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Contact journal email: thejspae@gmail.com

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ORIGINAL RESEARCH

Impact of late sowing on morphological and yield traits in 40s bread wheat

Muhammad Adnan[1], Abdullah Khan[1], Fida Mohammad[1], Fawad Ali[3], Quaid Hussain[2]

[1]Department of Plant Breeding and Genetics, The University of Agriculture Peshawar, Pakistan.
[2]Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Beijing China.
[3]Department of Plant Sciences, Quaid-I-Azam University, Islamabad-Pakistan.

Corresponding Author:

Muhammad Adnan
Email:
adnan.gxu786@gmail.com
Contact: +8618577966364)

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ABSTRACT

The unpredictability and large fluctuation of the climatic conditions in rainfed regions influences spring wheat yield and grain quality. These variations offer the opportunity for the production of better quality wheat. The effect of late sowing on wheat morphology and grain yield was studied in different 40s bread wheat at the research farm of PBG, The University of Agriculture Peshawar, Pakistan during 2013-14. Forty wheat genotypes were tested under normal and late sowing in 5 × 8 alpha lattice design with three replicates. Combined analysis of variance exhibited significant genotype by environment interactions for days to heading, flag leaf area, days to maturity, plant height, spikes m^{-2}, grains $spike^{-1}$,1000-grain weight, biomass yield, grain yield and harvest index. Days to emergence, headings, maturity ranged from 9 to 12, 111 to 121 and 155 to 164 days under normal while under late planting it ranged from 25 to 29, 95 to107 and 137 to 143 days. Mean data under normal planting ranged between 77 to 125cm; 25 to 41cm^2; 99 to 199; 10 to 13 cm 32 to 49; 52 to 88g; 8533 to 13667 kg, 1869 to 4681 kg; 21 to 35% whereas under late planting its range was 63 to 91 cm, 18 to 37 cm^2, 57 to 137, 8 to 12 cm, 22 to 52, 36 to 75g, 2400 to 7933 kg, 540 to 2739 kg and 20 to 42% for plant height, flag leaf area, spikes m^{-2}, spike length, grains $spike^{-1}$, 1000-grain weight, biomass, grain yield and harvest index, respectively. Wheat genotypes planted at late condition took maximum days to emergence, while less number of days were reacquired for wheat genotypes planted at normal sowing date to get mature. Late planting negatively affected all yield contributing traits like; spikes m^{-2} (29%), grains $spike^{-1}$ (18%) 1000-grain weight (29%), biomass (55%) and grain yield (50%). On the basis of the current exploration, it is obtained that genotype SRN 19111 was identified superior for 1000-grain weight, biomass yield and grain yield under normal planting, while genotype PR-107 exhibited higher grain yield under late planting. Therefore, these genotypes are recommended for further extensive testing.

KEYWORDS: Alpha lattice design, genotypes, sowing time, wheat yield and yield components

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1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major cereal crops in the world known for its high nutrient values (Adnan et al., 2017; Dutta et al., 2019). Cereal crops; wheat, rice and maize account for roughly half of the world's human caloric intake (Rosenthal and

Ort, 2012). Wheat is regarded as a staple food crop in most of the countries including Pakistan. World wheat production during 2009-10 decreased to 0.32% as compared to the previous cropping season (2008-09), whereas 5.4% decrease in production was estimated for the 2010-2011 season. This decrease in wheat production might be due to several reasons such as improper agronomic practices, poor management and unfavorable weather conditions such as high temperature, drought and salinity (Ali et al., 2019). The assessment and characterization of plant germplasm are vital for usage of genetic resources of any type (Ali et al., 2019; Vigna et al., 2011). Wheat genotype developed through modern technologies is having good yield and achieved to having good harvest index. However, there is still a gap to develop and select new wheat genotype which can resist the harsh environmental condition and giving high yield (Riazuddin et al., 2010). It has been observed that about 80 % of the wheat crop in Pakistan is late sown, while only 20 % of the wheat crop in sown at normal planting time. Planting of the wheat crop at normal sowing time could add about two million ton of wheat to the national production of wheat. The causes of delay in sowing of wheat may be due to the presence of previous crops in the field and the absence of quality and timely agriculture input because of these reasons at grain filling stage the wheat crop is induced to terminal high temperature due to which the yield of the wheat crop is reduced much. Production of tillers, kernel size, 1000-grain kernel weight, spike length, biomass yield, grain yield and harvest index are decreased that may vary from genotype to genotype. High temperature may also negatively affect other biochemical and physiological functions in wheat crop (Hamam and Khaled, 2009; Kattenberg et al., 1995; Reynolds et al., 2001). Delay in sowing of wheat after 10^{th} November may cause 42 kg ha^{-1}loss in yield (Khan, 2003). The probability of invasion of pest disease attacks, drought and high-temperature shocks are more for wheat genotype sown late. Therefore, the normal planting of wheat crop shows good results for obtaining good yield. Molecular and conventional approaches can play a vital role in the development of wheat genotypes that are high-yielding and having adoption to various environmental risks.

Production considerations like vegetative growth, grain yield and quality is more affected by planting time. The research work carried out on planting date may be helpful for wheat growers to obtain optimum yield from their farming. Late sowing increases the risk of yield loss (Ehdaie et al., 2001). Morphological and yield traits are directly related with the sowing time. Mid-November sowing produces the highest number of tillers m^{-2}, spike m^{-2}, 1000 grain weight and grain yield (Nasser 2009). Early or late sowing increases the risk of yield losses (Ehdate et al., 2001). Similarly biomass accumulation, grain yield, number of spikes m^{-1} and 1000 grain weight of wheat were increased with early (early November) sowing over late (December) sowing as reported by Aftab et al. (2004). The present study was carried out to find out the impacts of late sowing in morphological and yield traits in newly developed wheat genotypes.

2. MATERIALS AND METHODS

2.1 Experimental location and weather detail

The field experiment was carried out at the Research Farm of PBG, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan during 2013-14. The farm is located at 34.01_ N latitude, 71.35_ E longitude, at an altitude of 350 m above sea level in Peshawar valley. The site is situated about 1600 km North of the Indian Ocean and has a continental type of climate. Warsak canal from Kabal River was used for the irrigation system. The soil of this site is clay loam, containing low in organic matter (0.87%), potassium (121 mg kg^{-1}); phosphorus (6.57 mg kg^{-1}), alkaline (pH 8.2); and with calcareous nature (Khan et al. 2009, So et al. 2012). The minimum, maximum temperature and average rainfall data was collected from the metrological station and shown in Fig 1 a & b.

2.2 Experimental design and agronomic practices

Experimental material consisted of 40 genotypes (39 Advance lines and 1 local check). These genotypes were planted under normal and late planting dates to study the effects of planting time on different genotypes. Experimental material was received from the National Agriculture Research Center (NARC) Islamabad as National Uniform Yield Trial (NUYT) 2013-14. The experiment was laid out in 5 × 8 alpha lattice design with 3 replicates. Every genotype was planted in a 6-row plot, having row to row and plant to plant distance of 0.25 m and 5 m, respectively. Normal planting was done on November 20, 2013, while late planting was done on December 21, 2013. All other cultural practices were applied uniformly to the experiments sown under normal and late planting dates. Three to four irrigations were applied during the wheat growth period.

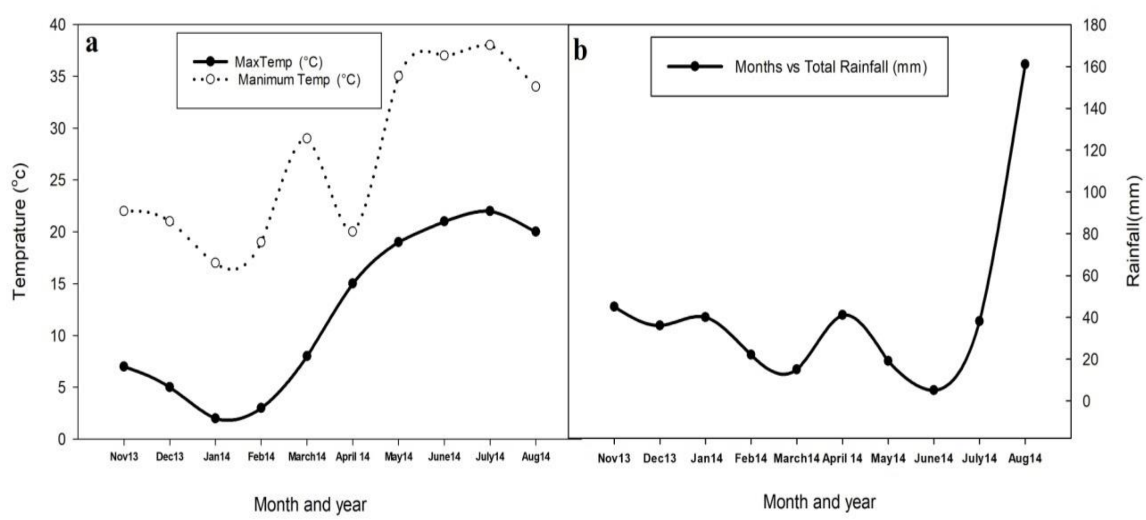

Figure 1. Mean monthly precipitation and air temperature during Nov 2013 to August 2014.

Table. 1. List of genotypes used in the experiment

Genotype	Code	Breeding center
1	109384	RARI- Bahawalpur
2	99172	RARI – Bahawalpur
3	99346	RARI – Bahawalpur
4	99114	RARI – Bahawalpur
5	DN-93	ARI- DI Khan
6	CT 09137	NIFA-Peshawar
7	SRN 09111	NIFA – Peshawar
8	V-09082	WRI – Faisalabad
9	V-09087	WRI – Faisalabad
10	V-10104	WRI – Faisalabad
11	V-10110	WRI – Faisalabad
12	V-11160	WRI – Faisalabad
13	SKD-11	WRI-Sakrand
14	NN-Gandum-I	NIBGE Fsd
15	NN-Gandum-II	NIBGE Fsd
16	TW96010	AZRI Bhakkar
17	TW96018	AZRI Bhakkar
18	SD-998	NIA Tandojam
19	NIA-MN-08	NIA Tandojam
20	CIM-04-10	NIA Tandojam
21	ESW-9525	NIA Tandojam
22	PR-103	CCRI-Pirsabak
23	PR-106	CCRI-Pirsabak
24	PR-107	CCRI-Pirsabak
25	RCA-1	RCA Seeds
26	V-11005	WRS-Tandojam
27	NR-413	NARC-Islamabad
28	NR-421	NARC-Islamabad
29	NR-409	NARC- Wheat
30	NR-419	NARC-Islamabad
31	UAF-9452	Univ. of Agri. Fiasalabad
32	Guard-C	Hybrid – Guard
33	SAWSN-02-102	AZRC-DI Khan
34	Janbazz	UOA Peshawar
35	TD-1	WRI-Sakrand
36	Pirsabak-13	CCRI-Pirsabak
37	Sehar-06	WRI-Faisalabad
38	V07096	WRI-Faisalabad
39	Aas-11	RARI- Bahawalpur
40	NARC-11	Wheat-NARC

2.3 Data collection and measurements

Days to emergence of wheat were observed in all plots by counting the number of days from sowing until the date when more than 70% of plants emerged. Days to heading were recorded by counting days in number from sowing until the date when more than 75% of tiller formed anthers. Data on plant height was recorded by taking the height of 5 tillers at random in each plot and the height from the base to the tip of each tiller was measured using a meter rod including awns and was then averaged. Flag leaf area was recorded at the panicle initiation stage. Days to physiological maturity of wheat were observed in all plots by counting the number of days from sowing until the date when more than 75% of tillers had become mature in all plots. Spikes m^{-2} was recorded by counting spikes in three middle rows and were then converted to m^{-2} using the same formula as spikes m^{-2}. Spike length was recorded at physiological maturity by using simple geometric ruler. Data on leaf chlorophyll content was recorded as a SPAD value using the Spad meter by putting the flag leaf on the scanner of the meter and hold for a while (Islam et al. 2014). Grains $spike^{-1}$ was noted by counting number of grains per spike in five randomly selected spikes in each plot and was averaged. Data on thousand grain weight was obtained by counting 1000 grains at random from the grain lot of each plot and were measured using an electronic balance. Biological yield was noted by harvesting three rows in the middle of each plot and were dried in an open field for one week and were then weighed and converted to kg ha^{-1} using equation 1. The grain yield was determined by threshing the harvested sample and the grain obtained was weighed and converted to kg ha^{-1}. Harvest index expressed in percentage is the ratio of seed yield to biological yield. It was determined by dividing seed yield by biological yield multiplied by 100.

BY(kg ha^{-1})= BY(kg) in four central rows× 10000/rows-row distance × number of number of rows × row length-------------(1)

2.3 Statistical Analysis

Analyses were carried and the significant means for various traits were separated with the application of LSD test. Sigma plot X7 was used for figure and data analysis was done through IBM-SPSS20 and Microsoft excel.

3. RESULTS

3.1 Phenology

Data on days to emergence, days to heading and days to maturity was considerably altered by wheat genotypes, normal and late planting. Days to emergence for wheat genotypes was recorded from 9 to 12 days under normal planting sowing and 25 to 29 days under late planting sowing condition (Table 2). Whereas among genotypes 109384, DN-93, V-10104, V-10110, V-11160 and NN-Gandum-I took minimum (9) days to emergence while genotype SD-998 took maximum (12) days to emergence. In contrast, genotypes DN-93 and UAF-9452 took minimum (25) days to emergence while genotype ESW-9525 took maximum (29) days to emergence under late planting condition. Average over normal and late planting days to emergence ranged from

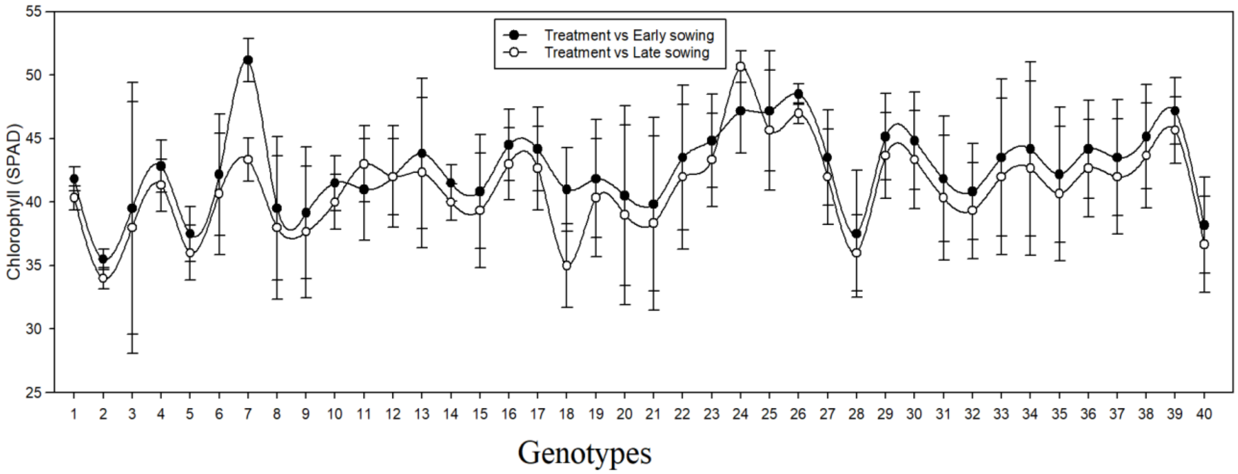

Figure 2. Chlorophyll content as influenced in different wheat genotypes and by sowing dates. Note: the serial numbers from 1 to 40 indicates the genotypes, for detail see table 1.

18 days for genotype NR-419 to 26 days for genotype PR-107. Mean values for 40 wheat genotypes for days to emergence were 10 and 26 days under normal and late planting sowing conditions, respectively (Fig. 3A).

Data on days to heading showed minimum (111) days to heading were observed for genotype PR-419 and maximum (121) days to heading were observed for genotypes CT 09137, SD-998 and NIA-MN08 under normal planting while at late planting minimum (95) days for genotypes TW96018 and PR-419 and maximum (107) days to heading were observed for genotypes DN-93 and ESW-9525 (Table 2). Genotype PR-419 took minimum days to heading under both normal and late planting conditions. The significant G×E interaction implies that the genotypes were observed to have different relative days to heading across planting dates. Mean genotypic performance across normal and late plantings for days to heading were 118 days in normal planting while 103 days in late planting(Fig. 3B). Thus in late sowing conditions the head in emergence 15 days were reduced as compared to normal sowing.

Data on day's physiological maturity under normal planting ranged from 155 to 164 days, while under late planting condition it ranged from 137 to 143 days (Table 2). Genotype UAF-9452 took minimum (155) days to physiological maturity while maximum (164) days to maturity were recorded for genotype NR-413 under normal planting. Under late planting condition minimum (137) days to maturity were recorded for genotype UAF-9452 while maximum (143) days to maturity were recorded for genotype NR-413. Interestingly the genotype UAF-9452 took minimum days while genotype NR-413 took maximum days to maturity under both normal and late planting. Mean days to maturity across two production environments ranged from 147 for genotype 99346 to 153 for genotype V-09082. Average days to maturity were 159 and 140 days under normal and late planting conditions, respectively (Fig. 3C). From the net difference in days to maturity it was notice that the wheat genotypes planted at late planting condition get mature 19 days earlier than wheat genotypes sown at their normal sowing condition.

