Journal of Physical, Biomedical and Biological Sciences
eISSN: 3005-6489
www.jpbab.com
https://doi.org/10.64013/jpbab.v2026i1.60
*J. Phys. Biomed. Biol. Sci., 2026; Volume, **5**: 60*
Original Research Article **Open Access**

MULTIVARIATE EVALUATION OF AGRONOMIC AND BIOMASS TRAITS IN WILD POPULATIONS OF *AMARANTHUS VIRIDIS* L.: IMPLICATIONS FOR POTENTIAL DOMESTICATION

KHALID MAR*, HASSAN MU

Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab, P.O. Box. 54590, Lahore, Pakistan
**Correspondence Author Email Address: m.ar.khalidofficial@gmail.com*

(Received, 16th January 2026, Accepted 18th June 2026, Published 27th June 2026)

Abstract *As an underutilized C4 pseudo-cereal and leafy germplasm, Amaranthus viridis L. is a nutritionally rich "superfood" with remarkable medicinal properties. Although it has been used globally throughout human history, it also frequently grows as a weed in major agricultural belts like the rice belt or Kalar track, such as in Pakistan, including areas of the districts Sialkot, Narowal, Sheikhupura, and Hafizabad. Amaranthus viridis L. poses a hazard to rice crops by competing for critical resources such as water, minerals, and nutrients. Crop yields decline as a result of this competition. A planned study was carried out to investigate the traits of Amaranthus viridis L. in three separate locations with various settings in order to address the problem. The findings demonstrated a significant relationship between characteristics such as shoot length, root length, inflorescence length, plant fresh weight, plant dry weight, moisture content, leaf length, leaf width, and leaf area. Conclusively, location 3 was found to be a favorable setting for Amaranthus viridis L. plants to thrive and develop vigorously. It is highly advised to acknowledge its production for future agronomic selection and domestication strategies over the desert regions and barren lands due to its soil-enhancing, resistant, medicinal, and nutritional benefits to ensure the utilization of nature's blessing for the welfare of mankind in all possible ways.*

Keywords: *Amaranthus Viridis; Morphological Traits; Multivariate Analysis; Correlation; Heritability; Genetic Advance; Biplot*

Introduction

Amaranth (*Amaranthus* spp.), a traditional dual-purpose plant that is valued as both food and medicine, has been a staple food and cultural symbol since pre-Columbian times, with significance comparable to that of maize and beans. This resistant crop has C4 photosynthetic efficiency, which enables it to grow in harsh environments, mature shortly, be disease-resistant, and adapt widely. It is referred to as a "poor man's vegetable" and "superfood" because of its low cost and high nutritional content, and it exhibits a variety of pharmacological activities that provide a plethora of health benefits. Additionally, amaranth is considered one of the 36 most promising crops in the world (Council, 1984) because it has the potential to mitigate world hunger (Jin et al., 2025). *Amaranthus viridis L.*, also known as green amaranth, is an annual herb that is classified as part of the Amaranthaceae family. 'Slender Amaranth' in English, 'Jangli Chaulai' in Urdu, 'Choloi' in Hindi, 'Marrissag' in Bengali, and 'Zhou Guo Xian' in Chinese are the common names for the Amaranthus viridis L. Although Amaranthus viridis possibly originated in Asia, it is currently a weed found worldwide in tropical and subtropical areas and is as common in places distant from temperate zones, such as those in Australia, Asia, North America, and Europe. It is also a significant and widespread weed in equatorial Africa. In some places, such as Nigeria, Gabon, and the Democratic Republic of the Congo, it is grown (Reyad-ul-Ferdous et al., 2015).

