

1. INTRODUCTION

Agriculture sector plays a crucial role in the socio-economic development of Pakistan. Accelerated growth in the sector is required to fulfill the requirement of the increasing population for food and agro-based industrial products [1]. As the average size of operational holding is decreasing day by day due to population pressure, therefore, the ultimate way of increasing production is to raise the productivity levels [2]. Crop productivity can be raised through the adoption of promising water conservation; soil fertility and health-improving technologies. As adoption of these technologies makes it possible to obtain higher crop production with low use of costly productive resources. This would also result in increasing income of farmers, input suppliers, small entrepreneurs, and other relevant stakeholders. While, majority of the farming community in the country is constrained by a lack of information, farm machinery, and finances to adopt these technologies [3, 4].

In brief, adoption of moisture conservation, soil fertility, and health-improving technologies could result in saving of precious inputs, higher crop

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productivity and farm income, labour opportunities for rural poor, reduction in poverty, low food prices, improvement in food security, and environmental protection and sustainability [5]. Similarly, a robust and positive effect of agricultural technology adoption on farm household well-being is reported [6].

It suggests that there is a large scope for enhancing the role of agricultural technology in 'directly' contributing to poverty alleviation. In this regard, effective dissemination of appropriate agricultural technologies to end-users is being emphasized [7]. In the same perspective, the role of Agricultural Service Providers (Agric. SPs) in the farming is very important, especially for small and medium land holder farmers, who have limited access to costly agricultural technologies, practices, and inputs. Even farmers who can financially afford to adopt high-cost technologies mostly lack the necessary technical know-how due to a low level of education and ineffectual agricultural extension system. This necessitates the role of well-trained Agric. SPs, equipped with the necessary knowledge, machinery, and farm tools. It is reported by Mehmood *et al.* and Hassan *et al.* that the provision of quality and well-timed services at judicious prices significantly improves agricultural production [8, 9]. Similarly, a positive impact on livelihood and income of Agric. SPs have also been reported [9]. Thus, they can prove to be helping hand for the farmers at their doorsteps in acquiring these technologies. They also provide post-adoption services for the proper functioning of these technologies through the provision of technical guidance, and necessary repair and maintenance services. Thus, their role is very much required at the grassroots level for the development of the sector.

Though many studies have been undertaken on watershed management, adoption of water conservation as well on soil fertility, and health-improving practices; however, only a few of these highlight the role of Agric. SPs in promotion of these technologies. Thus, this study has been undertaken to fill the research gap. In this reference, a cadre of Agric. SPs was developed for dissemination of water and soil-related technologies through USDA/ICARDA funded projects called 'Pakistan water dialogue-Diffusion and adoption through partnerships and action of the best watershed rehabilitation and irrigation practices and technologies to help rural farmers (Watershed Rehabilitation and Irrigation Practice Project)' and 'Dissemination, diffusion, and adoption of the best soil fertility and soil health management practices and technologies for the farmers of Pakistan (Soil Fertility and Health Improvement Project)'. These projects were executed in two phases; Phase-I from 2013 to 2016 and phase-II from 2017 to 2018.

These projects were executed through technical partner institutes/ project collaborators including Pakistan Council of Research in Water Resources (PCRWR), Islamabad and its regional offices in Lahore, Peshwar, Tandojam and Quetta; Pakistan Agricultural Research Council (PARC) and its research establishments including Climate, Energy and Water Research Institute (CEWRI), Agricultural and Biological Engineering Institute (ABEI), and Land Resources Research Institute (LRRI) located in PARC-National Agricultural Research Centre, Islamabad; Barani Agricultural Research Institute (BARI), Chakwal; Soil and Water Conservation Research Institute (SAWCRI), Chakwal; Water Management Research Centre (WMRC), University of Agriculture, Faisalabad (UAF); Soil Fertility Research Institute (SFRI), Lahore; South Asian Conservation Network (SACAN), Lahore; Institute of Water Resources Engineering and Management (IWREM), Mehran University of Engineering and Technology (MUET), Jamshoro, and Agriculture Extension Department, Sakrand, Sindh. Under these projects, a series of training programs were organized by the South Asian Conservation Network (SACAN), Lahore to prepare a force of Agric. SPs throughout the country in selected water conservation and soil health/ fertility improving technologies/ practices.