3.2 Growth traits

Data on wheat plant height were recorded significantly among genotypes and planting dates (Table 2). Generally, shorter plants were observed in late sowing as compared to the normal sowing date. Under normal planting conditions minimum (77 cm) plant height was observed for genotype TD-1 while genotype SAWN-02-102 showed maximum (125 cm) plant height. While genotype TD-1 showed minimum (63 cm) plant height and genotype SAWN-02-102 maximum (91 cm) plant height under late planting. Plant height in overall interaction value ranged from 63 cm for genotype TD-1 to 125 cm for genotype SAWN-02-102. Mean values across normal and late plantings were observed for genotype TD-1 to be 70 cm and 108 cm for genotype SAWN-04-102. Under normal planting the mean value was 105 cm while under late planting mean plant height of 80 cm was observed (Fig. 3D).

Spike length under normal planting varies from 10 to 13 cm and 8 to 12 cm under late planting condition (Table 2). Under normal planting genotype Guard-C showed minimum (10 cm) spike length while genotype 109384 showed maximum (13 cm) spike length. In contrast, genotype CT 09137 showed minimum (8 cm) spike length under late planting condition while genotype Aas-11 showed maximum (12 cm) spike length. Therefore, in normal sowing the genotype produced plants with maximum spike length. Mean value for spike length in overall interaction was ranged from 8 cm for genotype CT 09137 to 13 cm for genotype 109384. Mean over the two planting environments genotype Aas-11 produced plants with maximum spike length 13 cm while genotype CT 09137 produced plants with minimum spike length 9 cm. Mean for 40 wheat genotypes under normal and late planting were 12 and 10 cm respectively (Fig. 3E).

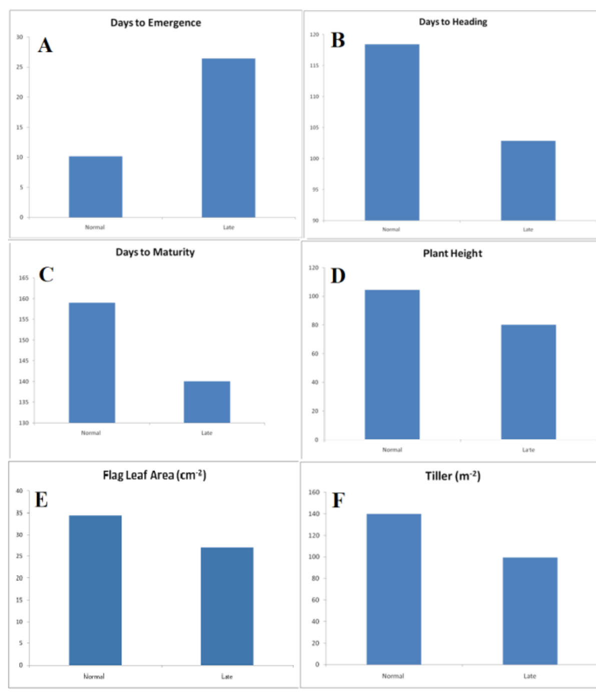

Figure 3. Impact of normal and late planting on yield and yield components of 40 breads wheat genotype.

Maximum (199) number of spikes m^{-2} were recorded for normal planting while minimum (57) spikes m^{-2} for late planting

(Fig. 3F). Genotype Guard-C showed maximum (199) spikes m^{-2} while genotype SAWSN-02-102 showed minimum (99) spikes m^{-2} under normal planting condition. Under late planting condition genotype CIM-04-10 showed minimum (57) number of spikes m^{-2} whereas genotype PR-107 showed maximum (145) number of spikes m^{-2}. The interaction mean value for number of spikes m^{-2} for normal and late planting ranged from 166 for genotypes Guard-C to 81 for genotype CIM-04-10 (Table 2). Mean values for 40 genotypes for spikes m^{-2} over normal and late planting were 140 and 100 respectively.

3.3 Physiological traits

The data recorded for flag leaf area under late planting ranged from 18 cm^2 to 37 cm^2, while under normal planting condition it ranged from 41 to 25 cm^2 (Table 2). Genotype DN-93 showed minimum flag leaf area of 18 cm^2, while genotype V-11005 exhibited maximum flag leaf area of 37 cm^2 under late planting while in normal planting condition minimum (25 cm^2) flag leaf area were observed for genotype NR-419, while maximum (41 cm^2) flag leaf area were recorded for genotypes PR-107 and RCA-1. The significant G × E interaction can be confirmed from the mean values for flag leaf area as the genotype showing maximum flag leaf area in normal sowing did not show maximum flag leaf area in late sowing which meant that the ranking of genotypes changed on the basis for their performance for flag leaf area under normal and late plantings. Based on the mean performance of genotypes under normal and late planting minimum flag leaf area was observed for genotype V-11160 (25 cm^2) while maximum for genotypes PR-107 and RCA-1 (38 cm^2). Averaged over 40 genotypes, flag leaf area was 27 and 34 cm^2 under normal and late planting conditions, respectively.

Data on chlorophyll content significant difference between genotypes and sowing dates. Between sowings dates early sowing showed higher chlorophyll content across the genotypes (Fig. 2). Among the genotypes no 7 in the Table 2 (SRN 09111) showed higher (51.1) chlorophyll content at early sowing (Figure 2), whereas during late sowing genotype number 24 resulted higher (50.6) chlorophyll content. The possible reason for this variation might be attributed to the genetic characteristics of the genotype as reported by (Kochak-Zadeh et al. 2013)

3.4 Yield components and yield traits

Data on number of grains $spike^{-1}$ were found significant for genotypes and planting date. Minimum number of grains $spike^{-1}$ (32) were observed in late sowing as average across 40 wheat genotype while normal sowing showed maximum (39) number of grains $spike^{-1}$ (Fig. 3F). Under late planting condition minimum (22) number of grains $spike^{-1}$ was observed for genotype CT 09137, while genotype Janbaz showed maximum (52) number of grains $spike^{-1}$. In contrast genotype CIM-04-10 produce maximum (59) number of grains $spike^{-1}$ while genotype Guard-C produce minimum (27) numbers of grain spike under normal planting (Table 2). Planting dates showed significant variation in number of grains $spike^{-1}$ in wheat genotypes. Number of grains $spike^{-1}$ in overall interaction value ranged from (22) for genotype CT 09137 to (59) for genotype CIM-04-10. Due to late planting 18% reduction occurred in number of grains spike^{-1}. Sial et al. (2005) reported reduction in grain numbers due to late sowing and temperature Stress. The significant G × E interaction can be confirmed from the mean values for number of grains spike^{-1} as the genotype showing maximum number of grains spike^{-1} in normal sowing did not produce plants with maximum number of grains spike^{-1} in late sowing which means that the ranking of genotype in changed on the basis for their performance for number of grains spike^{-1} under normal and late planting.

Minimum (8533) biomass was recorded for genotype TD-1 and maximum (13667) biomass for genotype SRN 09111 at normal planting condition while at late planting minimum (2400 kg) and maximum (7933kg) biomass for genotype SD-998 and NR-421 respectively. Maximum and minimum biomass was observed for normal and late plantings which showed that the interaction effect of genotype and normal and late planting was effective in causing variation in genotypes for biomass. The genotypic effect was also observed significant which show that the genotypes are significantly different from each other for biomass. Averaged over normal and late planting minimum (5767 kg ha^{-1}) biomass was observed for genotype TD-1 while maximum (9700 kg ha^{-1}) for genotype NR-421. At late planting condition 55% reduction in bio-mass yield was observed. The 40 wheat genotypes produce an average 10885 kg ha^{-1} at normal plantings while the same genotypes sown late produce an average yield of 5990 kg ha^{-1} (Fig. 4K). It is obvious from the result that the wheat genotypes sown at their normal sowing period produce biomass yield of 4895 kg ha^{-1} than the wheat genotypes sown at late condition.

Data on 1000 grain weight demonstrates slighter (52 g) weight for genotypes SD-998 and NIA-MN-08, whereas heavier (88 g) 1000 grains weight was recorded for genotype SRN 09111 at normal planting while at late planting (36 g) and (75 g) for genotypes NIA-MN-08 and V 07096 respectively (Table 2). Interestingly the genotype NIA-MN-08 has resulted minimum 1000-grain weight under normal and late plantings. In overall interaction value for 1000 grain-weight ranged from (36 g) for genotype NIA-MN-08 to (88 g) for genotype SRN09111. The mean calculated for normal and late planting for 1000-grain weight genotype NIA-MN-08 had minimum (44 g) 1000-grain weight of and genotype V 07096 had maximum (75 g) of 1000 grain-weight. The average value calculated for 40 wheat genotype was 70 and 50g for normal and late planting respectively (Fig. 4G). Thus the kernel weight of wheat genotypes was reduced grain because of late sowing condition and hence normal planting was superior to late planting. The averaged 1000-grain weight of wheat genotypes was 20 g more than the wheat genotypes sown at late planting condition. 29 % weight loss was noticed at late planting condition.

Data on grain yield ranged averaged from 540 kg ha^{-1} for genotype NIA-MN-08 to 5336 for genotype SRN 09111 (Table 2). Data showed that under normal planting genotype NIA-MN-08 resulted Minimum (1869 kg ha^{-1}) grain yield and genotype

SRN 09111 resulted in higher grain yield, while at late planting genotype NIA-MN-08 demonstrate lesser grain yield (540 kg ha^{-1}) and genotype PR-107 showed greater (2739 kg ha^{-1}) grain yield. Across normal and late plantings smallest amount of (1205 kg ha^{-1}) grain yield kg ha^{-1} was confirmed for genotype NIA-MN-08 and highest amount of (3329 kg ha^{-1}) grain yield for genotype V-11005. Normal planting produce more grain yield kg ha^{-1} than late planting (Fig. 4K).

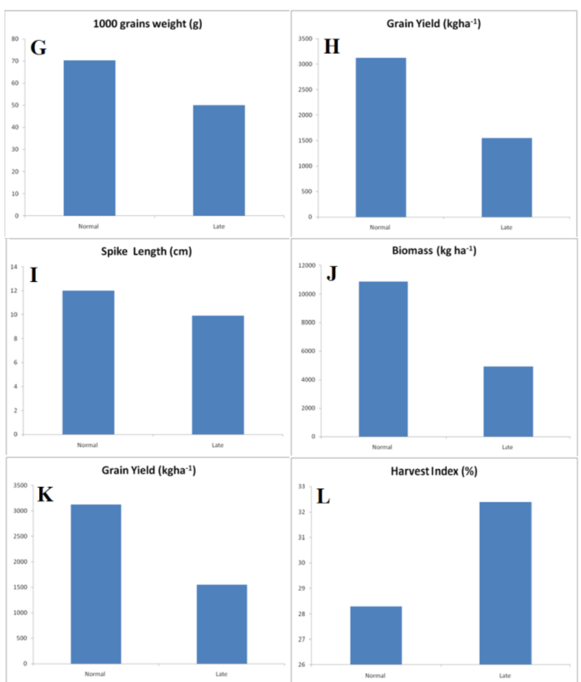

Figure 4. Impact of normal and late planting on yield and yield components of 40 breads wheat genotype.

Harvest index under normal planting ranged from 21 to 35 percent, while under late planting the harvest index ranged from 20 to 42 percent (Table 2). Genotype RCA-1 gave maximum harvest index of 35 percent whereas genotype SD-998 shows minimum harvest index of 21 percent under normal planting. Under late planting condition genotypes SD-998 and Guard-C gave maximum harvest index of 42 percent while genotype gave minimum harvest index of 20 percent (Fig. 4L).

4. DISCUSSION

Planting time is one of the major causes of low yield in wheat. In normal course, the sowing of wheat must be completed in the month of November in Pakistan. But in some cases, wheat grown after Cotton, Rice, Sugarcane and fodder crops in rotation may be delayed up-to December. This reveals negative impact on the grain yield in wheat. Research activities regarding the role of planting time and identification of wheat genotypes having better performance in such situation is highly desirable. Whereas among genotypes 109384, DN-93, V-10104, V-10110, V-11160 and NN-Gandum-I took minimum (9) days to emergence while genotype SD-998 took maximum (12) days to emergence. In contrast, genotypes DN-93 and UAF-9452 took minimum (25) days to emergence while genotype ESW-9525 took maximum (29) days to emergence under late planting condition. Average over normal and late planting days to emergence ranged from 18 days for genotype NR-419 to 26 days for genotype PR-107. Mean values for 40 wheat genotype for days to emergence were 10 and 26 days under normal and late planting sowing conditions, respectively. Whereas genotype PR-419 showed less (111) days to heading(111) and more (121) days to heading were recorded for genotypes CT 09137, SD-998 and NIA-MN08 under normal planting, while at late planting minimum (95) days for genotypes TW96018 and PR-419 and maximum (107) days to heading were observed for genotypes DN-93 and ESW-9525 (Table 2).

Table 2: Mean performance of wheat genotypes for spikes m^{-2}, spike length, grain $spike^{-1}$, 1000 grain weight, bio-mass yield kg ha^{-1}, grain yield kg ha^{-1} and harvest index for wheat genotypes under Normal and late plantings 2013-14.

	Normal				Late			
Traits	Ranges	Means	Best Genotype	LSD (0.05)	Ranges	Means	Best Genotype	LSD (0.05)
Days to emergence (no)	9-12	10	109384	0.8	25-29	26	UAF-9452	1
Days to heading (no)	95-121	118	NR-409	0.8	95-107	103	NR-409	1.4
Plant Height (cm)	76.93-125.2	104.41	V-09082	1.2	63.26-90.66	80.38	Janbaz	3.4
Flag leaf Area (cm^2)	25.26-40.86	34.40	PR-107	1.6	17.69-36.73	27.28	V-11005	2.1
Days maturity (no)	155-164	159	UAF-9452	4.6	137-143	140	UAF-9452	1
Spike m^{-2} (no)	99-199	140	Guard-C	7	56-145	100	CIM-04-10	7
Spike length (cm)	9.7-13.2	11.63	109384	0.7	8.31-11.56	9.92	Aas-11	0.6
Grains $spike^{-1}$	27-59	39	CM-04-10	2	22-53	32	Janbaz	2
1000- grain weight (g)	51.74-87.59	70	SRN 09111	1.9	36.27-75.46	50.24	V07096	2
Bio-mass yield (kg ha^{-1})	8533-13667	11089	SRN 09111	403	2400-7933.3	4895	NR-421	390
Grain yield (kg ha^{-1})	1869-5336	3127	SRN 09111	51	540-27389	1548.21	PR-107	68
Harvest index	21-35	28	RCA-1	1.4	20.4-41.6	32.4	V-9082	2.7

While at late planting minimum (95) days for genotypes TW96018 and PR-419 and maximum (107) days to heading were observed for genotypes DN-93 and ESW-9525 (Table 2). Genotype PR-419 took minimum days to heading under both normal and late planting conditions. Data on days to physiological maturity under normal planting ranged from 155 to 164 days, while under late planting condition it ranged from 137 to 143 days. Genotype UAF-9452 took minimum (155) days to physiological maturity while maximum (164) days to maturity were recorded for genotype NR-413 under normal planting. Under late planting condition minimum (137) days to maturity were recorded for genotype UAF-9452 while maximum (143) days to maturity were recorded for genotype NR-413. Average days to maturity were 159 and 140 days under normal and late planting conditions, respectively. It was observed that wheat genotypes sown at late planting condition emerged 16 days later than wheat genotypes sown at normal planting time to normal planting condition. The reason behind maximum days to emergence may be due to low temperature at late planting due to which wheat genotypes took maximum days to emerge as compared The same report was given by Benjamin (1990) and Gul et al (2012) as they observed that low temperature during emergence and seedling growth has detrimental effect on the crop establishment and productivity. Early emergence of head in wheat is one of the prime objectives in breeding programs of wheat because maximum time is available for grain filling. Late heading results in decrease grain size resulting in low grain weight (Irfaq et al, 2005). Among wheat genotype highly significant differences between genotype and environment interaction for days to heading are similar with the results of Razzaq et al. (1986), Subhan et al. (1991), Inamullah et al. (2007), Ilyas et al. (2013). Muhammad et al., (2007) conducted a research and observed significant result for days to heading across two environmental conditions. From the net difference in days to maturity it was notice that the wheat genotypes planted at late planting condition get mature 19 days earlier than wheat genotypes sown at their normal sowing condition. The wheat genotype took minimum days to get mature at late sowing because of continuity of race and to avoid from upcoming unfavorable condition. Nahar et al. (2010) reported up to 15% reduction in maturity period of wheat genotypes due to the effect of heat stress. Ilyas et al (2013) also found significant differences among wheat genotypes for days to maturity. Muhammad et al. (2007) also reported highly significant genotype by planting date interaction for days to maturity. The wheat genotype took minimum days to get mature at late sowing because of continuity of race and to avoid from upcoming unfavorable condition.