Amaranthus viridis L. can reach a height of one meter and is an erect or ascending herb that can also infrequently persist as a short-lived perennial. The stems have branching patterns and are angular and smooth, and may occasionally have hairs on the top portion. The petioles of *Amaranthus viridis L.*, alternating leaves, which are up to 10 cm long, are located along the stem. The leaf blades range in size from 2 to 8 cm in length and 1.5 to 6 cm in width, with a deltoid-ovate to rhomboid-oblong form. The apex is emarginated with a little mucro, and the leaf edges may appear sinuate. The short cuneate is the base of the leaves. Depending on the plant, the leaves may have a smooth surface, be pubescent, or be covered in hairs. *Amaranthus viridis* is characterized by thin clusters of axillary or primarily terminal spikes that are frequently paniculated and can grow up to 12 cm in length. In the lowest portion of the stem, these spikes may form profiled axillary clusters that are about 7 mm in diameter. This plant produces green, unisexual, subsessile blooms. Around them are bracteoles and lanceolate-ovate bracts. There are usually more female blossoms, even when male and female flowers coexist. Flower bracteoles are oblong in shape. Obovate in shape, with a length of about 1 mm. The bracteoles of flowers, on the other hand, are somewhat longer, measuring between 1 and 1.5 mm, and they resemble white membranes. The subglobose capsule that *Amaranthus viridis* develops is about 1.5 mm in diameter and is either the same size as or slightly bigger than the perianth. There is only one seed inside the capsule, which is subglobose, somewhat flattened, shiny black, and about 1 mm in diameter. The seed's border is sharp, and it could have a rough surface or subtle characteristics (Meeran et al., 2023**;** Reyad-ul-Ferdous et al., 2015).

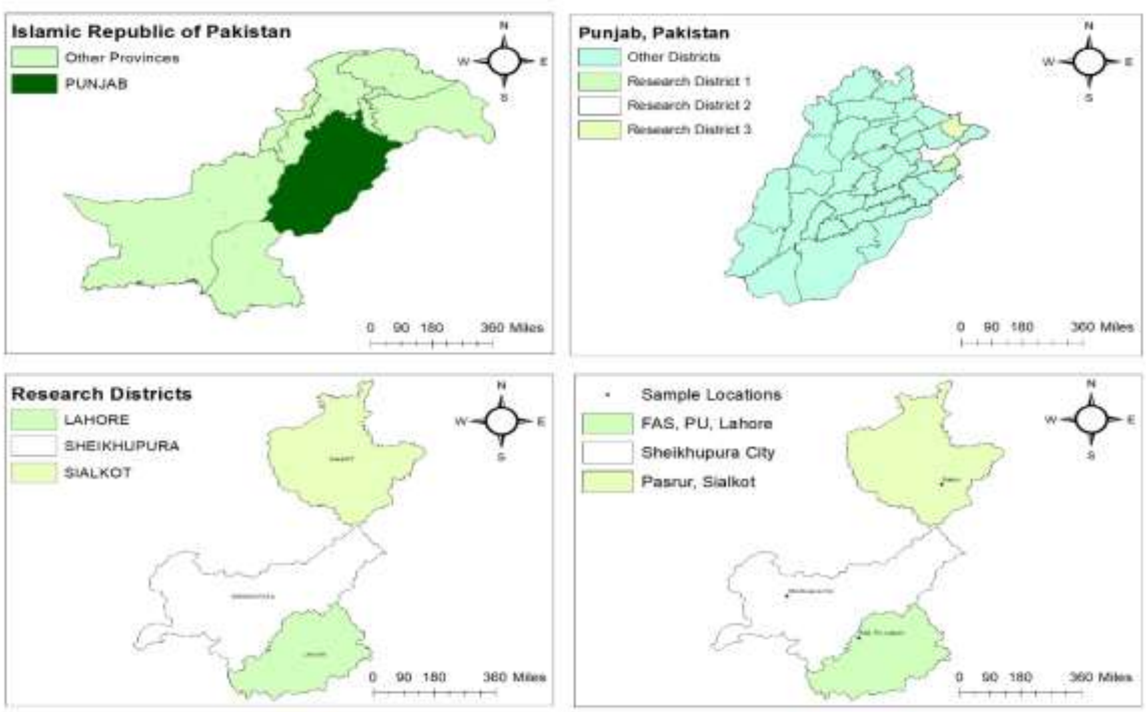
Amaranthus viridis L. has long been used to treat snake bites, ulcerative gingivitis, hematuria, blisters, swellings, and diarrhea (Jin et al., 2025; Kumar et al., 2022). According to the "Classified Materia Medica," it was utilized as early as the Song Dynasty to treat "black eye conditions and wind-heat of liver channel" (Jin et al., 2025). Conventional uses also include diuretics, vermifuges for venereal disorders, analgesics, anti-rheumatics, antiulcer, antiemetic, and improvements in appetite, laxative, anti-leprotic, respiratory, ocular, and asthmatic treatment. Additionally, the plant has antiviral, ribosome-inactivating protein, ßcarotene, and lactin characteristics that are antiproliferative and antifungal (Reyad-ul-Ferdous et al., 2015). Researchers acknowledge that additional research is necessary to confirm the veracity and efficacy of these assertions. The goal of ongoing research is to better comprehend the plant's potential health benefits and lay the groundwork for its many therapeutic applications (Meeran et al., 2023).