As such participatory technical skill-enhancing training programs are always helpful for Agric. SPs in understanding practicality of a technology, increase adoption probability, improve their confidence level at farmers' field, and enable them to adopt service provision as a business [10]. In the first phase of the project, Agric. SPs were trained in selected technologies. So that after acquiring the required technical knowledge, they can help technical partners of the project to disseminate information and promote adoption. During the second phase of these projects, SACAN linked trained Agric. SP with private and public sector organizations to facilitate them in delivery of services and thereby develop them as sustainable entrepreneurs by making available subsidy/ credit arrangements for them. During this phase, SACAN also organized skill attainment training for Agric. SPs in 'On-farm soil testing using soil testing kit' in Punjab Province, as it was not included in Phase-I. This study is based on the data for the period (2015-2017), as the adoption of technologies and demand for services of Agric. SPs started after completion of training programs organized by SACAN and the development of demonstration sites by the technical partners in 2013-14. As already stated, the study is targeted to highlight the role of Agric. SPs in the adoption of selected water and soil-related technologies among the farming communities, and determine monetary gains acquired by Agric. SPs through the provision of these services. Specific objectives of the study are: to study adoption of the technologies by the farming community through assistance of Agric. SPs; to determine income generation potential of the services provided by Agric. SPs to farming community; to find out constraints faced by Agric. SPs in promotion of new technology/services; and to inquire Agric. SPs about their perceptions about the future adoption potential of selected technologies.

2. MATERIAL AND METHODS

The study is based on primary data collected through field surveys conducted in years 2018-19 by using a pre-tested questionnaire in all the provinces of Pakistan (Khyber Pakhtunkhwa, Baluchistan, Sindh, and Punjab). A list of the technologies for which services were provided to farmers throughout the country, under both water conservation and soil fertility/health improvement projects are given in Table 1. SACAN, Pakistan organized numerous training programs through which 410 farmers were also trained as Agric. SPs in five selected water conservation technologies, and six soil fertility/ health improvement technologies. Few farmers were trained in more than one technology. Out of these, 178 farmers were trained in drip/bubbler irrigation and gypsum application, 72 in bed planting, 76 in ridge planting of crops, 78 both inland laser leveling and bed planting of crops, 21 in use of zero tillage drill, 34 each both in sales or delivery of biozote and use of wheat fertilizer placement drill, and 12 in use of soil testing kit. A sample of eighty farmers was drawn purposively to cover all these technologies as per details given in Table 1. Mean annual adoptions of the technologies per Agric. SP over three years i.e. 2015-17 has been determined by using expression 1.

$$\frac{Number\ of\ Adoptions}{(Number\ of\ Agric.\ SPs)\times(Timer\ Period\ in\ Years)} \quad (1)$$

Closed-ended questions regarding the constraints in large-scale adoption of the technologies were asked from the sample Agric. SPs. The response was measured according to a 5-point Likert scale, where strongly agree scores 5 and strongly disagree scores 1. A score of 2.5 was considered as the cut-off point i.e. if the mean value of responses to a statement is greater than 2.5 it means that respondents have a positive attitude toward this statement i.e. they agree with it. While,

Table 1. Technologies Promoted by Agric. SPs

S. No.	Moisture Conservation Technologies/ Irrigation Practices	Sample Size	S. No.	Soil Fertility and Health Improvement Technologies	Sample Size
1	Gypsum sales in Pothwar-Punjab	02	1	Fertilizer placement drill in Pothwar-Punjab	01
2	Laser land leveling in Sindh	10	2	Pak Seeder in Rice-wheat zone of Punjab	01
3	Ridge planting of crops in Punjab	12	3	Fertilizer prediction model in Punjab	03
4	Bed planting of crops in Punjab	13	4	Soil testing kit in Punjab	04
5	Drip/Bubbler irrigation in Pothwar-Punjab	14	5	Biozote sales/delivery in Punjab	07
-	-	-	6	Zero tillage drill for wheat in Balochistan	13
	Total	51		Total	29

if the mean value of responses to a statement is less than 2.5, respondents have a negative attitude toward this statement i.e. they disagree with it. Mean scores close to 5 indicate strong agreement while, on the other hand, mean scores close to 1 indicate strong disagreement with a statement. Similarly, sample Agric. SPs were asked about adoption potential of the technologies on basis of a 5-point Likert scale. Data was analyzed through MS Excel for descriptive statistics and graphical presentation of results.

3. RESULTS

Agricultural Services Providers' induced adoptions of various moisture conservation/ irrigation practices as well as, soil fertility and soil health improvement technologies are presented in detail in Table 2. During the period (2015-17), a mean number of adoptions per Agric. SP per annum was highest for use of gypsum for moisture conservation in rain-fed areas of Pothwar-Punjab (39.3), followed by ridge planting of crops in irrigated areas of Punjab (4.0), and land laser leveling in irrigated areas of Sindh (2.2). Findings of use of gypsum are in line with that of [11], as they validated through on-farm participatory research in the rainfed-Pothwar, that gypsum is effective in moisture conservation and results in higher wheat productivity. They reported considerable improvement in adoption by developing linkages of farmers, input dealers/ Agric. SPs and gypsum suppliers. Moreover, it is comparatively a low-cost technology. While adoptions of bed planting of crops in irrigated areas of Punjab (0.8) and drip/bubbler irrigation (0.2) in Pothwar-Punjab through Agric. SPs were much low. The results are in line with [10], they reported that despite efforts made for a long time, drip irrigation technology could not get much popularity in rain-fed areas of Punjab due to the non-availability of installation services; its repair, and maintenance. They stressed that it is capital-intensive technology and need above-average returns on investment to pay it back. Thus to achieve high returns, it was emphasized that farmers should be inculcated to shift from subsistence to high-value crop farming. Furthermore, the use of quality material matters much to prolong the operational life of drip irrigation systems. It cuts down the cost of production and increases profitability [12].