4.2 Growth traits

Plant height, Spike length and number of spikes m^{-2} were recorded significantly among genotypes and planting dates. Generally, shorter plants were observed in late sowing over normal sowing date. Genotype TD-1 results in shorter (77 cm) and genotype SAWN-02-102 showed taller (125 cm) plant height plant height under normal planting condition. Whereas under late planting genotype TD-1 showed minimum (63 cm) plant height and genotype SAWN-02-102 maximum (91 cm) plant height. Spike length under normal planting varies from 10 to 13 cm and 8 to 12 cm under late planting condition. Under normal planting genotype Guard-C showed minimum (10 cm) spike length while genotype 109384 showed maximum (13 cm) spike length. In contrast, genotype CT 09137 showed minimum (8 cm) spike length under late planting condition while genotype Aas-11 showed maximum (12 cm) spike length. Number of spikes m^{-2} were recorded from 57 to 199 spikes m^{-2}. Among genotypes, genotype Guard-C showed maximum (199) spikes m^{-2} while genotype SAWSN-02-102 showed minimum (99) spikes m^{-2} under normal planting condition. The possible reason for increasing in plant height among cultivars might be due to the differences in their genetic makeup. These results are in agreement with those obtained by Wahid et al. (2017). Genotypic differences were also observed by Ahmad et al. (1997). Similarly, Laghari et al. (2012) found similar result that 32.54% reduction in plant height took place due to late sowing when compares to normal sowing. Reduction of 25 cm occurred as a result of late planting. Rashid et al. (2004) and Knapp and Knapp (1978) have also observed that wheat genotypes sown at late planting results in reduction in plant height. Irfaq et al., (2005) also reported reduction in plant height of wheat genotypes due to late sowing and high temperature stress.

Difference in spike lengths of the genotypes was also reported by Kakar et.al. (2003). Gul et al (2012) also found that sowing of wheat genotype at normal time produce maximum number spikes m^{-2} than late sowing. These significant differences are pointing towards the presence of sufficient genetic variations among the genotype. The results are in accordance with the findings of Munir et al (1999) and Rajora (1999).

Flag leaf area under late planting ranged from 18 cm^2 to 37 cm^2, while under normal planting condition it ranged from 41 to 25 cm^2. Averaged over 40 genotypes, flag leaf area was 27 and 34 cm^2 under normal and late planting conditions, respectively .Genotype DN-93 showed minimum flag leaf area of 18 cm^2, while genotype V-11005 exhibited maximum flag leaf area of 37 cm^2 under late planting while in normal planting condition minimum (25 cm^2) flag leaf area were observed for genotype NR-419, while maximum (41 cm^2) flag leaf area were recorded for genotypes PR-107 and RCA-1. Similarly for chlorophyll content, among the genotypes SRN 09111 showed higher (51.1) chlorophyll content at early sowing (Figure 2), whereas during late sowing genotype PR-107 resulted higher (50.6) chlorophyll content. The possible reason for this variation might be attributed to the genetic characteristics of the genotype as reported by Kochak-Zadeh et al. (2013). Rane et al. (2007) reported that cooler climates favored the vegetative as well as the reproductive phases of wheat growth.

4.4 Yield components and yield traits

In wheat, grains per spike, biomass, 1000 grain weight and grain yield is one of the important primary yield contributing character. Number of grains $spike^{-1}$, biomass, 1000 grain weight and grain yield were found significant for genotypes and planting date. Under late planting condition minimum (22) number of grains $spike^{-1}$ was observed for genotype CT 09137, while genotype Janbaz showed maximum (52) number of grains $spike^{-1}$. Similarly less biomass was recorded for genotype TD-1 and higher biomass for genotype SRN 09111 at normal planting condition while at late planting minimum and maximum biomass were recorded for genotype SD-998 and NR-421 respectively. The 40 wheat genotypes produce an average 10885 kg ha^{-1} at normal plantings while the same genotypes sown late produce an average yield of 5990 kg ha^{-1}. Data on 1000 grain weight demonstrates slighter weight for genotypes SD-998 and NIA-MN-08, whereas heavier 1000 grains weight was recorded for genotype SRN 09111 at normal planting while at late planting lower and higher 1000 grains weight for genotypes NIA-MN-08 and V 07096 respectively. Grain yield showed that under normal planting genotype NIA-MN-08 resulted minimum grain yield and genotype SRN 09111 resulted in higher grain yield, while at late planting genotype NIA-MN-08 demonstrate lesser grain yield and genotype PR-107 showed greater grain yield. Across normal and late plantings smallest amount of grain yield kg ha^{-1} was confirmed for genotype NIA-MN-08 and highest amount of grain yield for genotype V-11005. The possible reason in the differences of bio-mass and yield attributes of the genotypes might be attributed to the genetic characteristic of each genotype as reported by Kakar et al. (2003). Akram et al. (2008) also found significant variation in wheat genotypes for 1000-grain weight. Abdullah et al. (2007) and Ansari et al. (1989) also found that late planting of wheat genotypes results in decrease of 1000- grain weight. Laghari et al (2012) and Irfaq et al (2005) reported biomass yield reduction in wheat genotypes because of late sowing. The finding of our research that the ranking of wheat genotypes according to yield production are changed by sowing the same wheat genotypes at two different environment are similar with the research findings of Cotes et al. (2006), Amin et al. (2005), Khalil et al. (2005), Garcya et al. (2003) and Kakar et al. (2003). Rane et al. (2007) reported that colder climates favored the vegetative as well as the reproductive phases of wheat growth

5. CONCLUSION

From this study it is concluded that late sowing of wheat negatively affect both morphological traits and grain yield and may results in 50 % of wheat yield loss. Hence timely sowing of wheat genotypes is highly suggested. The conducted research identified genotype SRN 19111 superior for 1000-grain weight, bio-mass yield and grain yield under normal planting, while genotype PR-107 exhibited higher grain yield under late planting. Therefore, these genotypes are recommended for further extensive testing and utilization in different wheat breeding programs.

Authors Contributions:
M.A and F.M conceived the main idea of research, M.A wrote the manuscript. A.K, F.A and Q.H revised the manuscript and provided suggestions. In addition M.A and AK assessed and analyzed the data, and performed data collection. All authors have read and agreed to the published version of the manuscript.

Data Availability statements: The data presented in this study are available on request from the corresponding author.

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REFERENCES

Abdullah, M., Aziz-Ur-Rehman, N. Ahmad and I. Rasul. Planting time effect on grainand quality characteristics of wheat. Pak. J. Agri. Sci. (2007). Vol. 44 (2): 200-202.

Adnan, M., F. Muhammad, Q. Hussain, I. Hussain and F. Ali. Heritability estimates and correlation analysis in bread wheat (Triticum aestivum L.) under normal and late plantings. Pure Appl. Biol. (2017). 6 (4): 1151-1160.

Ahmed, N., M.A. Chowdhry, I. Khaliq and M. Maekaw. The inheritance of yield and yield components of five wheat hybrid populations under drought condition. Indo. J. Agric. Sci. (1997). 8(2): 53-59.

Kashtwari, M., Mansoor, S., Wani, A.A., Najar, M.A., Deshmukh, R.K., Baloch, F.S., Abidi, I. and Zargar, S.M. Random mutagenesis in vegetatively propagated crops: opportunities, challenges and genome editing prospects. Molecular Biology Reports. (2021). pp.1-21.

Ali, I., Khan, A.A., Munsif, F., He, L., Khan, A., Ullah, S., Saeed, W., Iqbal, A., Adnan, M. and Ligeng, J. Optimizing rates and application time of potassium fertilizer for improving growth, grain nutrients content and yield of wheat crop. Open Agriculture. (2019). 4(1), pp.500-508.

Ali, F., Yilmaz, A., Chaudhary, H.J., Nadeem, M.A., Rabbani, M.A., Arslan, Y., Nawaz, M.A., Habyarimana, E. and Baloch, F.S. Investigation of morphoagronomic performance and selection indices in the international safflower panel for breeding perspectives. Turkish Journal of Agriculture and Forestry. (2020). 44(2), pp.103-120.

Amin, M., Mohammad, T., Khan, A.J., Irfaq, M., Ali, A. and Tahir, G.R. Yield stability of spring wheat (Triticum aestivum L.) in the North West Frontier Province, Pakistan. Songklanakarin J. Sci. Technol. (2005). 27(6), pp.1147-1150.

Ansari, B.A., M.M. Rajper, A.J., Malik and A.G., Magsi. (1991). Heritability estimates of plant height, yield and yield components in Triticum aestivum L. Pak. J. Agric. Engg. Vet. Sci. 7: 35-40.

Azam, S.M., Mohammad, F., Ahmad, I., Khalil, I.H., Jadoon, S.A. and Nasim, A. Divergence in F3 segregating bread wheat populations. International Journal of Basic and Applied Sciences. (2013). 13(3), pp.94-99.

Cotes, J.M., Crossa, J., Sanches, A. and Cornelius, P.L. A Bayesian approach for assessing the stability of genotypes. Crop Science. (2006). 46(6), pp.2654-2665.

Dutta, S., Kumar, M. and Mukhopadhyay, K. Identification and Validation of Leaf Rust Responsive Wheat isomiRs and their Target Genes in both Wheat and Puccinia triticina. Tropical Plant Biology. (2019). 12(4), pp.318-335.

Ehdaie, B., Alloush, G.A., Madore, M.A. and Waines, J.G. Genotypic variation for stem reserves and mobilization in wheat: II. Postanthesis changes in internode water-soluble carbohydrates. Crop science. (2006). 46(5), pp.2093-2103.

Del Moral, L.G., Rharrabti, Y., Villegas, D. and Royo, C. Evaluation of grain yield and its components in durum wheat under Mediterranean conditions: an ontogenic approach. Agronomy Journal. (2003). 95(2), pp.266-274.

Gul, H., Saeed, B., Khan, A.Z., Haleema, B., Parveen, L. and Badshah, N.L. Morphological and some yield attributes in cultivars of wheat in response of varying planting dates and nitrogen application. Emergence. (2006). 1000, p.2.

Hamam, K.A. and Khaled, A.G.A. Stability of wheat genotypes under different environments and their evaluation under sowing dates and nitrogen fertilizer levels. Australian Journal of Basic and Applied Sciences. (2009) 3(1), pp.206-217.

Ilyas, M., F. Mohammad, I.H. Khalil, M. Arif, W. Khan, Saifullah and S.M. Azam. Yield Potential of F4:7 Bread Wheat Lines under Normal and Late Plantings. International Journal of Basic & Applied Sci. (2013). 13(05). 32-37.

Inamullah, N.H., Shah, Z.H. and FU, K. An analysis of the planting dates effect on yield and yield attributes of spring wheat. Sarhad J. Agric. (2007). 23(2), pp.269-275.

Irfan, M., Muhammad, T., Amin, M. and Jabbar, A. Performance of yield and other agronomic characters of four wheat (Triticum aestivum L.) genotypes under natural heat stress. Int. J. Bot. (2005). 1(2), pp.124-127.

Kakar, K.M., Kakar, Z. and Shawani, M.I. Varietal dynamics of yield stability in wheat. OnLine Journal of Biological Sciences (Pakistan). (2003).

Kattenberg, A. Climate models: projections of future climate (No. CONF-960146-). American Meteorological Society, Boston, MA (United States). (1996).

Khan, A., Jan, M.T., Marwat, K.B. and Arif, M. Organic and inorganic nitrogen treatments effects on plant and yield attributes of maize in a different tillage systems. Pak. J. Bot. (2009). 41(1), pp.99-108.

Khan, A., Jan, M.T., Marwat, K.B. and Arif, M. Organic and inorganic nitrogen treatments effects on plant and yield attributes of maize in a different tillage systems. Pak. J. Bot. (2009). 41(1), pp.99-108.

Kochak-Zadeh, A., Mousavi, S. and Nejad, M. The effect of salinity stress on germination and seedling growth of native and breeded varieties of wheat. J. Novel Appl. Sci. (2013). 12, pp.703-709.

Laghari, K.A., Sial, M.A. and Arain, M.A. Effect of high temperature stress on grain yield and yield components of wheat (Triticum aestivum L.). Journal of Science, Technology and Development. (2012). 31, pp.83-90.

Laghari, K.A., Sial, M.A., Arain, M.A., Mirbahar, A.A., Pirzada, A.J., Dahot, M.U.

and Mangrio, S.M. Heritability studies of yield and yield associated traits in bread wheat. Pak. J. Bot. (2010). 42(1), pp.111-115.

Khan, M.I., Amin, M. and Shah, S.T. Agronomic evaluation of different bread wheat (Triticum aestivum L.) genotypes for terminal heat stress. Pak. J. Bot. (2007). 39 (7... Pak. J. Bot, 39(7), pp.2415-2425.

Munir, I., Swati, M.S., Muhammad, F., Ahmad, R. and Imtiaz, M. Heterosis in different crosses of wheat (Triticum aestivum L.). Sarhad Journal of Agriculture (Pakistan). (1999).

Qamar, M. Genetic variability among wheat cultivars and effect of planting date ongrain and straw yield under double cropping zone of northern areas ofPakistan. Sarhad Journal of Agriculture (Pakistan). (2004).

TAHIR, M., ANWAR, S.A.S., ALI, A. and ISMAIL, M. Assessment of heritability estimates for some yield traits in winter wheat (Triticum aestivum L.). Pak. J. Bot. (2011). 43(6), pp.2733-2736.

Rane, J., Pannu, R.K., Sohu, V.S., Saini, R.S., Mishra, B., Shoran, J., Crossa, J., Vargas, M. and Joshi, A.K. Performance of yield and stability of advanced wheat genotypes under heat stress environments of the Indo-Gangetic plains. Crop science. (2007). 47(4), pp.1561-1573.

Rashid, M.H., Samanta, S.C., Biswas, P., Mannan, M.A. and Zaman, A.K.M.M., Performance of thirty wheat genotypes under late sown conditions in southern region of Bangladesh. Asian Journal of Plant Sciences (Pakistan). (2004).

Razzaq, A., Zada, K. and Shah, P., 1986. Effect of sowing dates and varieties on yield and yield components of wheat in the Peshawar valley [Pakistan]. Sarhad Journal of Agriculture (Pakistan).

Reynolds, M. P. Application of physiology in wheat breeding. Cimmyt, 2001.

Riaz-ud-Din, M.S., Ahmad, N., Hussain, M. and Rehman, A.U. Effect of temperature on development and grain formation in spring wheat. Pak. J. Bot. (2010). 42(2), pp.899-906.

Rosenthal, D.M. and Ort, D.R. Examining cassava's potential to enhance food security under climate change. Tropical Plant Biology. (2012). 5(1), pp.30-38.

Shoran, J. Estimation of variability parameters and path coefficients for certain metric traits in winter wheat (Triticum aestivum L. em. Thell.). Indian J Genet. (1995). 55(4), pp.399-405.

Sial, M.A., Arain, M.A., Khanzada, S.H.A.M.A.D.A.D., Naqvi, M.H., Dahot, M.U. and Nizamani, N.A. Yield and quality parameters of wheat genotypes as affected by sowing dates and high temperature stress. Pakistan Journal of Botany(2005). 37(3), p.575.

So, M.Y., Sha, S., Antoniou, M., Wu, S.S. and Tan-Un, K.C. Mycotoxin Zearalenone induced apoptosis in BEAS-2B cells through generation of ROS and activation of JNK and p38 MAPKs signalling pathways. In 6th SETAC World Congress/SETAC Europe 22nd Annual Meeting. SETAC Europe. The Abstracts Book's web site is located at http://berlin.setac.eu/scientific_programme/d ownload_the_abstracts_book/?contentid=582.(2012).

Subhan, F., Haider, A. and Hatam, M. Effect of sowing dates on yield components of wheat cultivars. Sarhad Journal of Agriculture (Pakistan). (1991).

Vigna, B.B.Z., Jungmann, L., Francisco, P.M., Zucchi, M.I., do Valle, C.B. and de Souza, A.P. Genetic diversity and population structure of the Brachiaria brizantha germplasm. Tropical Plant Biology. (2011). 4(3), pp.157-169.

Wahid, S.A. and Al-Hilfy, I.H. Impact of sowing dates on the growth and straw yield of some bread wheat cultivars and their relationship with accumulated heat units. Arts, humanities and natural sciences conferences. (2017). (Arabic Language)

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ORIGINAL RESEARCH

Unprecedented response of wheat to irrigation levels and various rates of Nano-black carbon

Imran[1,2,3]*

[1]Department of Agronomy, The University of Agriculture, Peshawar, Pakistan
[2]Agriculture Officer, (Extension Wing), Office of the District Director Agriculture Extension, District Kurram, Merged Districts Khyber Pakhtunkhwa, Pakistan
[3]Rhizosphere laboratory, University of Cukurova, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Adana, Turkey,

Correspondence
Imran
*Corresponding author email: imranagrarian@aup.edu.pk

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ABSTRACT

In Khyber-Pakhtunkhwa, Pakistan, wheat yield is subjected to the availability of water and the proper rate of Nano-black carbon in the soil. Delay in the rain and unsuitable soil health cause severe yield reduction. Therefore this experiment was conducted to compare irrigation levels in relation to a different rate of Nano-black carbon to find out the high yielding fact that could enhance wheat productivity and food security. Three different irrigation levels (250-mm, 275-mm and 300-mm), were compared in early growth stages with five different rates of Nano-black carbon (5Mg ha^{-1}, 10Mg ha^{-1}, 15Mg ha^{-1}, 20Mg ha^{-1} and 25Mg ha^{-1}). All other agronomic practices were kept similar for each replication. Data was recorded on different growth parameters such as days to emergence, emergence m^{-2}, plant height, spike length, number of spikes m^{-2}, thousand-grain weight and grain yield. The study confirmed that almost all irrigation levels were prominent but a significant reduction in different parameters was observed with variation in Nano-black carbon that could ultimately affect soil health and productivity. It was concluded that the proper rate of Nano-black carbon can significantly enhance the development of the roots system which may ultimately increase the shoot growth and final yield. Wheat irrigation levels (250 mm) can properly save water and increase wheat productivity in combination with Nano-black carbon amendments.