Material and methods

Collection of Plant Material

Plants of *Amaranthus viridis* were collected from three different locations (Figure 1) across Punjab, Pakistan, including Pasrur (Sialkot), Sheikhupura, and University of the Punjab, Lahore, Pakistan. The geographical coordinates of these locations are '32.2625° N, 74.6576° E', '31.7117° N, 73.9957° E' and '31.5036° N, 74.3039° E', respectively. Plants collected were used as samples. After that, required measurements were made, data were collected, and the entire plant material was then exposed to shadow drying.

Figure 1: Research Locations on Map



Shoot Length (cm)

The shoot length of the plant was measured by using a 'cm' measurement scale from the point where the shoot attaches to the roots of the plant up to the point where the inflorescence of the plant emerges.

Root Length (cm)

The root length of the plant was measured by using a 'cm' measurement scale below from the point of attachment of the root and shoot.

Inflorescence Length (cm)

The inflorescence length of the plant was measured by using a 'cm' measurement scale above the point of attachment of the shoot and the inflorescence part of the plant.

Plant Weight Fresh (g)

The fresh weight was determined as soon as possible after it was taken out of the field in order to keep the weed sample from drying out. The sample's weight was determined using an electronic/digital weight balance.

Plant Weight Dry (g)

The weed samples, after taking the desired measurements, were subjected to shade drying for up to 48 hours, and then the dry weight of the sample was determined using an electronic/digital weight balance.

Moisture Content (%)

The following formula was used to record the overall moisture percentage of the plant samples, including the inflorescence.

$$\text{Moisture Content (\%)} = (\text{Fresh Weight} - \text{Dry Weight})/\text{Fresh Weight} \times 100$$

Number of Leaves (Per Longest Shoot)

The number of leaves present on one of the shoots was counted manually. This shoot was the same length as the shoot length.

Leaf Length (cm)

The leaf length was measured using the 'cm' measurement scale from the point where the leaf blade emerges from the petiole to the tip/apex of the leaf. One of the largest leaves of the plant was selected and considered the representative of the whole.

Leaf Width (cm)

The leaf width was measured using the 'cm' measurement scale from the widest/broader portion of the same largest leaf selected as the representative.

Leaf Area (cm^2)

The three parameters, including the leaf length (L), leaf width (W), and the correction factor named the 'Montgomery parameter (c),' were multiplied to calculate the leaf area (A). The value of the Montgomery Parameter ranges between 0.5 and 0.785, depending upon the leaf structure and the plant species. Montgomery parameter's value for Amaranthus viridis was calculated as '0.6419' (Schrader et al., 2021; Shi et al., 2019). The following formula was used for the estimation of leaf area

$$\text{Leaf Area} = \text{Montgomery Parameter} \times \text{Leaf Length} \times \text{Leaf Width}$$

$$A = cLW$$

Results and discussions

Analysis of Variance (ANOVA) was performed on the data, and the 95% p-value ($p<0.05$) was used to differentiate the mean differences. Tukey HSD was used to determine better performing location among all three distinct locations being studied for the analysis of morphological traits. The Pearson correlation coefficient was used to determine the link between the morphological traits. Heritability, Genetic Advance (GA), Phenotypic Coefficient of Variance (PCV), and Genotypic Coefficient of Variance (GCV) were also assessed (Ogechi and Joseph, 2017; Sogbohossou and Achigan-Dako, 2014).

The morphological traits being studied are abbreviated in the data analyzed. Hence, the abbreviations for each of the ten traits are; root length (RL), shoot length (SL), Inflorescence length (IL), plant weight fresh (PWF), plant weight dry (PWD), moisture content (MC), number of leaves (NOL), leaf length (LL), leaf width (LW) and leaf area (LA).