In the case of soil fertility and soil health-improving technologies, fertilizer placement drill for wheat in Pothwar was most promising with the highest mean number of services (28.7) and mean adoption area from the year 2015-17 (18.0 acre), followed by biozote sales/ delivery (5.6). The positive impact of the use of fertilizer placement drills on wheat productivity has been reported by [13]. Many researchers including [14, 15] reported that the use of biozote resulted in better seed germination and healthier crops of wheat and

Table 2. Adoption of technologies by farmers through Agric. SPs

Technologies/Practices	Agric. SPs (No.)	Total Adoptions (2015-17)		Annual Mean per Agric. SP		Mean Area per Adoption (Acre)
		Farmers (No.)	Area (Acre)	Farmers (No.)	Area (Acre)	
Moisture Conservation/ Irrigation Practices						
Gypsum sales/delivery	02	236	392	39.3	65.3	1.7
Laser land leveling	10	66	385	2.2	12.8	5.8
Ridge planting	12	114	735	4.0	20.4	5.1
Bed planting	13	33	188	0.8	4.8	5.7
Drip/Bubbler	14	09	71	0.2	1.7	7.9
Total	51	488	1771	3.2	11.6	3.6
Soil Fertility and Soil Health Improving Technologies						
Fertilizer placement drill	01	86	1552	28.7	517.3	18.0
Pak Seeder	01	03	48	1.0	16.0	16.0
Biozote sales/delivery	07	118	489	5.6	23.3	4.1
Zero tillage drill	13	12	99	0.3	2.5	8.3
Total	22	219	2186	3.3	33.2	10.0
Fertilizer prediction model*	03	126	367	42.0	122.3	2.9
Soil testing kit*	04	10	11	2.5	2.8	1.1
Total	07	136	378	19.4	54.0	2.8

Source: Field Survey 2018, Pakistan *Data just for the year 2017 rice. However, they stated that the main hurdles in large-scale adoption of the technology are farmers' unawareness about the benefits of the technology, their low educational level, and its unavailability in local markets in Punjab province. Similarly, Agric. SPs' inability to maintain a cold chain in the delivery may result in the non-effectiveness of the product. They [15] stressed that input dealers/ Agric. SPs, local representatives, technical and extension institutions should be engaged in the commercialization of the technology. The fertilizer prediction model, and soil testing kit were in the first year of diffusion and adoption in the year 2017. The fertilizer prediction model also provided good business to Agric. SPs with 42 services per Agric. SP in just one year of business.

An increasing trend in the mean number of adoptions per Agric. SP for all the technologies over time has been observed. In the first two years of the second phase of projects (2015 and 2016). The adoption was quite low, as diffusion (dissemination of the information) of the technologies and capacity building of Agric. SPs through training was the prime focus of project activities (Table 3). Though adoption of the technologies gained a little hike in the year 2016. In the third year (2017), the institute that was responsible for the capacity building of Agric. SPs i.e. SACAN, Lahore, along with technical partner institutions put maximum efforts into the promotion of technologies. Consequently, a considerable number of adoptions of all the technologies were achieved. In the year 2017, the number of adoptions for soil moisture conservation technologies increased by about four folds and, that of soil fertility/ health-improving technologies by two and a half folds than that in the year 2015. Similarly, due to a consistent increase in the number of adoption, the area on which technologies were adopted by the farmers through services provided by Agric. SPs also increased over time (Table 4), specifically in the third year.

During the study period (2015-17), in the case of watershed rehabilitation technologies, the highest change in adoption rates by number (24.5 times) and area (58.0 times) were observed for laser land leveling in Sindh province over that of the year 2015, and among the soil fertility and soil health-improving technologies, the highest change in adoption rate occurred for zero tillage drill in Balochistan province (9.0 times by number and 43.5 times by area over that of the year 2015).

On the whole, the area under the adoption of watershed rehabilitation technologies increased by six-folds, and that of soil fertility and soil health-improving technologies doubled during 2015 to 2017. Though, Pak-seeder for sowing wheat crop in the rice-wheat zone of Punjab could not gain much popularity and was adopted on a limited area, a registered increase of just 1.4 times over that of the year 2016. Similarly, changes in gypsum application area in Pothwar, ridge planting, and bed planting of crops in Punjab were comparatively small as compared to that in the year 2015 viz. 3.7, 4.5, and 4.6 times, respectively. Similarly, areas under application of biozote, and fertilizer placement drill