KEYWORDS: Wheat (*Triticum aestivum* L.), Nano-black carbon, growth, yield, drought, irrigation regimes

1. INTRODUCTION

Wheat (*Triticum aestivum L.)* belongs to the family *Gramineae* and is consumed as a major grain crop of the world (Imran, 2021a). It is also known as the king of cereal and supplies about 60% of the calories and protein of the average diet (Khalil and Jan, 2012). It is ranked 1st among other cereal crops in Pakistan and occupies about 66% of the annual food cropped area (Anonymous, 2012). In Pakistan, wheat is grown on an area of 9204 thousand hectares with an annual production of 9260 thousand hectares with an average yield of 2752kg ha^{-1} (Tunio et al. 2016). Despite higher yield potential, the average yield of wheat in Pakistan is low as compared with advanced countries (Imran, 2021a; Imran et al., 2021a; Imran et al., 2020a). Several factors are responsible for the low productivity of wheat in Pakistan like edaphic factors, cultural practices, genetic factors and environmental conditions. In the current scenario, improved cultural practices and proper nutrient management are the major issues in wheat production. In Pakistan wheat are raising under both, irrigated and rain-fed condition, whereas in Khyber Pakhtunkhawa, almost 70% of wheat is grown under rainfed condition(Imran, 2015a; Imran, 2021b; Imran et al., 2021c). Efforts are in progress to breed for high yield, better protein quality, biotic and abiotic stresses resulting in the development of many cultivars (Al-Maskri et al., 2012). Irrigation levels having the potential for early seedling vigor use available soil water efficiently, resulting in better dry matter accumulation and higher grain yield (Awan et al.,2005). The best irrigation level allows the cropto produce a higher yield under unfavorable conditions (drought, salinity and extreme temperature) (Imran et al., 2020b; Imran et al., 2018; Imran et al., 2021b).

Grain yield of wheat can be reduced with the reduction in irrigation level, whereas the addition of biochar might improve water use efficiency and plant essential nutrients uptake (Jan et al., 2012). Previously it is reported that biochar with different irrigation regimes may show high efficiency and booted yield (Ali et al., 2020; Ali et al., 2021; Imran et al., 2020c; Imran et al., 2021d; Imran et al., 2021e; Imran, 2015b). Donaldson et al. (2013) reported that irrigation regimes resulted in increased wheat straw production and generally higher grain yield compared with various carbon sources. Furthermore, Patel et al. (2010) and Aslam et al. (2013) tested newly introduced irrigation levels, concerning yield and observed that the highest yield (5484kg ha^{-1}) was obtained with the integration of organic sources and the highest irrigation level (Tunio et al. 2016). Likewise, yield reduction of 27% and 52% was noted by Ali et al. (2014) when wheat crop was sown with and without organic amendments. In addition, Tahir et al. (2011) concluded that regardless of irrigation levels, better yields were obtained when wheat was treated with organic sources application before irrigation. Shah and Akmal (2002) reported that grain yield decreased by 28 percent when irrigation was delayed by 45 days in the season. Furthermore, early irrigation favored in high tillering and ultimately in respect of grain and biological yield.

The current study aimed to investigate the effects of different irrigation levels with five different Nano-black carbon rates on wheat crop growth parameters such as days to emergence, emergence m^{-2}, plant height, spike length, number of spikes m^{-2}, thousand-grain weight and grain yield. Furthermore, we aimed to evaluate the suitable level of Nano-black carbon under less irrigation to find out the high yielding fact that could enhance wheat productivity and food security

2. MATERIALS AND METHODS

2.1 Experimental Site

Wheat performance under different irrigation levels and various rates of Nano-black carbon for grain yield was studied under field conditions during the winter season of 2018-19 and 2019-20. The soil of the experimental site was clay loam with less organic matter and slightly acidic. Experiments were conducted in Randomized Complete Block Design with split plots arrangement having three replications. Nano-black carbon was allotted to the main plots while Irrigation levels were assigned to sub-plots. Each experimental unit was 1.5 m x 3.3, accommodating 11 rows equally spaced at 30cm. Initially, all sowings were done at a uniform seeding rate at the rate of 100 kg ha^{-1}. However, the desired population was maintained by manual thinning. Phosphorous at the rate of 90 kg ha^{-1} was applied in the form DAP. All the phosphorous and ½ of the nitrogen was applied at the time of sowing and Nano-black carbon was applied at the time of seedbed preparation. The remaining ¼ nitrogen was top-dressed with first irrigation and ¼ with second irrigation. The crop was sown on a well-prepared seedbed using a seed rate of 120 kg ha^{-1}. All other agronomic practices were kept normal and uniform for all the treatments

Following factors were studied during the experiment, Nano-black carbon were applied to the main plot at the rate of 5 Mg ha^{-1}, 10 Mgha^{-1},15 Mg ha^{-1}, 20 Mg ha^{-1}and 25 Mg ha^{-1}while irrigation level were treated to sub-plots at the rate of 250 mm, 275 mm and 300 mm respectively.

2.2 Procedure for Recording Data

Data regarding days to emergence was recorded by counting the days taken from the date of sowing to the date when 50% emergence occur in each plot. Data on emergence m^{-2} was recorded in two central rows by using the following formula. Data on the number of tillers was recorded by counting the numbers of tillers in central two rows of each plot and was then converted into numbers of tillers m^{-2}. Data on plant height was recorded by measuring randomly selected 10 plants in each plot from the base of the plant to the tip of spikes excluding awns at physiological maturity. Data on spike length was recorded by using a plastic scale of length 30cm.Data regarding grain spike^{-1} was recorded by counting wheat grains in randomly selected five spikes in each plot and was averaged accordingly. Data on grain yield was recorded by harvesting the two central rows in each plot and was sun-dried, weighed, and then converted to kg ha^{-1}. Data regarding thousand grains weight was recorded on the sensitive electronic balance after counting a thousand grains for each plot.

2.3 Statistical Analysis

The recorded data were statistically analyzed according to the analysis of variance techniques used for randomized complete block design and the least significant difference (LSD) was used at a 5% level of significance (P≤0.05) upon significant F-test through the procedure described by Jan et al. (2009).

3. RESULTS AND DISCUSSION

3.1 Days to Emergence (m^{-2})

Data regarding days to emergence (DE) in response to different irrigation levels and Nano-black carbon showed significant differences in emergence timing (Table 1). Earlier emergence was noted with 5 Mg ha^{-1}followed by 10 Mg $ha^{-1}$and 15 Mg ha^{-1} having at par values while delayed emergence was recorded with 25 Mg ha^{-1}followed by 20 Mg ha^{-1}respectively (Fig 1). The reason for emergence deviation might be due to environmental factors which influence enzyme activation and rupturing of seed coat and ultimately seed emergence. The optimum temperature activates most of the enzymes in aleoron layer of the seed coat which activates the embryo of the seed and leads to early emergence. The possibility of early emergence in 5 Mg ha^{-1} might be due to optimum temperature in field conditions and enhanced emergence as compared to other Nano-black carbon. The delayed emergence might be due to environmental stress in which seeds do not react well and become dormant due to harsh components of the environment (minimum temperatures, rainfall, humidity, winds and sunshine, etc) and may take more days to emergence. Similarly, in the case of wheat Irrigation levels, earlier emergence was produced by 275mm irrigation followed by 250 mm irrigation level whereas the delayed emergence was noted 300 mm irrigation. This might be the genetic character of the variety and leading to early or late emergence. These results are in connection with the findings of Imran et al. (2020) who reported that differences in the total roots of a young seedling of different wheat Irrigation levels were affected by different moisture levels and Irrigation levels. While their interactions were non-significant. These results are supported by Shah et al. (2011) who reported that reduction in moisture content reduces the seminal root length. The difference in root length is the cause of survival of a seedling in stresses and its re-growth potentials under abnormal situations. The better the root grows with a relatively longer length may result inthe better establishment of the seedling and withstand against abnormal situations e.g. drought, high temperatures and salt stress to convert the seedling to a healthy plant for production (Khan et al., 2014).

3.3 Emergence (m^{-2})

Data regarding emergence per m^{-2} of different wheat Irrigation levels (Table 2) showed significant differences in seedling emergence of different wheat Irrigation levels as affected by different Nano-black carbon. More seedlings were counted with seeds sown on 20 Mg ha^{-1} followed by 5 Mg ha^{-1} and 15 Mg ha^{-1} having at par values while less number of emerged seedlings were counted with 10Mg ha^{-1} and then by 25 Mg ha^{-1} respectively (Fig 2). The reason for emergence deviation might be due to environmental factors which influence enzyme activation and rupturing of seed coat and ultimately seed emergence.

The optimal temperature activates the majority of the enzymes in the aleoron layer of the seed coat, wakes up the embryo of the seed, and results in early emergence. When compared to other Nano-black carbon rates, the prospect of early emergence in 5 Mg ha^{-1}

Table 1. Days to emergence as influenced by different Nano-black carbon rates and irrigation levels (Data pooled over the both years)

Nano-black carbon (Mg ha^{-1})	Irrigation levels			Mean
	250 mm	275 mm	300 mm	
5	7.333	6.333	7.333	7.000d
10	9.000	10.333	9.333	9.556c
15	9.000	9.333	9.333	9.222c
20	12.333	10.000	11.333	11.222b
25	20.667	21.333	24.667	22.222a
	11.667ab	11.467b	12.400a	

LSD value ($P \leq 0.05$) for Irrigation levels = 0.6
LSD value ($P \leq 0.05$) for Nano-black carbon = 0.8

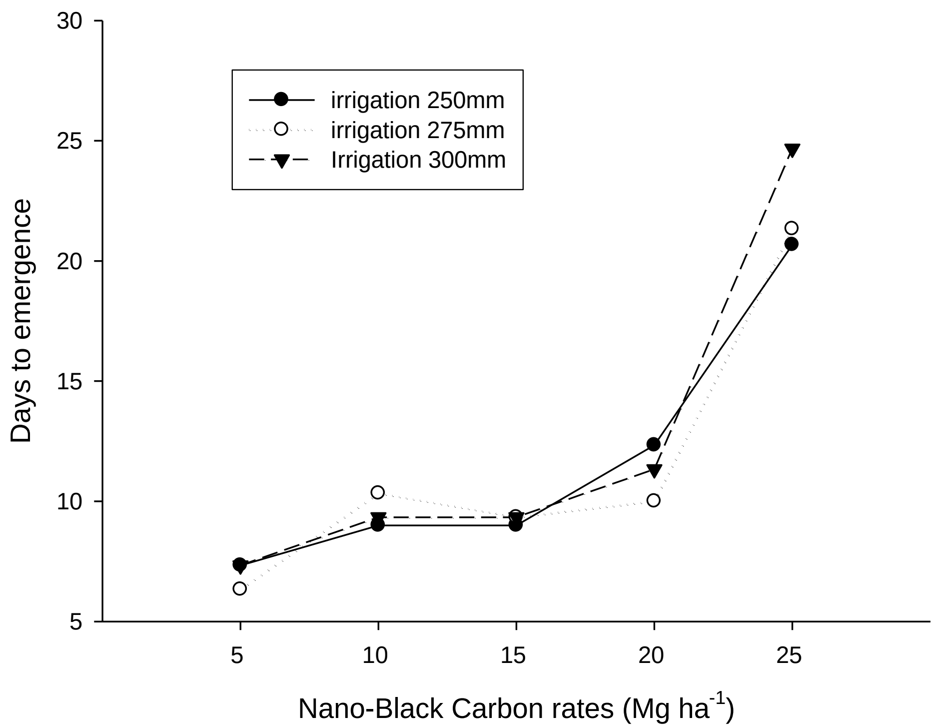

Figure 1. Response of days to emergence to different irrigation levels and Nano-black carbon

may boost emergence. The emergence may be due to environmental stress, in which seeds do not react effectively and go latent as a result of severe environmental components, and it may take additional days for the seeds to emerge. Similarly, more seedlings were counted with 250mm irrigation, followed by 275mm irrigation, however minimal emergence (m-2) was found with 300mm irrigation having statistically equal values with 275mm irrigation. This might be due to the genetic characteristics of the variety, resulting in early or late emergence. Different moisture levels and Irrigation levels have an impact on Irrigation levels. Even though their encounters were insignificant. According to literature, the root is the most important part of a plant and has strived to be the best until the availability and search for water to support plant development (Ozham and Hajibabaei, 2014). The more roots a plant has, the healthier it is, the better it can withstand drought and wind, and the seedling has a better chance of survival. Our findings are confirmed by the findings of Abdoli and Saeidi (2012), who revealed that decreasing the moisture level reduces the radical weight of different wheat irrigation levels. Water stress inhibits the mobilization of starchy endosperm in various species (Bouaziz and hicks 1990). Increasing early growth has the potential to increase soil N (Pang et al., 2014) and P (Ryan et al., 2014) absorption, hence improving crop nutrient-use efficiency and weed competitiveness (Coleman et al., 2001).

3.4 Number of Tillers (m^{-2})

Data on the number of tillers per m-2 revealed substantial changes in the number of tillers per m-2 of different irrigation levels as impacted by varied Nano-black carbon concentrations (Table 3). The different Nano-black carbon effect was non-significant for the number of tillers m^{-2} showing statistically at par value for all Nano-black carbon. The Irrigation levels effect was significant and more tillers were counted with 275 mm irrigation followed by 250 mm irrigation whereas the tinniest tillers m^{-2} were recorded with 300 mm irrigation (Fig 3). Another possible reason could be due to environmental factors which influence enzyme activation. The optimum temperature may enhance plant growth and development. The possibility might be due to optimum temperature in field conditions and enhanced plant lateral growth and branches initiations. These results are in connection with the findings of Imran et al. (2020) who reported that differences in the total roots of a young seedling of different wheat irrigation levels were affected by different moisture levels and Irrigation levels. While their interactions were not significant.

The possible reason could be increased early growth has the potential to increase soil N (Pang et al., 2014) and P (Ryan et al., 2014) absorption, hence improving crop nutrient-use efficiency and weed competitiveness (Coleman et al., 2001).

Table 2. Emergence m^{-2} as influenced by different Nano-black carbon rates and irrigation levels (Data pooled over the both years).

Nano-black carbon (Mg ha^{-1})	Irrigation levels			Mean
	250 mm	275 mm	300 mm	
5	188.33	135.00	139.00	154.11a
10	77.00	83.67	62.00	74.22b
15	128.00	105.33	73.67	102.33b
20	200.67	166.00	158.67	175.11a
25	104.00	87.33	110.00	100.44b
	139.60a	115.47b	108.67b	

LSD value ($P \leq 0.05$) for wheat Irrigation levels = 0.7
LSD value ($P \leq 0.05$) for Nano-black carbon = 0.9

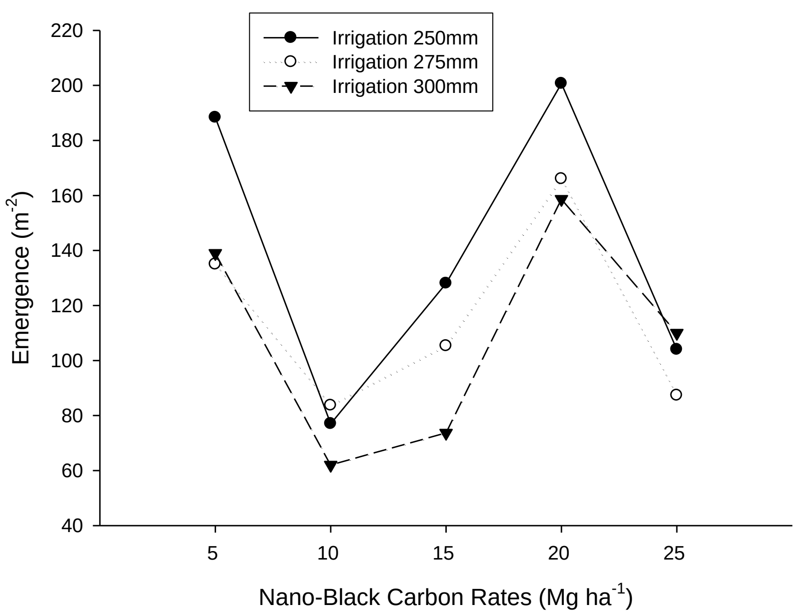

Figure 2. Response of emergence m^{-2}to different irrigation levelsandNano-black carbon

More robust crops collect more light, maximizing crop growth rates and biomass, especially with late planting or in settings with shorter crop duration (Takahashi and Gotoh, 1996; Regan et al., 1997). Appropriate variety selection based on seedling performance is thus essential for higher yield and crop growth under abnormal conditions, such as drought stress, which is most commonly encountered by wheat crop in the country as well as in the province where more than 66 percent of the area is rain-fed.