Analysis of Variance

The findings are displayed in Table 1, which shows conclusive results between the three locations being observed and studied for the *Amaranthus viridis*. The average shoot length was recorded as (42.7±0.3873 cm), root length as (15.389±0.6955 cm), inflorescence length as (11.422±0.4823 cm), fresh plant weight as (30.667±0.7071 g), dry plant weight as (10.556±0.1521 g), moisture content as (63.887±0.5442 %), number of leaves (16.11±0.5092), leaf length as (6.7111±0.2037 cm), leaf width as (4.8±0.1683 cm) and the leaf area as (20.894±1.2903 cm²). Table 2 shows the analysis of variance of genetic components of the morphological traits mentioned above. *Amaranthus viridis* can withstand harsh, hot, and dry climates is further supported by its higher moisture content percentage, and its ability to survive under conditions of water deficit suggests that it may present difficult conditions for crop plants in terms of water, minerals, nutrients, and space, which could have an impact on crop yield and production. The species' robustness is demonstrated by its greater plant fresh weight (Meeran et al., 2023).

Tukey's Comparison

Tukey HSD comparison shows all-pairwise comparisons test of all the morphological traits for locations under study. There are significant differences among the means of the traits illustrating the overall location performance. The better performing locations in each trait are also determined using the comparison. The results of Tukey HSD comparison are illustrated using a clustered bar chart, which shows the performance and grouping of the locations (Figure 2).

Figure 2: Trait performance of morphological traits of *Amaranthus viridis* L. in sampled locations

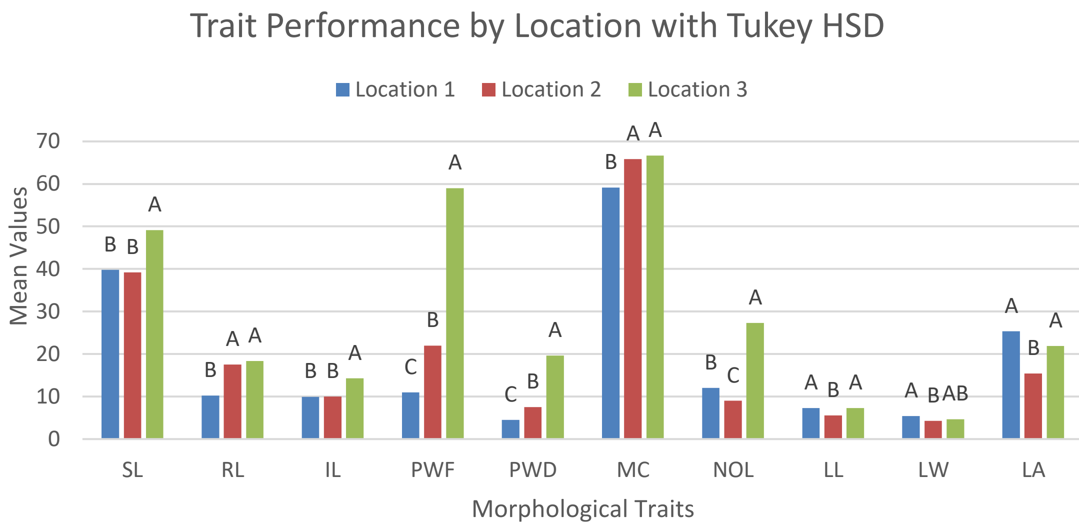


Figure 2. The Tukey comparison divides the three locations into three homogeneous groups, A, B, and C, as the good, moderate, and low performing, respectively. Any group in between these three, as AB means performance in between the group A and B. Location 3 significantly differs in all the morphological traits being studied except the three named leaf length, leaf width, and leaf area, while Location 1 performs significantly better than the other two locations, in mentioned three traits. Location 2 leads location 1 in all traits except shoot length and number of leaves, where location 1 leads while lagging behind location 3 in the mentioned three traits.

Correlation Analysis (Pearson)

The link between the several morphological traits of Amaranthus viridis is examined using Pearson correlation analysis, which reveals strong positive or significant negative correlations among the traits under study. The correlation analysis given in Table 1 shows significant, positive, strong to moderate correlation of shoot length with inflorescence length, fresh plant weight, dry plant weight and number of leaves, root length with fresh plant weight, dry plant weight and moisture content, inflorescence length with shoot length, fresh plant weight dry plant weight and number of leaves, fresh plant weight with shoot length, root length, inflorescence length, dry plant weight, moisture content and number of leaves, dry plant weight with shoot length, root length, inflorescence length, fresh plant weight, moisture content and number of leaves, moisture content with root length, fresh plant weight and dry plant weight, number of leaves with shoot length, inflorescence length, fresh plant weight and dry plant weight, leaf length with leaf width and leaf area, leaf width with leaf length and leaf area, leaf area with leaf length and leaf width whereas shows significant but negative, strong to moderate correlation of root length with leaf width, moisture content with leaf width and leaf width with root length and moisture content.