Table 3. Adoption rate of technologies

Technologies/Practices	**Mean adoptions per Agric. SP (No.)**			**Change over 2015**	
	2015	**2016**	**2017**	**No. per year**	**No. of times**
Best Watershed Rehabilitation and Irrigation Practices					
Gypsum sales/delivery	45	80	111	33.0	2.5
Laser land leveling	2	15	49	23.5	24.5
Ridge planting	14	35	65	25.5	4.6
Bed planting	4	11	18	7.0	4.5
Drip/Bubbler	1	3	5	2.0	5.0
All	66	144	248	91.0	3.8
Soil Fertility and Health Improvement					
Fertilizer placement drill	20	23	43	11.5	2.2
Pak Seeder	-	1	3	1.5*	3.0*
Biozote sales/delivery	20	48	50	15.0	2.5
Zero tillage drill	1	2	9	4.0	9.0
All	41	74	105	32.0	2.6

Source: Field Survey 2018, Pakistan Over year 2016

Table 4. Adoption area of technologies

Technologies/Practices	Mean Area per Agric. SP (acre)			Change over 2015	
	2015	2016	2017	Area per year	No. of times
Best Watershed Rehabilitation and Irrigation Practices					
Gypsum sales/delivery	51	154	187	68.0	3.7
Laser land leveling	5	90	290	142.5	58.0
Ridge planting	92	228	415	161.5	4.5
Bed planting	23	59	106	41.5	4.6
Drip/Bubbler	4	24	43	19.5	10.8
All	175	555	1041	433.0	5.9
Soil Fertility and Health Improvement					
Fertilizer placement drill	400	501	650	125.0	1.6
Pak Seeder	-	20	28	8.0*	1.4*
Biozote sales/delivery	88	198	203	57.5	2.3
Zero tillage drill	2	10	87	42.5	43.5
All	490	729	968	239.0	2.0

Source: Field Survey 2018, Pakistan * Over year 2016

Table 5. Income of Agric. SPs through the provision of farm service in the year 2017 (Percent)

Technologies	Income Ranges (Rs.)					
	Nil	1000-50000	50001-100000	100001-150000	150001-200000	above 200000
Drip/Bubbler Irrigation	14	0	0	14	29	43
Gypsum	0	50	0	0	0	50
Bed Planter	7	31	31	23	8	0
Ridge Planter	0	0	42	25	8	25
Laser Land Leveler	0	0	30	20	10	40
Biozote Sales/Delivery	14	58	0	0	14	14
Pak Seeder	100	0	0	0	0	0
Zero Tillage	23	39	23	15	0	0
Fertilizer Prediction Model	0	100	0	0	0	0
Soil Testing Kit	0	75	0	0	0	25
Fertilizer Placement Drill	14	0	0	14	29	43

Source: Field Survey 2018, Pakistan

in Punjab province could not expand to great extent viz. just by 2.3 and 1.6 times over that is year 2015, respectively.

Income earned by Agric. SPs through the provision of farm services to promote selected technologies under both projects in the year 2017 are categorized into different ranges in Table 5. Water-saving technologies, drip/bubbler irrigation, gypsum sales/delivery, and laser land leveling have good income generation potential, as income of more than forty percent of the sample Agric. SP was more than Rs. 200,000. Likewise, services in bed planting and ridge planting generated considerable returns as near about one-third Agric. SPs (31 % in case bed planting and 42 % in case of ridge planting) obtained income in the range of Rs. 50,001 to Rs. 100,000. These services have comparatively low-income potential compared to others, as these are seasonal in nature and limited only to crop sowing seasons. However, multi-crop ridge/ bed planters can be introduced through Agric. SP, these may result in better returns for them.

In soil fertility improving technologies, services in fertilizer placement drill has good income generating potential, as it generated income above Rs. 200,000 for 43 % Agric. SPs in the year 2017. Biozote sales/delivery, zero tillage, and fertilizer prediction model generated a low level of income i.e. Rs. 1,000 to Rs. 50,000 for most of the Agric. SPs (39 % in zero tillage drill, 58 % in biozote sales/ delivery and 100 % in fertilizer prediction model). Twenty-three percent of Agric. SP giving services of zero-tillage drill in Balochistan reported providing free of cost services to their fellow farmers due to their cultural norms and values. As they promoted the technology free of cost for the welfare of their community. Similarly, in the case of Pak Seeder in the rice-wheat cropping zone of Punjab, Agric. SPs reported providing services to their neighboring farmers without charging payments. Agric. SPs providing services for two or more technologies have more chances to earn a substantial income. Thus, their success in the business can be increased through capacity building by enhancing skills in effective entrepreneurship management to seek business in two or more technologies.

Income earned by Agric. SP through the provision of farm services in the year 2017, irrespective of technology types has been categorized in Figure 1. One-fifth of Agric. SPs' earned income above Rs. 200,000. This is quite encouraging, as subsistence farmers (having land holding up to 2 ha) in the country earn income usually in the range of Rs. 200,000 to 300,000 annually. In total, forty-six percent of the Agric. SPs reported to earn income above Rs. 100,000 for the provision of farm services. Thus, service provision to fellow farmers by Agric. SPs have substantial income generation potential and help promote promising water-saving; soil fertility, and health-improving technologies/ practices in the country.