3.5 Plant Height (cm)

Data regarding plant height (Table 4) divulge that the highest plant height was recorded with 10 Mg ha^{-1} followed by statistically similar values of 15 Mg ha^{-1} and 5 Mg ha^{-1}. The dwarf plants were produced when the wheat Irrigation levels were sown on 25 Mg ha^{-1} and 20 Mg ha^{-1} respectively. Among the irrigation levels, the highest plant height was noted in irrigation level of 300 mm followed by 250 mm having statistically similar values. The lowest plant height was produced by 275 mm irrigation rate respectively. The possibility might be due to optimum temperature in field conditions and enhanced emergence as compared to other Nano-black carbon (Fig 4). The explanation might be related to environmental fluctuations in temperature, rainfall, humidity, and so on, which cause extra days to develop. Similarly, more seedlings were counted with 50 mm irrigation level, followed by 275 mm, although minimal emergence (m-2) was detected with 300 mm irrigation level having statistically identical values with 275 mm. This might be due to the genetic characteristics of the variety, resulting in early or late emergence. These findings are consistent with those of Imran et al. (2020), who discovered that variations in the total roots of a young seedling of various wheat Irrigation levels were influenced by moisture and Irrigation levels. Even though their encounters were insignificant. Variety Hashim-08 has the greatest root number in the group, followed by Pak-2013, DN-84, and Pirsabak-2005. While Kpk-2015 has the lowest amount of roots reported. It is reported that root is the fundamental portion of a plant and has striven to the best till the availability and search of water to sustain plant growth (Ozham and Hajibabaei, 2014). When the quantity of roots rises, the crop becomes more resilient to drought and wind, and the seedling has a better chance of surviving even when less water is available. More vigorous crops collect more light, maximizing crop growth rates and biomass, especially when planting late or in settings where crop duration is limited (Takahashi and Gotoh, 1996; Regan et al., 1997). Appropriate variety selection based on seedling performance is thus essential to be taken into account for higher yield and crop growth under abnormal circumstances, such as drought stress, which is most commonly faced by wheat crops in the country as well as in the province where more than 66 percent of the area is rain-fed.

3.6 Spike Length (cm)

Mean values of the data revealed that the highest spike length was recorded with 15 Mg ha^{-1} followed by statistically similar values of 5 Mg ha^{-1} and 10 Mg ha^{-1}(Table 5). Minimum spike length was recorded in those plots which were sown on 25 Mg ha^{-1} or either 20 Mg ha^{-1}. Among the Irrigation levels, lengthy spikes were produced with 300 mm followed by statistically similar 250 mm and 275 mm irrigation levels (Fig 5). The possibility might be due to optimum temperature in field conditions and enhanced emergence as compared to other Nano-black carbon.

Table 3. Number of tillers m^{-2}as influenced by different Nano-black carbon rates and irrigation levels (Data pooled over the both years).

Nano-black carbon (Mg ha^{-1})	Irrigation levels			Mean
	250 mm	275 mm	300 mm	
5	200.33	229.33	144.67	191.44
10	184.00	223.00	140.67	182.56
15	171.00	233.00	148.33	184.11
20	191.33	184.00	187.67	187.67
25	217.33	179.33	169.67	188.78
	192.80ab	209.73a	158.20c	

LSD value (P≤ 0.05) for wheat Irrigation levels = 10.3
LSD value (P≤ 0.05) for Nano-black carbon = 11.2

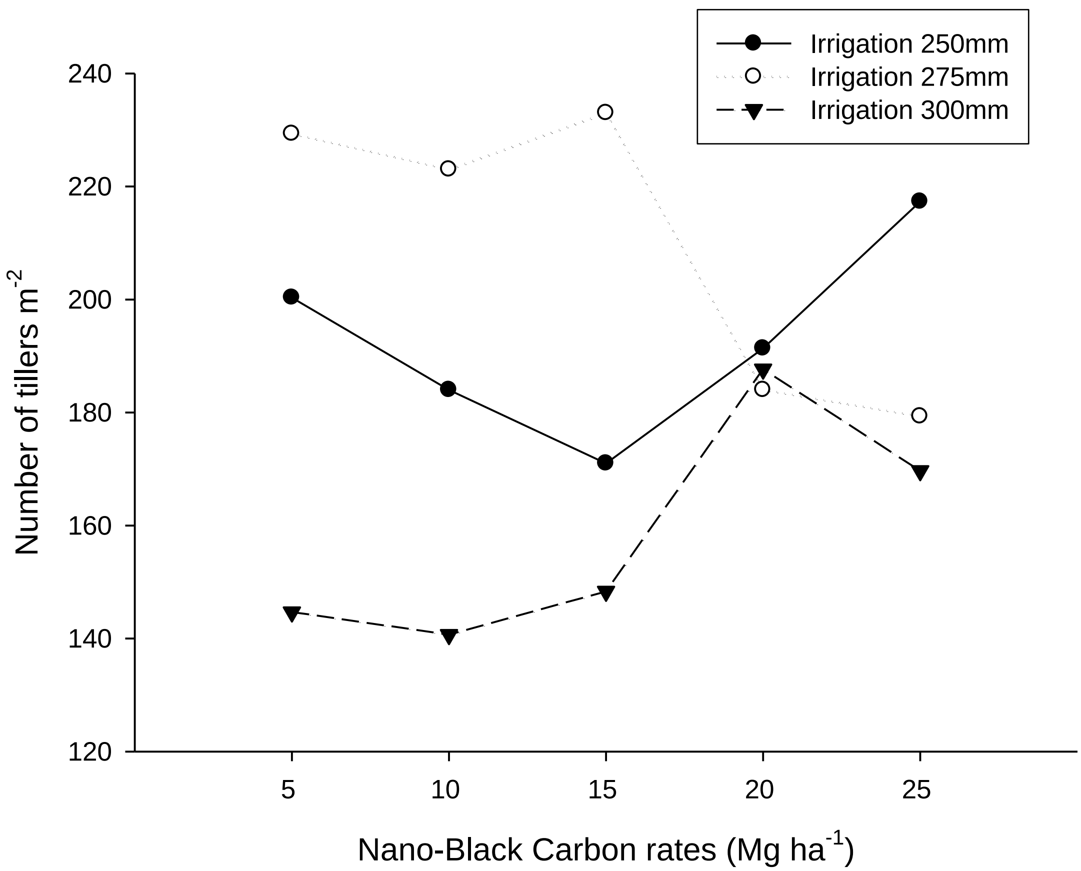

Figure 3. Response of number of tillers m^{-2}to different Irrigation levelsandNano-black carbon

Table 4. Plant height (cm) as influenced by different Nano-black carbon rates and irrigation levels (Data pooled over the both years).

Nano-black carbon (Mg ha^{-1})	Irrigation levels			Mean
	250 mm	275mm	300 mm	
5	101.83	90.32	94.30	95.48ab
10	107.21	97.42	112.75	105.79a
15	90.51	94.95	107.54	97.67ab
20	92.11	92.11	96.77	93.66b
25	86.52	80.65	92.72	86.63b
	95.64ab	91.09b	100.82a	

LSD value ($P \leq 0.05$) for wheat Irrigation levels = 10.3
LSD value ($P \leq 0.05$) for Nano-black carbon = 11.2

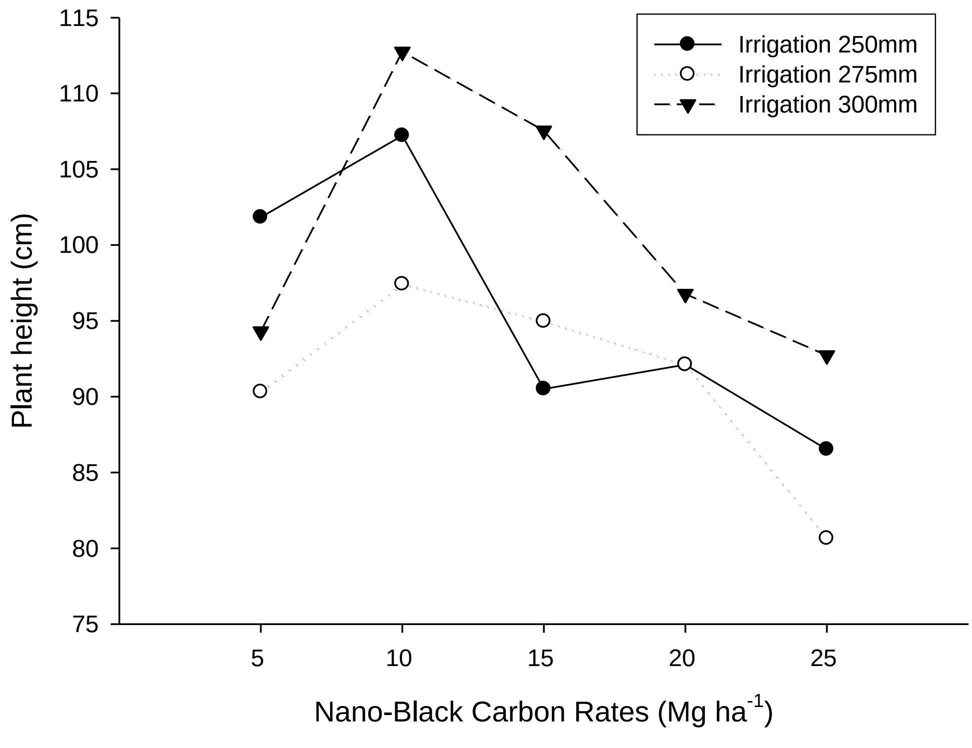

Figure 4. Response of plant height to different irrigation levels and Nano-black carbon

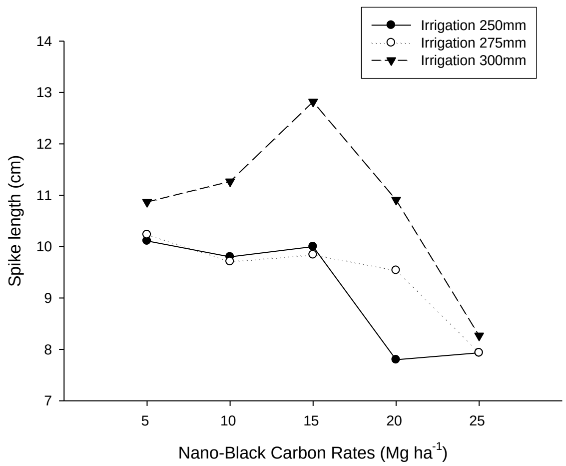

Figure 5. Response of spike length to different Irrigation levels and Nano-black carbon.

The enlarged plant might be attributed to temperature, and similarly, more seedlings were counted with 250 mm irrigation, followed by 275 mm, and the least emergence (m-2) was reported with 300 mm irrigation. These findings are consistent with the findings of Imran et al. (2020), who discovered variances in the total roots of early seedlings of different wheat varieties. Different moisture levels and Irrigation levels affectedthe irrigation levels. While their encounters were insignificant. Increasing crop nutrients, vigorous crop growth, and biomass have the potential to boost crop nutrients, vigorous crop growth, and biomass, especially with late planting or in conditions where crop duration is shorter. Drought stress is the most prevalent problem that wheat faces and 66 percent of the area are used to support plant development.

3.6 Thousand Grain Weight (g)

Data regarding thousand-grain weight of different wheat Irrigation levels as influenced by different Nano-black carbon(Table 6). The mean values divulge that more thousand-grain weight was produced by those plots which were sown on 5 Mg ha^{-1} followed by 10Mg ha^{-1} whereas minimum thousand seed weight was recorded with 20 Mg ha^{-1} followed by 15 Mg ha^{-1} and 25 Mg ha^{-1} respectively (Fig 6). The Irrigation levels were non-significant for thousand seed weight and noted that all the cultivars produced statistically similar thousand-grain weight. The fluctuation in thousand-grain weight with different Nano-black carbon might be due to optimum temperature in field conditions and enhanced emergence as compared to other Nano-black carbon. Plant phenology can be improved by using nano-black carbon and irrigation. These findings are related to the work of Imran et al. (2020), who discovered that variances in the total roots of a young seedling were impacted by varying moisture levels.

Biochar increases soil N and P to improve crop nutrient utilization efficiency and weed competitiveness (Coleman et al., 2001). In the case of Irrigation levels comparison, the highest grain yield was recorded with the sowing of the cultivar 250 mm followed by 300 mm having statistically similar produce. More vigorous crops collect more light, maximizing crop growth rates and biomass, especially when planting late or in settings where crop duration is limited (Takahashi and Gotoh, 1996; Regan et al., 1997).

Table 5. Spike length (cm)as influenced by different Nano-black carbon rates and irrigation levels (Data pooled over the both years).

Nano-black carbon (Mg ha^{-1})	Irrigation levels			Mean
	250 mm	275mm	300 mm	
5	10.110	10.233	10.867	10.403ab
10	9.800	9.710	11.267	10.259ab
15	10.00	9.843	12.820	10.888a
20	7.800	9.537	10.910	9.416b
25	7.933	7.933	8.267	7.857c
	9.129b	9.339b	10.826a	

LSD value (P≤ 0.05) for wheat Irrigation levels = 10.3
LSD value (P≤ 0.05) for Nano-black carbon = 11.2

Table 6. Thousand grain weight (g) as influenced by different Nano-black carbon rates and irrigation levels (Data pooled over the both years).

Nano-black carbon (Mg ha^{-1})	Irrigation levels			Mean
	250 mm	275 mm	300 mm	
5	46.667	53.333	46.667	48.889a
10	46.667	33.333	40.000	40.000b
15	33.333	30.000	36.667	33.33bc
20	33.333	33.333	30.000	32.222c
25	36.667	33.333	30.000	33.333bc
	39.333a	36.667a	36.667a	

LSD value (P≤ 0.05) for wheat Irrigation levels = 10.3
LSD value (P≤ 0.05) for Nano-black carbon = 11.2

Table 7. Grain yield (kg ha^{-1}) as influenced by different Nano-black carbon rates and irrigation levels (Data pooled over the both years).

Nano-black carbon (Mg ha^{-1})	Irrigation levels			Mean
	250 mm	275 mm	300 mm	
5	4404.2	4579.3	4656.2	4546.6 a
10	3757.6	1777.8	3609.2	3048.2 b
15	3339.9	1764.2	1670.1	2258.1 bc
20	2122.6	1631.0	1973.3	1908.9 cd
25	1710.6	888.7	1333.4	1310.9 d
	3067.0a	2128.2b	2648.4ab	

LSD value (P≤ 0.05) for wheat Irrigation levels = 10.3

LSD value (P≤ 0.05) for Nano-black carbon = 11.2

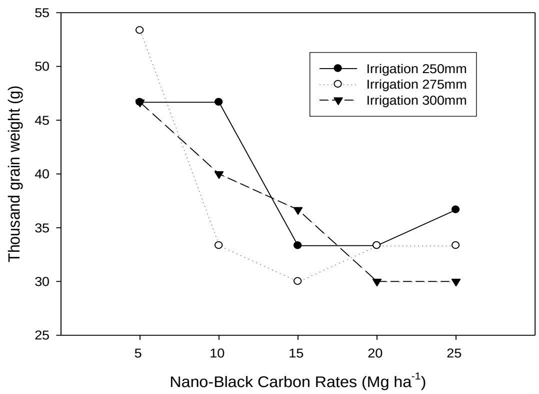

Figure 6. Response of thousand-grain weight to different Irrigation levels and Nano-black carbon

Appropriate variety selection based on seedling performance is thus essential to be taken into account for higher yield and crop growth under abnormal circumstances, such as drought stress, which is most commonly faced by wheat crop in the country as well as in the province where more than 66 percent of the area is rain-fed. Despite the fact that their encounters were insignificant.

3.7 Grain Yield (kg ha^{-1})

Data regarding grain yield of different wheat Irrigation levels as influenced by different Nano-black carbon (Table 7). Mean data revealed that the highest wheat grain yield was produced by those plots which were sown on 5 Mg ha^{-1} Followed by 10 Mg ha^{-1} having at par value with 15 Mg ha^{-1}. The lowest grain yield was produced when the plots were sown on 25 Mg ha^{-1} followed by 20 Mg ha^{-1} respectively (Fig 7). The lowest grain yield was noted with the sowing of the cultivar 275 mm. Generally, it was concluded that the most productive cultivar was 250 mm having the supreme grain yield as compared to other sown cultivars.

Among the Nano-black carbon, either 5 Mg ha^{-1}for wheat production was noted the outmost promising factor to overcome on food hunger and inconsistency.

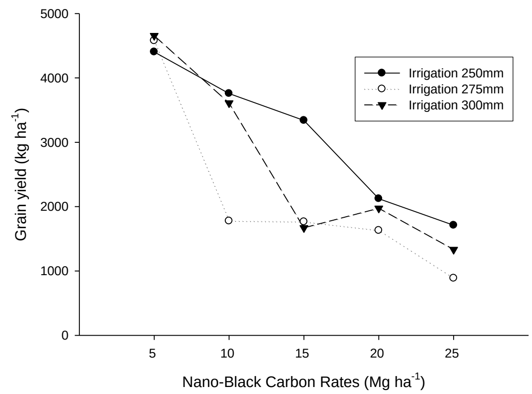

Figure 7. Response of grain yield to different Irrigation levelsandNano-black carbon

Imran et al. (2020) stated that plant phenology may be enhanced by employing Nano-black carbon and watering. They also demonstrated that more vigorous crops are taken into account for greater yield and crop growth in atypical situations. Variety Hashim-08 has the greatest root number of the bunch, followed by Pak-2013, DN-84, and Pirsabak-2005. While Kpk-2015 has the fewest roots. According to reports, the root is the most important part of a plant and has strived to be the best until the availability and search for water to support plant development (Ozham and Hajibabaei, 2014). The bigger the root number, the healthier the plant, the better it will be able to withstand drought and wind, and the seedling will have a better chance of survival if water is made accessible at any stage throughout the crop's growth.