Table 1: Correlation (Pearson) among different morphological traits of *Amaranthus viridis L.*

Traits	SL	RL	IL	PWF	PWD	MC	NOL	LL	LW
RL	0.5098								
IL	0.9732*	0.5588							
PWF	0.9523*	0.7186*	0.9223*						
PWD	0.9642*	0.6942*	0.9388*	0.9982*					
MC	0.5027	0.943*	0.4925	0.7299*	0.698*				
NOL	0.9916*	0.4195	0.9599*	0.9173*	0.931*	0.4241			
LL	0.5271	-0.3676	0.5135	0.2605	0.2966	-0.439	0.5968		
LW	-0.063	-0.698*	-0.0823	-0.3215	-0.3008	-0.7151*	0.0178	0.7349*	
LA	0.2339	-0.5853	0.2131	-0.0474	-0.0172	-0.6281	0.3148	0.9256*	0.9354*

* = significant at 5% probability level

The * values show the significant correlation, which is determined by the p-value, such as 0.05 in the current analysis, while the signs +, - show the direction of correlation, either positive or negative. A p-value smaller than 0.05 shows a significant correlation, while a p-value greater than 0.05 shows a non-significant correlation.

. p-value < 0.05 shows significance, p-value > 0.05 shows non-significance

The value greater than 90% has a strong correlation, values between 60-89% have a high/good correlation, values below 50% up to 20% show a moderate correlation, while values below 20% show a poor but significant correlation. In other terms, correlation ranges between -1, 0, and 1, which shows the strength and direction of correlation. The correlation factor or parameter factor 'r' whose value is nearer to -1 or 1 is considered to be a strong negative or strong positive correlation, respectively, and values that equal 0 or are nearer to it are considered to have no correlation or a weak degree of correlation, respectively. These numbers indicate how the morphological traits correlate with each other and how well the plant can withstand hot, severe environmental conditions (Shehzadi et al., 2022). This capability is explained by the formation of organic compounds and their increased rate of photosynthetic activity, both of which support its vigorous growth and development (Meeran et al., 2023).

Assessment of Genetic Components

Genotypic variance (GV), Genotypic coefficient of variance (GCV%), Phenotypic variance (PV), Phenotypic coefficient of variance (PCV%), Environmental coefficient of variance (ECV%), Heritability (h^2_{bs}), Heritability Percentage (h^2_{bs}%) and Genetic advance (GA%) percentage are calculated using mean sum of squares (MS), grand mean (GM) and error in variance (EV). Table 2 shows the results for the morphological traits of the plant being studied. These factors help the breeders to analyze the influence of the genetics of a plant and the environmental effects on a particular trait. Transfer of traits or maintaining characters over generations and their expressions are also calculated using these factors. This helps ease the selection procedure.

Table 2: Genetic Components/Factors

Traits	M.S	G.M	GV	GCV %	PV	PCV %	EV	ECV %	h^2bs%	GA%
SL	94.36	42.70	31.30	85.62	31.75	86.23	0.45	10.27	98.58	113.43
RL	59.47	15.39	19.34	112.11	20.79	116.24	1.45	30.71	93.02	148.52
IL	18.63	11.42	5.98	72.35	6.68	76.46	0.70	24.72	89.55	95.85
PWF	1897.00	30.67	631.83	453.91	633.33	454.44	1.50	22.12	99.76	601.32
PWD	193.53	10.56	64.49	247.16	64.56	247.30	0.07	8.08	99.89	327.43
MC	51.15	63.89	16.75	51.21	17.64	52.55	0.89	11.79	94.96	67.84
NOL	290.11	16.11	96.44	244.67	97.22	245.65	0.78	21.97	99.20	324.13
LL	2.95	6.71	0.94	37.45	1.07	39.85	0.12	13.62	88.32	49.61
LW	0.93	4.80	0.28	24.22	0.37	27.64	0.09	13.31	76.82	32.09
LA	76.25	20.89	23.75	106.62	28.75	117.30	4.99	48.89	82.63	141.25

Fresh Plant Weight has the highest and Leaf Width has the lowest GCV%, PCV%, as well as h2bs%, GA%, while Leaf Area has the highest and Dry Plant Weight has the lowest ECV%. This shows the influence of different genetic and environmental impacts on the expression of morphological traits in the given sample and their strength to be transferred through heritability to next generation, the offspring. Moreover, these agronomic biomass traits are being governed by additive gene action, because of high broad-sense heritability and genetic advance. This means a Breeder can simply perform mass selection for high-yielding trait production for agricultural cultivation.