Constraints in promotion of technologies faced by Agric. SPs in the context of crop farmers' characteristics in the country are presented in Table 6. Lack of awareness in the farming community about promising water-saving; soil fertility and health-improving technologies, lack field experience, and hands-on training of Agric. SPs and their inability to cover farmers' demand specifically for technologies having seasonal nature of service are major constraints in the wider adoption of selected technologies.

While other factors, like lack of resources to invest in farming, social/cultural non-acceptance of technologies, traditional attitude, and small land holdings of farmers also hinder large-scale adoption. Mean of the sample Agric. SPs' Likert-scale responses by technology is four and indicate that adoptions of drip/ bubbler irrigation, bed planting of crops, use of fertilizer placement drill, and Pak Seeder are constrained much due to these farming issues than other selected technologies.

Moreover, constraints for wider adoption of individual technologies also vary. In case of gypsum and biozote (sales/ delivery); ridge planting and zero tillage drill inability of Agric. SPs to cover farmers' demand is a major constraint in large scale adoption. In the case of fertilizer placement drill; lack of awareness, limitation on resources to invest, and social/ cultural non-acceptance are main constraints in adoption. While, adoption of Pak-seeder is much limited, mainly due to small land holdings and lack of field expertise on the part of service providers. The use of fertilizer prediction models and soil testing kits is constrained by a lack of awareness. Adoption of laser land leveling technology is not gaining impetus for large-scale adoption due to a dearth of financial resources to invest on the part of Agric. SPs as well as farmers, and lack of expertise by Agric. SPs in service provision. Adoption of drip/ bubbler irrigation and bed planting of crops is constrained by all the factors listed in Table 6. According to Agric. SPs perceptions, ridge planting, laser land leveling, Pak Seeder, zero tillage drill, fertilizer prediction model, and fertilizer placement drill have great adoption potential. In the case of gypsum delivery/ sales and soil testing kit technology, one-half of the respondents expressed high optimism while

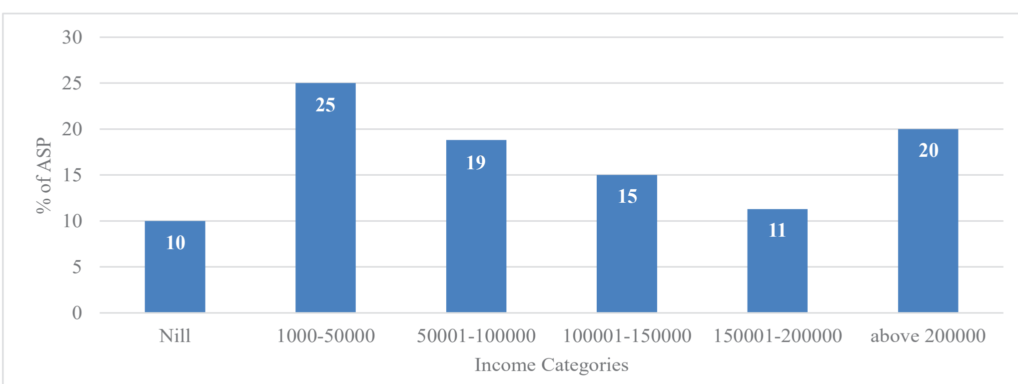

Fig. 1. Income earned by Agric. SPs through farm services Source: Field Survey 2018, Pakistan

Table 6. Constraints in promotion of technologies

Technologies	Lack of awareness	Lack of resources to invest	Sociocultural non-acceptance	Traditional attitude	Small land holdings	Lack field experience and hands on training	Inability to cover demand	Average
Drip/Bubbler Irrigation	4	4	4	4	4	4	4	4
Gypsum Sales/Delivery	3	2	2	2	2	2	4	2
Bed Planter	4	4	4	4	4	4	4	4
Ridge Planter	3	3	2	2	2	4	4	3
Fertilizer Placement Drill	5	5	5	3	3	3	3	4
Biozote Sales/Delivery	5	2	3	2	2	3	4	3
Pak Seeder	4	3	3	3	4	4	4	4
Zero Tillage	4	3	3	3	2	3	4	3
Fertilizer Prediction Model	5	3	3	3	3	3	3	3
Soil Testing Kit	4	3	3	4	3	3	4	3
Laser/ land leveler	2	4	1	2	2	4	3	3
Average	4	3	3	3	3	4	4	-

the other half were highly affirmative about the adoption potential of these technologies. In the case of drip/bubbler irrigation half of the Agric. SPs (50 %) strongly agreed, more than one-third (36 %) agreed that the technology has the potential to be adopted on a large scale, while the remaining (14 %) were uncertain about the adoption potential of the technology. About one-third of Agric. SPs (62 %), providing services for bed planting of crops (rice and wheat) strongly agreed about the up-scaling potential of the technology, eight percent each was agreed and disagreed with the adoption potential of the technology, and twenty-two percent were uncertain about further adoption prospects of the technology. In biozote supply/sales more than half (57 %) strongly agreed, 28 % disagreed and 15 % were uncertain about the wider adoption of the technology.