4. CONCLUSION

According to the findings of this study, a correct rate of Nano-black carbon can considerably improve the development of the root system, which may ultimately boost shoot growth and final yield. The current investigation found that wheat Irrigation levels (250 mm) may germinate properly even at low moisture and varying rates of Nano-black carbon. Variation in sowing date decreases growth and other yield-related qualities in a linear fashion. Different Irrigation levels responded differently to different Nano-black carbon, indicating that there is potential for additional development in genotype. It is necessary to develop such irrigation levels that can be grown in every environment and have changeable Nano-black carbon capacity to resist diverse environmental influences.

Data Availability statements: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

A R M Al-Tawaha., A Al-Tawaha., S N Sirajuddin, Imran, A M Qaisi…Ecology and adaptation of legumes crops: A review. IOP Conf. Series: Earth and Environmental science. (2020).p 492 doi:10.1088/17551315/492/1/012085

Abdel Rahman M. Al-Tawaha, Nusrat Jahan Imran. Growth, Yield And Biochemical Responses In Barley To DAP And Chitosan Application Under Water Stress. Journal Of Ecological Engineering. (2020).

Abdoli, M. and M. Saeidi. Effects of water deficiency stress during seed growth on yield and its components, germination and seedling growth parameters of some wheat cultivars. Int. J. Agric. Crop Sci. (2012). 4(15): 110-118.

Abdoli, M. and Saeidi, M. Effects of water deficiency stress during seed growth on yield and its components, germination and seedling growth parameters of some wheat cultivars. Inter J Agric Crop Sci. (2012). *4*(15):1110-1118.

Ali, I., He, L., Ullah, S., Quan, Z., Wei, S., Iqbal, A., Munsif, F., Shah, T., Xuan, Y., Luo, Y. and Tianyuan, L. Biochar addition coupled with nitrogen fertilization impacts on soil quality, crop productivity, and nitrogen uptake under double-cropping system. Food and Energy Security. (2020). 9(3), p.e208.

Ali, I., Ullah, S., He, L., Zhao, Q., Iqbal, A., Wei, S., Shah, T., Ali, N., Bo, Y., Adnan, M. and Jiang, L. Combined application of biochar and nitrogen fertilizer improves rice yield, microbial activity and N-metabolism in a pot experiment. PeerJ. (2020). 8, p.e10311.

Al-Maskri, A.H., M. Sajjad and S.H. Khan. Association mapping: a step forward to discovering new alleles for crop improvement. Int. J. Agric. Biol. (2012). 14: 153–160.

Amanullah, Inamullah, Mona Alwahibi, Mohamed Elshikh,Jawaher Alkahtani, Asim Muhammad, Shah Khalid, Imran, Manzoor Ahmad, Nangial Khan, Saif Ullah, Izhar Ali. 2020. Phosphorus and Zinc Fertilization Improve Zinc Biofortification in Grains and Straw of Coarse vs. Fine Rice Genotypes. Agronomy (2020). 10, 1155;doi:10.3390/agronomy10081155

Amanullah., Inamullah., Jawaher, A., MohamedSolman Elshikh, Mona S. Alwahibi, Asim Muhammad, Imran and Shah Khalid. 2020. Phosphorus and Zinc Fertilization Improve Productivity and Profitability under Rice-Wheat Cropping System. Agronomy. (2020). 10, 1085; doi:10.3390/agronomy10081085

Amir Zaman Khan, Imran, Asim Muhammad, Aiman Khalil, Hasina Gul, Habib Akbar and Said Wahab. Impact of fertilizer priming on seed germination behavior and vigor of maize. Pure and Applied Biology. (2016). Vol. 5, Issue 4, pp744-751.http://dx.doi.org/10.19045/bspab.2016.50093

Araus, J.L., G.A Slafer., M.P. Reynolds and C. Royo. Plant Breeding And Drought In C3 Cereals,What Should We Breed For? Annuals of Bot. (2002). 89: 925-940.

Atta, M., T. Mahmood and R.M. Trethowan. Relationship between root morphology and grain yield of wheat in north Western Australia. Aus. J. Crop Sci. (2013). 7(13): 2108-2115.

Awan, S.I., M.F.A. Malik and M. Siddique. Study on seedling traits for drought tolerance in wheat under moisture stress conditions. SAARC J. Agric. (2005). 3: 247–254.

Baloch, M.J., J. Dunwell, A.A. Khakwani, M. Dennet, W.A. Jatoi and S.A. Channa. Assessment of wheat cultivars for drought tolerance via osmotic stress imposed early seedling growth stages. J. Agric. Res. (2012). 50(3): 299-310.

Bouaziz, A. and Hicks, D.R. Consumption of wheat seed reserves during germination and early growth as affected by soil water potential. Plant and soil. (1990). 128(2):161-165.

Cauvain, S. and C.P. Cauvain. Bread making. CRC press (2003). p. 540.

Coleman, R.K., G.S Gill and G.J Rebetzke. Identification of quantitative trait loci (QTL) for traits conferring weed competitiveness in wheat (Triticum*aestivum* L.).Aus. J. Agric. Res. (2001). 52: 1235-1246.

Dhandas, S.S., G.S Sethi and R.K Behl. Indices of drought tolerance in wheat genotypes at early stages of plant growth. J. Agron. And Crop Sci. (2004). 190: 6-12.

Emam, Y. and E. Bijanzadeh. Water uptake and hydraulic conductivity of seminal and adventitious roots of five wheat cultivars at early growth stage. J. Agr. Sci. Tech. (2012). 14: 1605-1616.

Fischer, R.A. Growth and yield limitation to dry land wheat yield in Australia; a physiological framework. J. Aus. Inst. Agric. Sci. (1979). 45: 83-94.

Gulnaz, S., S.H. Khan, M. Shahzad, W. Nasim and M. Sajjad. Genetic evaluation of spring wheat (*Triticum aestivum* L.) germplasm for yield and seedling vigor traits. J. Agric. Soc. Sci. (2012). 8: 123–128.

Imran, A. Assessment of chemical and manual weed control approaches for effective weed suppression and maize productivity enhancement under maize-wheat cropping system. Gesunde Pflanzen. (2021b). 74, 9-26.

Imran, A. Influence of compost application and seed rates on production potential of late sown maize on high elevation in Swat-Pakistan. J Env Earth Sc5. (2015a). 36-40.

Imran, A., Arif, M., Shah, Z., and Bari, A. Soil application of Trichoderma and peach (Prunus persica L.) residues possesses biocontrol potential for weeds and enhances growth and profitability of soybean (Glycine max). Sarhad Journal of Agriculture. (2020b). 36, 10-20.

Imran, A., Bari, A., and Ali, R. Peach sources, phosphorous and beneficial microbes enhance productivity of soybean. Soy Res. (2018). 16, 39-48.

Imran, A., Hussain, I., Ali, I., Ullah, S., Iqbal, A., AR-Al Tawaha, A., Al-Tawaha, D., Sangeetha, J., Saranraj, A. R. P., and Al Sultan10, W. Agricultural soil reclamation and restoration of soil organic matter and nutrients via application of organic, inorganic and bio fertilization (Mini review). OIC conference series. (2021b).

Imran, A., Khalid, Shah, Arif, M., Fahad, S., and Al-Tawaha, A. R. M. The Application of Biochar for the Mitigation of Abiotic Stress-Induced Damage. In "Sustainable Soil and Land Management and Climate Change", (2021c). pp. 45-56. CRC Press.

Imran, A., Mahmood, T., Sajid, M., Altawaha, A. R., Al-Tawaha, A. R., and Qaisi, A. M. Improvement of Rice Quality via Biofortification of Selenium, Iron, and Zinc and Its Starring Role in Human Health. Rice Research for Quality Improvement: Genomics and Genetic Engineering, (2020c). 699.

Imran, A., Naveed, S, Khan, I., Sajid, M., Mahmood, T., Hussain, I., Ilyas, M., Ali, I., Ullah, S., Kamal, A., and Altawaha, A. Relationship of soil physico chemical properties with elevation and geographical directions. In "IOP Conference Series: Earth and Environmental Science". (2021d). Vol. 788, pp. 012172. IOP Publishing.

Imran, A.A. Khan, I.U. Khan and S. Naveed. Weeds density and late sown maize productivity influenced by compost application and seed rates under temperate environment. Pak. J. Weed Sci. Res. (2016). 22(1): 169-181.

Imran, A.A. Khan, Inamullah and Luqman. Weeding stages and their effect on yield and yield components of rice in upper Swat, Pakistan. Pak. J. Weed Sci. Res. (2015). 21(4): 555-563.

Imran, Abdul Bari, Roshan Ali, Naeem Ahmad, Zulfiqar Ahmad, Muhammad Idrees Khattak, Akhtar Ali, Fayaz Ahmad, Inayatullah Khan And Shahida Naveed. Traditional Rice Farming Accelerate CH4 &N2O Emissions Functioning As A Stronger Contributors Of Climate Change. Int J Environ Sci Nat Res. (2017). DOI: 10.19080/ARTOAJ.2017.09.555765

Imran, Amanullah and M. Al-Tawaha, A.R. The Productivity of subsequent wheat enhanced with residual carbon sources and phosphorus under improved irrigation system. Communications in Soil Science and Plant Analysis. (2020). 51(10), pp.1306-1314.

Imran, Amanullah, A. Bari and R. Ali. Peach sources, phosphorous and beneficial microbes enhance productivity of soybean. Soy. Res. (2018). 16(2): 39-48.

Imran, Amanullah, Abdel Rehman Al-Tawaha. Carbon sources application increase wheat yield and soil fertility. Communication in soil science and plant analysis. (2021).

Imran, Amanullah, Ali khan, A., Mahmood, T., Al Tawaha, A. R., and Khanum, S. Adequate Fertilization, Application Method and Sowing Techniques Improve Maize Yield and Related Traits. Communications in Soil Science and Plant Analysis. (2021a). 52, 2318-2330.

Imran, Amanullah, and M. Al-Tawaha, A. R. (2020a). The Productivity of Subsequent Wheat Enhanced with Residual Carbon Sources and Phosphorus under Improved Irrigation System. Communications in Soil Science and Plant Analysis51, 1306-1314.

Imran, Amanullah, Muhammad Arif, Zahir Shah and Abdul Bari. Integration of Peach (*Prunus persica* L.) residues, beneficial microbes and phosphorus enhance phenology, growth and yield of soybean. ISSN 1068-3674, Russian Agricultural Sciences, 2020, Vol. 46, No. 3, pp. 223–230. © Allerton Press, Inc., 2020.

Imran, Amanullah, Muhammad Arif, Zahir Shah and Abdul Bari. Soil application of *Trichoderma* and Peach (*Prunus persica* L.) residues possesses biocontrol potential for weeds and enhances growth and profitability of soybean (Glycine max). Sarhad Journal of Agriculture. (2020). 36 (1) 10-20. DOI | http://dx.doi.org/10.17582/journal.sja/2020/36.1.10.20

Imran, Amanullah.. Review. Global impact of climate change on water, soil resources and threat towards food security: evidence from Pakistan. Adv Plants Agric Res. (2018) 8(5):350–355. DOI:10.15406/apar.2018.08.00349

Imran, I., Amanullah, A., and M. Al-Tawaha, A. RThe productivity of subsequent wheat enhanced with residual carbon sources and phosphorus under improved irrigation system. Communications in Soil Science and Plant Analysis. (2021e). 51, 1306-1314.

Imran, K., Asad Ali Weeding stages and their effect on yield and yield components of rice in upper Swat-Pakistan. Pakistan Journal of Weed Science Research. (2015b). 21.

Imran, Shahida Naveed, Asad Ali Khan And Inayat Khattak. Impact Of Phosphorus Levels And Seed Rates On Growth And Yield Of Late Sown Maize On High Elevation In Swat, Pakistan. Pakistan J. Agric. Res. (2015). Vol. 28 No. 4:406-413

Imran, Zada H, Naveed S, Khattak I And Ahmad S. Variable Rates Of Phosphorous Application Influenced Phenological Traits Of Green Gram (*Vignaradiata* L.) J Agri Res (2016). 1(3).

Imran. The bioavailability of phosphorus in composite vs. hybrid maize differ with phosphorus and boron fertilization. Phosphorus, Sulfur, and Silicon and the Related Elements. (2021a). 1-13.

Izhar Ali, Asad Ali Khan, Imran, inamullah, Aman Khan. Humic acids and Nitrogen levels optimizing productivity of green gram (vigna radiate L.). Russian Agriculture Sciences. (2019). 45(1), 43-47.

Jajarmi, V. Effects of Water Stress On Germination Indices in Seven Wheat Cultivar 2009. World Acad. Sci. Eng. Tech. (2007).

Khan, M.A., B. Gul andD.J.weber. Action Of Plant Growth Regulators And Salinity on the seed germination of *ceratoideslanata*. Can. J. Bot. (2004). 82:37-42.

Khan, M.Q., S. Anwar and M.I. Khan, Genetic variability for seedling traits in wheat (*Triticum aestivum* L.) Under moisture stress conditions. Asian J. Plant Sci. (2002). 5: 588–590.

Khola Rafique, Seogchan Kang, Aziz-Ud-Din, Tariq Mahmood, Imran, Inamullah and

Hamida Mahmood. First Report of Vascular wilt on Lentil (Lens culinaris Medikus) caused by Fusarium redolens in Pakistan. Plant Disease. (2020). 11-19 doi.org/10.1094/PDIS-11-19-2519-PDN

Lopez, C., R. Richards and G. Arquhar. Variation in Early Vigour between Wheat and Barley. Crop Sci. (1995). 35: 472-479.

Manschadi, A.M., G.L. Hammer, J.T. Christopher and P.deVoil. Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivumL.*). Plant Soil. (2008). 303: 115–129.

MINFA. Ministry of Food and Agriculture Economic Wing, Islamabad. Govt. Pakistan. (2010).

Misra, A.N., A.K. Biswal and M. Misra, Physiological, biochemical and molecular aspects of water stress responses in plants and the biotechnological applications. Proc. Nat. Acad. Sci. India. (2002). 72B: 115–134.

Narayan, S., A. Mohan, K.S. Gill and P.V.V. Prasad. Variability of Root Traits in Spring Wheat Germplasm. Plos One. (2014). 9(6).

Neill, R. The Drinks Bible for the 21st Century. Octopus Pub. Group Cassel Illustrated (2002). p. 112.

Osmont, K.S., R. Sibout and C.S. Hardtke. Hidden branches: developments in root system architecture. Annual Res. Plant Bio. (2007). 58:93–113.

Palmer, J.J. How to Brew. Defenstrative Pub. Co. (2001). p. 233.

Pang, J., J.A. Palta, G.J. Rebetzke, x. Chapman and S. Fukau. Evaluation of reduced-tillering (tin) wheat lines in terminal water deficit environments. J. Expt. Bot. (2013). 64: 3439-3451.

Perry, M.W. and M.F.d'Antuono. Yield improvement and associated characteristics of some Australian spring wheat cultivars introduced between 1860 and 1982. Crop and Pasture sci. (1989). 40: 457-472.

Rauf, M., M. Munir M. UI-Hassan, M. Ahmed and M.Afzai. Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage.Afric. J. Biotech. (2007). 8: 971-975.

Regan, K.L., K.H.M. Siddique, D. Tennant and D.G. Abrecht. Grain yield and water use efficiency of early maturing wheat in low rainfall Mediterranean environments. Aus. J. Agric. Res. (1997). 48: 595-604.

Richards, R.A. and Z. Lukacs. Seeding VigourIn Wheat-Sources Of Variation For Genetic And Agronomic Improvement. Aus. J. Agric. Res. (2002). 53: 41-50.

Ryan, P., M. Liao, M. Delhaize, James, R.A.A. Richardson, K. Wiligama, W. Spielmeyer and G.J. Rebetzke. Effect of vigour and dwarfing genes on early phosphorus acquisition in wheat. Plant and Soil: (in press). (2014).

Sajjad, M., S.H. Khan and A.S. Khan, Exploitation of germplasm for grain yield improvement in spring wheat (*Triticum* aestivum L.). Int. J. Agric. Biol. (2011). 13: 695–700.

Shah, A., R. Gohar, S. Khalid and M. Akmal. Seminal root of maize Irrigation levels in relation to reduction in the substrate moisture content. Pak. J. Bot. (2011). 43(6): 2897-2902.

Siddique, K., D. Tennant, M. Perry and R. Belford. Water use and water use efficiency of old and modern wheat cultivars in a

Mediterranean-type environment. Crop and pasture sci. (1990). 41: 431-447.

Takahashi, T. and K. Gotoh. Yield improvement with high biomass in spring wheat genotype and its response to environments. In: Ishlii, R. Horie, T. Eds. Proceeding of 2nd Asian crop science conference. Asian Crop Sci. Assoc. Tokyo. (1996). 466-467.