PCA Biplot

The Principal Component Analysis resulted in PC1 accounting for 96.4%, while PC2 accounts for 3.6% of the observed variations in all morphological traits being studied (Figure 3). It has been found from results that the location 3 was reported as more suitable and highly productive for the growth and development of weed plants.

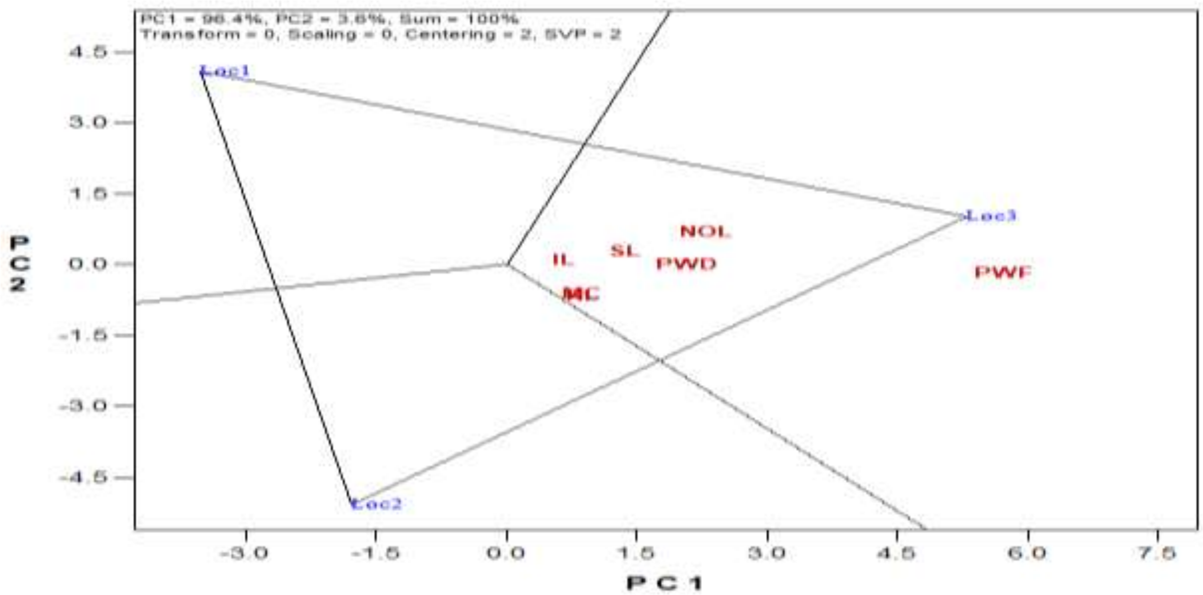


Figure 3. Principal biplot for locations and studied traits of *Amaranthus Viridis*

Conclusion

Location 3 among the sample locations is significantly highly productive for the production of *Amaranthus Viridis L.* Furthermore, high heritability and genetic advance give an opportunity to Breeders to work for its high yielding potential and domestication. Therefore, it is highly recommended to study and analyze population of *Amaranthus Viridis L.* through agricultural practices to minimize the loss and enhance the productivity.

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Declaration

Data Availability statement

All authenticated data have been included in the manuscript.

Consent for publication

Not applicable

Declaration of Competing Interests

The authors declare that they have no conflict of interest.

Author Contribution Statement

Muhammad Abdul Rehman Khalid collected data and conducted research and wrote article. Muhammad Abdul Rehman Khalid and Muawaz Ul Hassan make final editing was carried out and approved for final publication.

Acknowledgements

No external support or assistance was involved. All work reflects the efforts of the authors.

Informed Consent

Not applicable.

Ethical Statement

Not applicable