4. DISCUSSION

It has been declared that the participatory skill development of farmers is a necessary condition for the promotion of water conservation and soil fertility-related technologies. In this regard, the necessary condition has been fulfilled in the country. However, sufficient condition needs to be fulfilled by further follow-up by technical institutions [10]. Thus, the linkage between Agric. SPs and technical institutions promoting these technologies are required to be further developed and strengthened. Similarly, strengthening agriculture research institutes and extension services have been suggested to improve the technical knowledge of service providers [9].

Thus, both horizontal and vertical coordination is stressed, as collaboration among Agric. SPs themselves, allows access to technology at lower cost. While, cooperation of Agric. SPs-technical partners/ collaborators including technology suppliers in the private sector tends to reduce opportunistic behavior and market uncertainties [16]. In this reference, the imposition of a cap on rental charges by the government through a regulation mechanism has also been suggested [8]. In this way Agric. SPs can play a crucial role in knowledge dissemination and field demonstration, as well as in farmers' participatory research and training programs to popularize these technologies and enhance their adoption.

Agric. SPs reported barriers to the sustainability of work are low adoption rates, lack of public support, and enterprise-specific obstacles. Some of them have a plan for long-term sustainability such as training others or continuing to provide services without any type of support. They cited personal benefits such as self-improvement i.e. job experience and more earning, personal interest in the work being done, or seeing the community adopt new technologies. While, few researchers, including [17] also stressed improving the education status of the farming community to develop their behavior to augment the adoption of approved scientific technologies, enhance production and sustain food security in the long run. Similarly, the development of human capital characteristics for the adoption of sustainable agricultural practices is also emphasized [18, 19]. As it is stated that human capital-specific characteristics and entrepreneurial behaviour have a significant positive effect on the adoption of crop production technologies, specifically at small scale farms [18]. Thus, these human capital characteristics and coordination among stakeholders should be the prime focus, while devising programs to enhance the adoption of water conservation; soil fertility, and health-improving technologies. It is suggested to adopt the local service providers (LSP) model by involving local support organizations (LSOs) for agricultural advisory and related services. In the LSP model, local actors (farmers, business owners, breeders, etc.) are trained to provide services (knowledge, technology, training, etc.) to fellow farmers. Intangible benefits of the model are related to increased awareness and self-confidence of farmers to interact with Agric. SPs, and also in their problem-solving activities. This may enable farmers to express their technology needs and participate in designing, testing, and disseminating appropriate technologies. The system helps poor farmers being unable to afford inputs and services to put the advice into practice [20]. The most appropriate scale to adopt the model in the country is recommended at the Union Council level [8].

Similarly, it is reported that social organizations provided good quality extension and education-related services to the rural communities [21]. It is said that farmers rate LSO extension services providers high because they are accountable for the services they provide [22]. The increasing role of Information Communication Technologies (ICTs), especially mobile phones and the internet in this reference should also be considered [23]. To institutionalize the private sector for agricultural service provision on the model of metro cab system should also be envisaged. Where farmers can have better access to services well in time, and Agric. SPs may have more business. An added advantage of the system is an opportunity for the farmers to rate the services of Agric. SPs. Farmers' ability to get group deals to acquire services from Agric. SPs on low rates is another possibility. In this reference, a commission from helping farmers acquire the input/ services they need than having farmers pay for them seems a model with higher potential for financial sustainability [20]. It is always advisable to start with simple activities, having high chances of success, and quickly demonstrate success. Finally, as farmers' needs are constantly changing with time and socioeconomic attributes; thus, Agric. SPs need to periodically upgrade in knowledge, skills and attributes to keep pace with emerging challenges and dynamics of service provision.

5. CONCLUSION

Agriculture service providers (Agric. SPs) have contributed significantly to the dissemination and adoption of promising water-saving; soil fertility and health-improving technologies under USDA-ICARDA initiatives in the country. Demand for their services is increasing with time. Even, for a few technologies Agric. SPs are unable to meet the service demand of the farming community. Agric. SPs-induced adoption of selected technologies has sensitized the farming community about the economic importance of the adoption. Which will boost the adoption of these technologies in near future. Considerable adoptions through services of Agric. SPs are reported for use of gypsum for moisture conservation, biozote application for wheat and rice crops sales, fertilizer placement drill for wheat, and laser land leveling. Fertilizer placement drill and ridge sowing of wheat in Sindh was the most promising technologies and are adopted on a large area. Many socioeconomic factors affect the large-scale adoption of these technologies. Lack of awareness in the farming community about promising water-saving; soil fertility and health-improving technologies, lack field experience and hands-on training of Agric. SPs and their inability to cover farmers' demands well in time are major constraints in the large-scale adoption of selected technologies. Agric. SPs perceive that ridge planting, laser land leveling, Pak/ Happy Seeder for wheat, zero tillage drill for wheat, fertilizer prediction model, and fertilizer placement drill for wheat have great adoption potential in the country. Similarly, economic returns to sustain the livelihood of Agric. SPs are much essential to improve the delivery of services to the farming community. There is a need to technically access feasibility to use a few of the technologies for multiple crops like ridge and bed planters. The adeptness of Agric. SPs in service delivery can be enhanced through their capacity building by improving skills in effective entrepreneurship management, seeking more sales and services, provision of after-sale repair and maintenance services, and by seeking business in multiple technologies.