Ward, P.R., D.J.M. Hall, S.F. Micin, K. Whisson, T.M. Willis, K. Treble and D. Tennant. Water use by annual crops. Role of dry matter production. Crop and Pasture Sci. (2008). 58: 1159-1166.

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ORIGINAL RESEARCH

Impact of Phosphorous and Zinc Levels on the Productivity of Green Gram (*Vigna radiate* L.)

Aman Khan[1], Haq Nawaz[1], Abdul Rab[1], Muhammad Asim[1], Ismail Khan[2], Fazal Munsif[1]*

[1]The University of Agriculture Peshawar, Amir Muhammad Khan Campus Mardan
[2]College of water conservancy, Shenyang Agricultural University, Shenyang, 110866, China

Correspondence
Fazal Munsif
Corresponding Author: munsiffazal@gmail.com

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ABSTRACT

Mung bean is one of the important Kharif pulses in Pakistan and is grown mainly for its edible seeds; therefore, fertilizers management is an important factor for improving mungbean growth and yield. A field experiment was conducted during the summer of 2013 at Palato Farm of the University of Agriculture Peshawar, Amir Muhammad Khan Campus Mardan, to determine the effect of phosphorus (P) and Zinc (Zn) on the yield and yield component of mungbean. The experiment consisted of four levels of P (0, 25, 50, and 75 kg ha^{-1}) and four levels of Zn (0, 5, 10, and 15 kg ha^{-1}). Data associated with the number of leaves and plant height illustrated that the higher number of leaves $plant^{-1}$ (8.8) by an average was observed when P was applied at the rate of 75 kg ha^{-1} followed by 0 kg phosphorous (P) ha^{-1} (8.7) and Zn (Zn) application at the rate of 10 kg ha^{-1} produced a maximum number of leaves $plant^{-1}$ (9) followed by 15 kg ha^{-1}(8.8) where 0 kg ZN ha^{-1} resulted in (7.7). Similarly, Zn significantly affected plant height, while P and interaction between P and Zn levels were non-significant. The higher plant height (95.1 cm) was observed when P was applied at the rate of 75 kg ha^{-1}, followed by 50 kg P ha^{-1} (93.6 cm). Higher plant height (95.8cm) was recorded when ZN was applied at the 5 kg ha^{-1} followed by 10 kg ha^{-1}(95.1cm). Higher numbers of nodules (13.1) were observed with the application of 50 kg P ha^{-1} followed by 75 kg P ha^{-1} (12.3), while the lowest (10.6) nodules were observed in the control plot. P application at the rate of 25 kg ha^{-1} produced a higher grain yield than 75 and 50 kg ha-1 and Zn application at the rate of 5 kg ha-1 produced a higher grain yield than 10 and 15 kg ha^{-1}. Therefore, a lower rate of P 25 kg ha^{-1} and Zn 5 kg ha^{-1} is recommended for a higher yield of mungbean in the agro-ecological condition of Mardan.

KEYWORDS: Mungbean, Zinc, Phosphorous, Yield and Yield components

I. INTRODUCTION

Mungbean (Vigna radiate L.), also called green gram, is an important summer-growing pulse crop in Pakistan (Hakim et al., 2021; Ali et al., 2019; Ahmad et al., 2003). It contains 24.5% protein and 59.9% carbohydrates. It also contains 75 mg calcium, 8.5 mg iron, and 49 mg β-carotene per 100g of split dal (Hakim et al., 2021; Shakya et al., 2019). The foliage and stem are good sources of fodder for livestock. Its seed is more palatable nutritive, digestible, and non-flatulent than other pulses (Teferie et al., 2020; Tarafder et al., 2020). The unique and common feature of mungbean is the root nodules that contain aerobic bacteria called rhizobia which fix atmospheric nitrogen in the root and thus enhance soil fertility (Singh et al, 2021; Ashraf et al., 2003). It is also a substitute for animal protein and forms a balanced diet when used with cereals (Detzel et al., 2021). In Pakistan, mungbean is cultivated as a minor crop and used as food. The area under mungbean in Pakistan was around 141 thousand hectares, with 93 thousand tons in 2011 (Hakim et al., 2021; GOP, 2012).

Fertilizers management is one of the important factors for improving the growth and yield of mung-bean (Ali et al., 2019; Iqbal et al., 2021; Ullah et al., 2020). Phosphorous is an essential component of ADP, ATP, the cell wall, and DNA and plays a key role in promoting plants storage and structural activities (Aimen et al., 2021). P is an important element that significantly affects plant growth and metabolism (Amanullah et al., 2022; Sadiq et al., 2017; Bashir et al., 2011) and is a component of DNA and RNA, involved in cell division (Brady and Weil, 2004). Nodule establishment and its function are important sinks for P, and nodules usually have the highest P content in the plant (Sulieman et al., 2015). It is supposed that P is effectively translocated into grain at high rates since P is necessary for producing protein, phospholipids and phytin in bean grain (Rahman et al., 2008). Poor nodulation and poor plant vigour are observed in beans grown in P deficient soils (Bindraban et al., 2020). Among other essential factors, an appropriate supply of micronutrients is also required for crops' proper growth and yield. The importance of Zn as a micronutrient in crop production has increased in recent years (Amanullah et al., 2020; Thapa et al., 2021). Hence Zn is considered the most yield-limiting micronutrient (Arunachalam et al., 2013).

The Zn application essentially is being employed in the functional and structural components of several enzymes (Amanullah et al., 2020; Read et al., 2019), such as carbonic anhydrase, alcohol dehydrase, alkaline phosphatase, phospholipase, carboxypeptidase (Read et al., 2019) and RNA polymerase (Romheld and Marschner., 1991). Furthermore, plants that emerged from seeds with lower Zn could be susceptible to biotic and abiotic stresses (Rehman et al., 2018). Zn enriched seeds perform better concerning seed germination, seedling growth, and yield of crops (Haider et al., 2020). In addition, Zn acts as an activator of several enzymes in plants and is directly involved in the biosynthesis of growth substances such as auxin, which produces more plant cells (Umair et al., 2020; Gobarah et al., 2006).

Furthermore, Zn enhanced photosynthesis at the early growth of plants, improved nitrogen fixation, grain protein, and yields of mungbean plants (Umair et al., 2020). In Pakistan, Zn scarcity in the soil is the first most widespread problem. In Khyber Pukhtunkhwa, the extent of Zn deficient soils ranges from 21% to 77%. 42% of agricultural fields of Mansehra and Swat have a Zn deficiency. On average, 37% of fields are deficient in Zn (Ahsin et al., 2020).

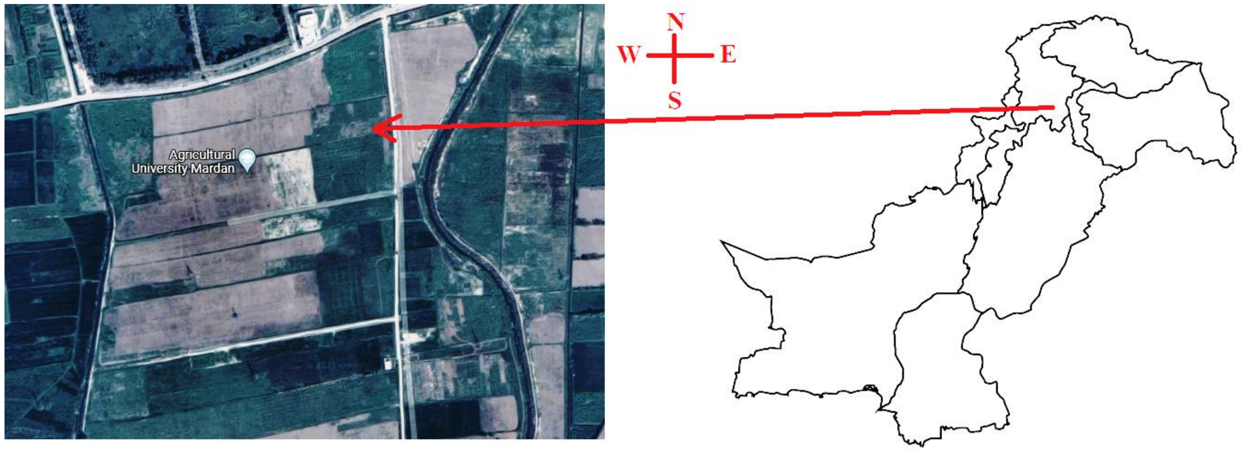

Figure 1. Map representing an experimental location in Mardan city, KPK Province, Pakistan, using Google maps.

Therefore, the present experiment was designed to study the effect of different levels of P and Zn on the yield of mungbean and find out the best combinations of P and Zn for higher yield and yield components of mungbean under the agro-climatic condition of Mardan.

2. MATERIALS AND METHODS

2.1 Experimental design

A field experiment was conducted during the summer of 2013 at Palato Farm of the University of Agriculture Peshawar, Amir Muhammad Khan Campus Mardan (Figure 1), to determine the effect of P and Zn on the yield and yield component of mungbean. The experiment was conducted in a randomized complete block design with three replications, and the plot size was 2 m x 1.8 m. Mungbean variety "Ramzan" was sown in lines having five rows 35cm apart on July 2, 2013. The experiment consisted of four levels of P (0, 25, 50 and 75 kg ha^{-1}) and four levels of Zn (0, 5, 10, and 15 kg ha^{-1}). SSP and $ZnSO_4$ will be used as the source of P and Zn, respectively and applied as a whole during seedbed preparation. All other agronomic practices, such as weeding, irrigation, plant protection measures, etc., were normal and uniform for all the experimental units.

2.2 Data collection and measurement

The number of plants m^{-2} were recorded from three central rows by counting the number of plants within the metering rod of each row and was converted to m^{-2}. Data on the number of branches $plant^{-1}$ was recorded by selecting five plants randomly from each treatment, and the number of branches were counted from base to top and were averaged. The number of leaves $plant^{-1}$ was recorded by selecting five plants randomly from each treatment, and the number of leaves was counted from base to top and averaged. Data on plant height was recorded by randomly selecting five plants from each treatment and measuring its height from base to tip and then averaged to record plant height and averaged them. The number of pod plant^{-1} was recorded by selecting five plants randomly from each plot and then were picked from it, and the number of pods was counted, and then the mean was calculated. Data on the number of grain pod^{-1} was recorded by counting the number of grains from five randomly selected pods in each plot and then averaged. Numbers of nodules plant-1 were recorded by selecting three plants randomly from each treatment, and the number of nodules were counted on roots and averaged. Data on Biological yield (kg ha^{-1}) was recorded by harvesting three central rows in each plot and kept in the field for sun drying. It was weighed with the help of scale and converted into kg ha^{-1} by this formula.

(Weight of bundles/ No. of rows, *Row length * row to row distance) x 10000

Data on grain yield was recorded by harvesting one square meter area from each plot and then were threshed, cleaned, dried, and weighed. The dried grains were weighed with the help of electronic balance and then converted to kg ha^{-1}. Data on thousand grains weight (g) was recorded by counting 1000 grains from each plot and then were weighed with the help of a sensitive electrical balance. The harvest index was calculated using the formula.

Harvest Index (%) = (Grains yield / biological yield) x100

2.3 Statistical analysis

The data were statistically analyzed using analysis of variance techniques appropriate for randomized complete block design. Means were compared using LSD test at 0.05 level of probability when the F-values were found significant (Jan *et al*., 2009).

3. RESULTS

3.1 Phenology and Physiology

Data recorded on the number of branches of plant^{-1} are presented in figure 2-A. In comparison, P and Interaction between P and Zn levels was found non-significant. The mean value of the data indicated that a higher number of branches plant^{-1} (1.88) was recorded when P was applied at the rate of 75 kg ha^{-1} followed by 50 kg P ha^{-1} (1.83) where 25 kg P ha^{-1} results number of branches plant^{-1} (1.17). The higher number of branches plant^{-1} (1.93) was recorded when Zn was applied at the rate of 10 kg ha^{-1} followed by 15 kg ha^{-1}(1.92), .where 0 kg Zn ha^{-1} resulted in the lower number of branches plant^{-1} (1.58).

Data recorded on the number of plants m^{-2}, are presented in figure 2-B. Statistical analysis of the data showed that Zn had a significant influence on the number of plants m^{-2} while P and interaction between P and Zn levels were found non-significant. The mean value of the data indicated that a higher number of plants m^{-2} (22.9) was recorded when P was applied at the rate of 75 kg ha^{-1}, followed by a control plot (21.6), whereas 25 kg P ha^{-1} had the least number of plant m^{-2} (20.3). The higher number of plants m^{-2} (24) was recorded when Zn was applied at the rate of 15 kg ha^{-1} followed by 10 kg ha^{-1}(23), where the least number of plant m^{-2} (17) were noted in the plot where no Zn was applied.

Data recorded on the number of leaves plant^{-1} are shown in figure 2-C. Statistical analysis of the data shows that P and Zn significantly influence the number of leaves plant^{-1}. While Interaction between P and Zn levels was found non-significant. The mean value of the data indicated that a higher number of leaves plant^{-1} (8.8) was observed when P was applied at the rate of 75 kg ha^{-1} followed by 0 kg phosphorous ha^{-1} (8.7), where 50 kg P ha^{-1} resulted in the lower number of leaves plant^{-1} (8). The higher number of leaves plant^{-1} (9) was recorded when Zn was applied at the rate of 10 kg ha^{-1} followed by 15 kg ha^{-1}(8.8), where 0 kg Zn ha^{-1} resulted in (7.7) lower number of leaves plant^{-1}.

Data associated with plant height are presented in figure 2-D. Statistical analysis of the data shows that Zn significantly affects plant height while P and Interaction between P and Zn levels were found non-significant. Mean data shows that higher plant height (95.1) was observed when P was applied at the rate of 75 kg ha^{-1} followed by 50 kg P ha^{-1} (93.6), where 0 kg P ha^{-1} results from low plant height (88.6). Higher plant height (95.8) was recorded when Zn was applied at the 5 kg ha^{-1} followed by 10 kg ha^{-1}(95.1). Where 0 kg Zn ha^{-1} resulted in lower plant height (81.9).

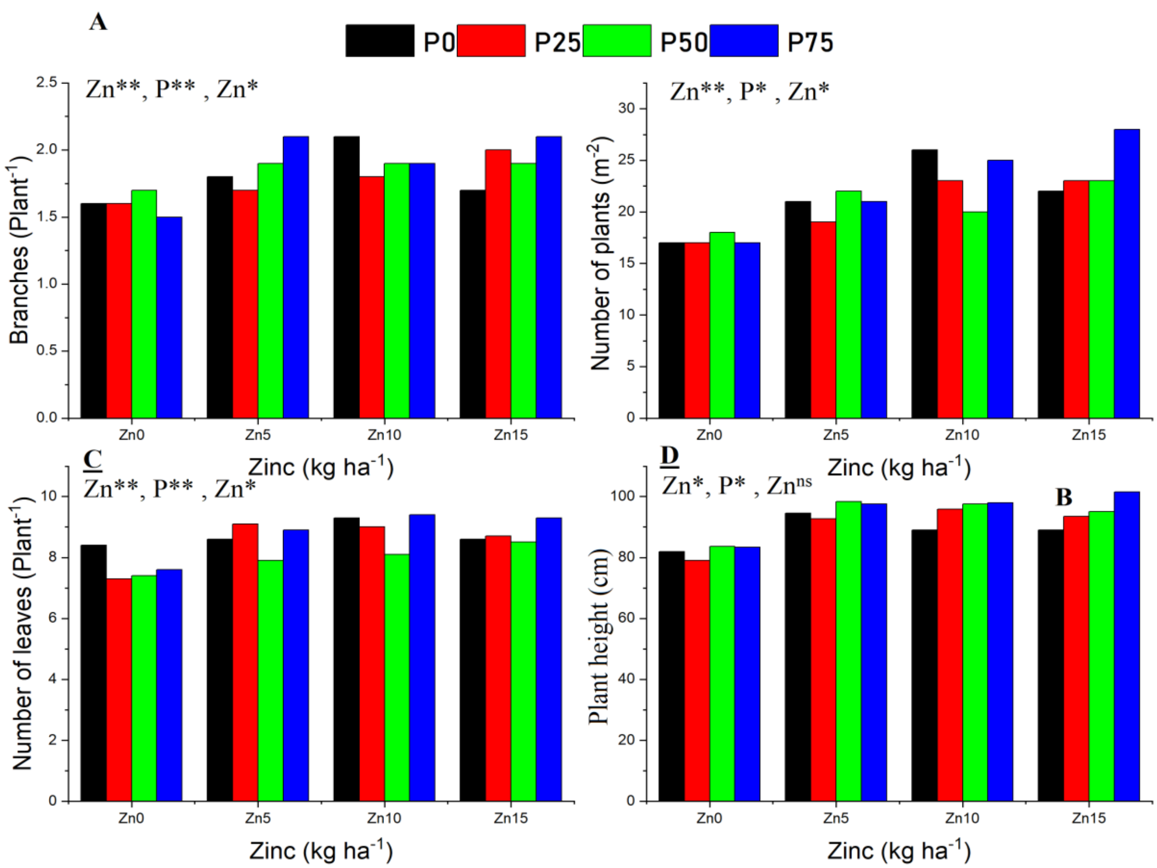

Figure 2. Effect of P and Zn on the number of plants m^{-2}, number of branches plant^{-1} , number of leaves plant^{-1} and plant height (cm) of mungbean.