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7. CONFLICT OF INTEREST

There is no conflict of interest among the authors in the findings of the study and description of results.

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Obituary

Dr. Abdul Quadeer Khan (N.I. & Bar, H.I.) (1936-2021)

It is with profound grief to inform all colleagues, friends, associates and well wishers that Dr. Abdul Quadeer Khan (N.I. & Bar, H.I.), Fellow of the Pakistan Academy of Sciences (PAS), a versatile metallurgist and nuclear scientist of Pakistan and Founding Chairman, Dr. A.Q. Khan Research Laboratories, Kahuta, breathed his last on October 10, 2021 at the KRL Hospital, Islamabad. His funeral was held in the Faisal Mosque, Islamabad and he was buried in H-8 Graveyard, Islamabad. As one of the most highly decorated scientist of Pakistan, Dr. Abdul Quadeer Khan has left behind a rich harvest of sweet memories and marvelous achievements as inspiration for all those who know him and for the generations to come. The nation will always remember him as Mohsin-e-Pakistan.

Dr. Abdul Quadeer Khan was born on 1st April 1936 in Bhopal, British India. In 1952, he migrated to Pakistan along with his parents and resided in Karachi. In Islamabad, he was residing in his own residence located along Hillside Road. He received his early education in Karachi. He graduated from University of Karachi: B.Sc., 1960; Technical University, Berlin, West Germany: Metallurgical Engineering Courses, 1962-1963; Delft Technological University, Delft, Holland: M.Sc., 1967; University of Leuven, Belgium: and Doctor of Engineering, 1972. His main areas of research included Metallurgy, Materials Science, Nuclear Technology with keen interest in Physical Metallurgy, especially Structure-Property Relationships in High Strength Alloys, Ballistic Missiles and Enrichment Technologies.

In 1976, on the request of Late Zulfiqar Ali Bhutto, the then Prime Minister of Pakistan, Dr. Abdul Quadeer Khan came to Pakistan and laid the foundation stone for Pakistan's Nuclear Programme. Since then, the scientific career of this distinguished scientist spans over 30 years of government service, during which he held many important positions such as Project Director, Dr. A. Q. Khan Research Labs, Kahuta, Pakistan, 1976-1998; and Chairman, Dr. A. Q. Khan Research Labs, Kahuta, Pakistan, 1998-2001. He also held very prestigious positions of Special Advisor to the Prime Minister on Strategic Program, KRL Affairs, 2001-2004; President, Pakistan Academy of Sciences, 1997-2002; Project Director, GIK Institute of Engineering Sciences & Technology, Topi, Pakistan, 1990-2002; Chief Coordinator for Guided Missile Program, 1990-2000; Senior Metallurgist/Deputy Manager, FDO Eng. Consultants, Amsterdam, Holland, 1972-1975; Research Fellow, Leuven University, Belgium, 1968-1972; Research Assistant to Professor Burgers at Delft Technological University, Holland, 1967-1968; Inspector of Weights & Measures, Govt. of Pakistan, 1959-1961; Practical Trainee with Siemens Pakistan Eng., 1958-1959; Tech. Asst., Star Eng. Works, Karachi, Pakistan, 1956-1958.

Dr. Abdul Quadeer Khan had a long and distinguished career in the field of metallurgy and allied disciplines. He was highly renowned for his pioneering research contributions in the field of uranium enrichment using the versatile high speed gas centrifuge technique in Pakistan. He played a pivotal role in Pakistan's nuclear Programme. The establishment of Kahuta Research Laboratories in fact speaks of his rank in the nuclear science arena and which lead to the 28 May, 1998 Nuclear Explosions by Pakistan in response to Indian nuclear tests. His sole efforts indeed paved the way for Pakistan to become the 7th nuclear state in the entire World and the first nuclear state in the

Muslim World.

In recognition of his outstanding contributions in the field of science and technology, Dr. Abdul Quadeer Khan received several prestigious civil awards, Nishan-i-Imtiaz and Bar, Govt. of Pakistan, 1996, 1998; Hilal-i-Imtiaz, Govt. of Pakistan, 1989; as well as Academic Awards in the form of Honorary Degrees such as: D.Sc., NED University of Engineering & Technology, 2014; D.Sc., M.A. Jinnah University, Karachi, 2012; D.Sc., University of Karachi, 1993; D.Sc., Baqai Medical University, Karachi, 1998; D.Sc., Hamdard University, Karachi, 1999; D.Sc., Gomal University, D.I. Khan, 1999; D.Sc., University of Engineering & Technology, Lahore, 2000; D.Sc., Sir Syed University, Karachi, 2001; D.Sc., Baluchistan University, Quetta, 2003; He was also a distinguished Alumnus of the famous Technical University of Berlin, Germany; the Delft University of Technology, The Netherlands; and University of Leuven, Belgium; During his scientific career, he was awarded with 66 Gold Medals and 3 Gold Crowns from various National Organizations; He is the only Pakistani who has been acclaimed as Mohsin-e-Pakistan by the whole nation. A large number of Institutes, Auditoriums and Research Centers have been named after Dr. A. Q. Khan within Pakistan and abroad.

Dr. Abdul Quadeer Khan was also honoured with membership of various outstanding national and international bodies, including Fellow: Pakistan Academy of Sciences, Islamic World Academy of Sciences, Kazakh National Academy of Sciences, Korean Academy of Sciences, Pakistan Institute of Metallurgical Engineers, Pakistan Institute of Engineers, Institute of Central and West Asian Studies; Chartered Engineer and Member, The Institute of Materials, London; Member, American Society of Metals (ASM), The Metallurgical Society of the American Institute of Mining, Metallurgical and Petroleum Engineers, Canadian Institute of Metals (CIM), Japan Institute of Metals (JIM).

Dr. Abdul Quadeer Khan was elected as Fellow of the Pakistan Academy of Sciences in 1988. Throughout his association with the Academy, Dr. Abdul Quadeer Khan highly supported for the build-up and upgradation of the Pakistan Academy of Sciences. During his six years' tenure as President - Pakistan Academy of Sciences from 1997-2002, he significantly facilitated for renovation of the Academy Secretariat Building as well as creation of a versatile Auditorium, Fellows Lodge, and Garages/Transport Parking within the Academy premises. He also facilitated provision of significant amount of Rupees 20 Million as Endowment Fund to the Academy for sustainability of its scientific and academic work.

Throughout his scientific career, Dr. Abdul Quadeer Khan remained a strong source of encouragement and support for his fellow colleagues, the associated scientific community including scientists, engineers and researchers in strategic scientific institutions as well as students in universities, colleges and schools and above all the common masses. His effective and efficient management enabled Pakistan to achieve wonders in the field of defense and cutting-end technology for scientific and academic purposes. His intellectual contributions to Pakistan were enumerable and his determination unmatched. He will always be remembered for his deeply professional attitude, problem solving capabilities, organizational and management skills, excellent judgment and calm good cheer. As a towering national hero, Dr. Khan has left us and the nation with deep sorrow and grief. There are no words to describe how much he will be missed by the nation. Our thoughts and heartfelt condolences go out to the family, friends and colleagues around the world. May ALLAH (SWT) give strength to his family, dears and nears to bear this great and irreparable loss and reward Dr. Khan for his services to the nation and bless him the highest place in Jinnah (Aamin).

On behalf of Pakistan Academy of Sciences, Islamabad

Instructions for Authors

Manuscript Format

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3. R.K. Robert, and C.R.L.Thompson. Forming patterns in development without morphogen gradients: differentiation and sorting. *Cold Spring Harbor Perspectives in Biology* 1(6) (2009).
4. D. Fravel. Commercialization and implementation of biocontrol. *Annual Reviews of Phytopathology* 43: 337359 (2005).

b. Books

5. W.R. Luellen. Fine-Tuning Your Writing. *Wise Owl Publishing Company, Madison, WI, USA* (2001).
6. U. Alon, and D.N. Wegner (Ed.). An Introduction to Systems Biology: Design Principles of Biological Circuits. *Chapman & Hall/CRC, Boca Raton, FL, USA* (2006).

c. Book Chapters

7. M.S. Sarnthein, and J.D. Stanford. Basal sauropodomorpha: historical and recent phylogenetic developments. In: The Northern North Atlantic: A Changing Environment. P.R. Schafer, & W. Schluter (Ed.), *Springer, Berlin, Germany*, pp. 365–410 (2000).
8. J.E. Smolen, and L.A. Boxer. Functions of Europhiles. In: Hematology, 4th ed. W.J. Williams., E. Butler and M.A. Litchman (Ed.), *McGraw Hill, New York, USA*, pp. 103–101 (1991).

d. Reports

9. M.D. Sobsey, and F.K. Pfaender. Evaluation of the H2S method for Detection of Fecal Contamination of Drinking Water, Report WHO/SDE/WSH/02.08, *Water Sanitation and Health Programme, WHO, Geneva, Switzerland* (2002).

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