3.2 Grain yield and yield traits (kg ha^{-1})

Results showed that P and Zn levels significantly affected the number of pods (figure 3A). While Interaction between P and Zn levels was found non-significant (Figure 3). The mean value of the data indicated that higher numbers of pods (10.3) were observed when P was applied at the rate of 25 kg ha^{-1} followed by 75 kg P ha^{-1} (10), where the control plot resulted in the lower number of pods (9.2). For Zn, higher numbers of pods (10.2) were observed when Zn was applied at the 5 kg ha^{-1} followed by 10 kg Zn ha^{-1}(10.1). Control plots resulted in lower numbers of pods (9). Similarly the grains pod^{-1} were also affected by Zn and P application (Figure 3B). In comparison, P and interaction between P and Zn levels were found non-significant. Mean data shows that a maximum number of grains pod^{-1} (10.1) was observed when P was applied at the rate of 50 kg ha^{-1} followed by 25 kg P ha^{-1} (10). Whereas P application at the rate of 50 kg ha^{-1} resulted in a lower number of grains (9.6). The higher number of grain pod^{-1} (10.3) was recorded when Zn was applied at the rate of 5 kg ha^{-1} followed by 10 kg ha^{-1}(10), whereas the control plot resulted in the lower number of grains (8.7).

Maximum numbers of nodules (13.1) were observed with the application of 50 kg P ha^{-1} followed by 75 kg P ha^{-1} (12.3), while the lowest (10.6) nodules were observed in the control plot (Figure 3C). Similarly, higher numbers of nodules (12.7) were observed with the application of 15 kg Zn ha^{-1} and followed by the control plot (11.7), while the lowest (11.1) nodules were observed on 10 kg Zn ha^{-1}. Results on grain yield showed that that a higher grain yield (826.7 kg ha^{-1}) was observed when P was applied at the rate of 25 kg ha^{-1}, followed by 50 kg P ha^{-1} (792.5 kg ha^{-1}), where a lower grain yield (737.4 kg ha^{-1}) were obtained from the control plot. Higher grain yield (835.0 kg ha^{-1}) was recorded when Zn was applied at the rate of 5 kg ha^{-1} followed by 15 kg ha^{-1} (800 kg ha^{-1}). Whereas lower grain yield (719.9 kg ha^{-1}) was recorded at plot had 10 kg Zn ha^{-1}.

Maximum (4493.4 kg ha^{-1}) biological yield was observed with the application of 25 kg P ha$^{-1,}$ while the lowest (3543.1 kg ha^{-1}) biological yield was observed for 75 kg P ha^{-1} (Table 1). Similarly, Zn has a maximum biological yield (4511.1 kg ha^{-1}) with the application of 15 kg ha^{-1}, while the lowest (3630.3 kg ha^{-1}) was recorded in the control plot. Higher (39.4) thousand-grains weight was observed with the application of 25 kg P ha^{-1}.followed by (38.8g) at the rate of 75 kg p ha^{-1} (Table 2). While the lowest (36.8g) thousand grains weight were observed for the control plot. Similarly, Zn has a significant influence on thousand grains weight, having a higher thousand grains weight (42.3g) for application of 15 kg Zn ha^{-1}, while the lowest (34.4g) thousand grains weight was recorded in the control plot. P and Zn had significant effects, while their interaction was non-significant. The higher harvest index (21.73%) was observed when P was applied at the rate of 75 kg ha^{-1} followed by 0 kg ha^{-1} (20.54%), where 25 kg ha^{-1} resulted in a lower harvest index (19.30%) (Table 3).. For Zn higher harvest index (23.0%) was observed when Zn was applied at the rate of 5 kg ha-1, followed by a control plot (21.3%).

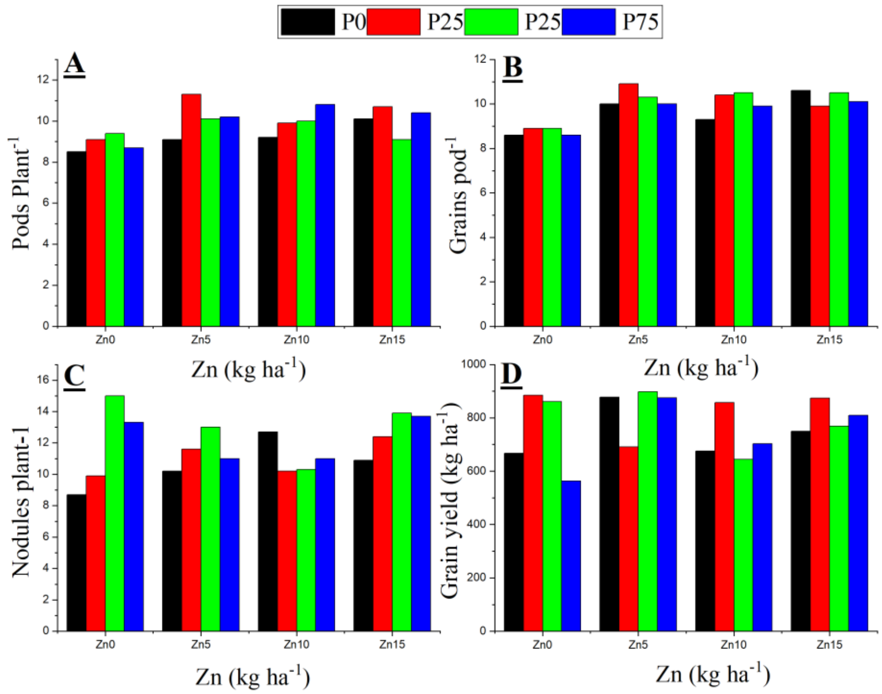

Figure 3. Effects of P and Zn application on pods plant^{-1} , grains pod^{-1}, nodules plant^{-1} and grain yield (kg ha^{-1}) of mungbean.

Table 1. Effects of P and Zn on biological yield of mung bean.

Zn (kg ha^{-1})	P(kg ha^{-1})				
	Control	25	50	75	Mean
Control	3233.1	4203.9	4301.1	2782.9	3630.3b
5	3515.4	4456.7	4001.7	3147.7	3780.4b
10	3885.2	4680.1	3659.4	3476.7	3925.3ab
15	4328.1	4632.9	4318.2	4765.0	4511.1a
Mean	3740.5b	4493.4a	4070.1ab	3543.1b	

Table 2. Effects of P and Zn on thousand-grain weight (gram) of mungbean.

Zn (kg ha^{-1})	P(kg ha^{-1})				
	Control	25	50	75	Mean
Control	29.7	38.0	34.2	35.7	34.4d
5	37.3	38.0	37.3	37.1	37.4c
10	38.7	39.3	40.0	39.0	39.3b
15	41.3	42.3	42.2	43.4	42.3a
Mean	36.8b	39.4a	38.4a	38.8a	

Table 3. Effects of P and Zn on harvest index of mung bean.

Zn (kg ha^{-1})	P(kg ha^{-1}) control	25	50	75	Mean
Control	21.2	23.3	20.2	20.5	21.3ab
5	25.1	16.0	22.7	28.5	23.0a
10	17.9	18.4	18.1	20.8	18.8b
15	18.0	19.6	18.5	17.2	18.3b
Mean	20.54a	19.30a	19.89a	21.73a	

Mean followed by different letters are found significant at 5% level of probabilities.

Furthermore, the application of 15 kg Zn ha^{-1} resulted in a lower harvest index (18.3%).

4. DISCUSSION

Micronutrient insufficiency is the primary cause of low crop development and production in arable soils (Imtiaz et al., 2011). Due to intensive agricultural practices, unwise use of mineral nutrition, breeding of high yielding and advanced varieties, and removal of huge quantities of nutrients at every crop harvest with lower nutrients returns to soils, the degree and extent of nutrient deficiency in arable soils has recently had serious consequences, resulting in lower micronutrients including zinc (Zn) in soil (Kanwal et al., 2019). Therefore to we conducted a field experiment on different Zn rates in combination with P rates to determine its effect on mungbean growth, yield and yield components. Our results showed that P and Zn significantly effected number of plants m^{-2} , number of branches plant^{-1} , number of leaves plant^{-1} and plant height (cm) pods plant^{-1} , grains pod^{-1}, nodules plant^{-1} and grain yield (kg ha^{-1}) , biological yield, thousand grains weight, and harvest index. The plots treated with 15 kg ha^{-1} Zn in combination with 50 kg P ha^{-1} Resulted in higher number of plants m^{-2}, more branches plant^{-1} , maximum leaves plant^{-1} and maximum plant height (cm), higher number of pods plant^{-1}, more grains pod^{-1},extra nodules plant^{-1} and higher grain yield (kg ha^{-1}). The possible explanation for these increments might due to the Zn promotes nodulation and nitrogen fixation in leguminous crops (Masood, et al., 2022; Shahrajabian et al., 2022; Gough et al., 2021). Furthermore Zn is important in the formation of auxin, which increases cell volume and increases plant height (Wang et al., 2016; Oguchi et al., 2004). Cakmak et al. (2000) reported that Zn is essential for active enzymatic activity, root cell elongation, and reducing free radical damage to the cell. Another possible explanation for these results that when Zn and P applied to soil improved soil physical and chemical properties which consequently improved mungbean growth, yield and yield component (Singh, et al., 2013). A previous study documented that compared to control, Zn increased branches numbers in plants and leaf area of mungbean (Haider et al., 2021). Nair studied the genetic diversity of mungbean for iron and zinc and discovered a large potential for improvement through biofortification, finding 20–40 g Zn concentration kg^{-1} for dry mungbean seed, which was virtually identical in our work. Overall the results showed that the addition of Zn and P fertilizer to soil can improve growth, yield and yield component of mungbean.

5. CONCLUSION

Our results showed that the growth, yield and yield components of mungbean were improved in Zn and P fertilizer application. On the basis of our results it is concluded that P application at the rate of 25 kg ha^{-1} produced a higher grain yield as compared to 75 and 50 kg ha^{-1}. Whereas, Zn application at the rate of 5 kg ha^{-1} produced a higher grain yield than 10 and 15 kg ha-1 hence lower rate of 5 kg ha-1 is recommended for higher yield of mungbean in agro-ecological condition Mardan.

Authors Contributions:
A.M and F.M conceived the main idea of research, A.M wrote the manuscript. H.N, A.R and I.K revised the manuscript and provided suggestions. In addition A.M and A.R assessed and analyzed the data, and performed data collection. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability statements: The data presented in this study are available on request from the corresponding author.

REFERENCES

Ahmad, R.I.A.Z., Ikraam, M., Ullah, E.H.S.A.N. and Mahmood, A.S.I.F., Influence of different fertilizer levels on the growth and productivity of three mungbean (Vigna radiata) cultivars. *International Journal of Agriculture & Biology*, *5*(3), (2003) pp.335-338.

Ahsin, M., Hussain, S., Rengel, Z. and Amir, M. Zinc status and its requirement by rural adults consuming wheat from control or zinc-treated fields. *Environmental geochemistry and health*. (2020). *42*(7), pp.1877-1892.

Aimen, A., Basit, A., Bashir, S., Aslam, Z., Shahid, M.F., Amjad, S., Mehmood, K., Aljuaid, B.S., El-Shehawi, A.M., Zuan, A.T.K. and Farooq, S. Sustainable phosphorous management in two different soil series of Pakistan by evaluating dynamics of phosphatic fertilizer source. Saudi Journal of Biological Sciences.(2021).

Amanullah, Alwahibi, M.S., Elshikh, M.S., Alkahtani, J., Muhammad, A., Khalid, S., Ahmad, M., Khan, N., Ullah, S. and Ali, I. Phosphorus and Zinc Fertilization Improve Zinc Biofortification in Grains and Straw of Coarse vs. Fine Rice Genotypes. Agronomy. (2020) 10(8), p.1155.

Amanullah, Yar, M., Khalid, S., Elshikh, M.S., Akram, H.M., Imran, Al-Tawaha, A.R.M., Ahmad, M., Muhammad, A., Ullah, S. and Ali, I. Phenology, growth, productivity, and profitability of mungbean as affected by potassium and organic matter under water stress vs. no water stress conditions. Journal of Plant Nutrition. (2022). 45(5), pp.629-650.

Arunachalam, P., Kannan, P., Prabukumar, G. and Govindaraj, M. Zn deficiency in Indian soils with special focus to enrich Zn in peanut. African Journal of Agricultural Research, (2013). 8(50), pp.6681-6688.

Bashir, K., Ali, S. and Umair, A. Effect of different phosphorus levels on xylem sap components and their correlation with growth variables of mash bean. Sarhad Journal of Agriculture, (2011). 27(4), pp.595-601.

Bindraban, P.S., Dimkpa, C.O. and Pandey, R. Exploring P fertilizers and fertilization strategies for improved human and environmental health. Biology and Fertility of Soils (2020). 56(3), pp.299-317.

Brady, N.C. and R.R. Weil. 2004. The Nature and Properties of Soils. 13th Ed.. Pearson

Cakmak, I. Tansley Review No. 111: possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New phytologist. (2000). 146(2), pp.185-205.

Detzel, A., Krüger, M., Busch, M., Blanco-Gutiérrez, I., Varela, C., Manners, R., Bez, J. and Zannini, E. Life cycle assessment of animal-based foods and plant-based protein-rich alternatives: an environmental perspective. Journal of the Science of Food and Agriculture. (2021)

Gobarah, M.E., Mohamed, M.H. and Tawfik, M.M. Effect of P fertilizer and foliar spraying with Zn on growth, yield and quality of groundnut under reclaimed sandy soils. J App Sci Res. (2006). 2(8), pp.491-496.

Gough, E.C., Owen, K.J., Zwart, R.S. and Thompson, J.P., Arbuscular mycorrhizal fungi acted synergistically with Bradyrhizobium sp. to improve nodulation, nitrogen fixation, plant growth and seed yield of mung bean (Vigna radiata) but increased the population density of the root-lesion nematode Pratylenchus thornei. Plant and Soil, (2021). pp.1-22.

Haider, M.U., Hussain, M., Farooq, M. and Nawaz, A. Optimizing Zn seed priming for improving the growth, yield and grain biofortification of mungbean (Vigna radiata (L.) wilczek). Journal of Plant Nutrition. (2020). 43(10), pp.1438-1446.

Haider, M.U., Hussain, M., Farooq, M., Ul-Allah, S., Ansari, M.J., Alwahibi, M.S. and Farooq, S., 2021. Zinc biofortification potential of diverse mungbean [Vigna radiata (L.) Wilczek] genotypes under field conditions. PLOS ONE. (2021). 16(6), p.e0253085.

Hakim, S., Imran, A. and Mirza, M.S. Phylogenetic diversity analysis reveals Bradyrhizobium yuanmingense and Ensifer aridi as major symbionts of mung bean (Vigna radiata L.) in Pakistan. Brazilian Journal of Microbiology, (2021). 52(1), pp.311-324.

Imtiaz, M., Rashid, A., Khan, P., Memon, M.Y. and Aslam, M. The role of micronutrients in crop production and human health. Pak. J. Bot. (2010). 42(4), pp.2565-2578.

Iqbal, A., Xie, H., He, L., Ahmad, S., Hussain, I., Raza, H., Khan, A., Wei, S., Quan, Z., Wu, K. and Ali, I. Partial substitution of organic nitrogen with synthetic nitrogen enhances rice yield, grain starch metabolism and related genes expression under the dual cropping system. Saudi Journal of Biological Sciences. (2021). 28(2), pp.1283-1296.

Masood, F., Ahmad, S. and Malik, A. Role of Rhizobacterial Bacilli in Zinc Solubilization. In Microbial Biofertilizers and Micronutrient. (2022). pp. 361-377). Springer, Cham.

Nair, R.M., Yang, R.Y., Easdown, W.J., Thavarajah, D., Thavarajah, P., Hughes, J.D.A. and Keatinge, J.D.H., 2013. Biofortification of mungbean (Vigna radiata) as a whole food to enhance human health. Journal of the Science of Food and Agriculture. (2013). 93(8), pp.1805-1813.

Oguchi, K., Tanaka, N., Komatsu, S. and Akao, S. Methylmalonate-semialdehyde dehydrogenase is induced in auxin-stimulated and zinc-stimulated root formation in rice. Plant cell reports. (2004) 22(11), pp.848-858.

Rahman M.M., Bhuiyan M. M. H., Sutradhar G.N.C., Rahman M. M. and Paul A.K. Replication Proteins. Ann. Rev., Biochem. (2008) 61, 897-946.

Read, S.A., Obeid, S., Ahlenstiel, C. and Ahlenstiel, G. The role of Zn in antiviral immunity. Advances in nutrition. (2019). 10(4), pp.696-710.

Rehman, A., Farooq, M., Ozturk, L., Asif, M. and Siddique, K.H. Zn nutrition in wheat-based cropping systems. Plant and Soil. (2018). 422(1), pp.283-315.

Sadiq, G., Khan, A.A., Inamullah, A.R., Fayyaz, H., Naz, G., Nawaz, H., Ali, I., Raza, H., Amin, J., Ali, S. and Khan, H.A. Impact of P and potassium levels on yield and yield components of maize. Pure and Applied Biology (PAB). (2017). 6(3), pp.1071-1078.

Shahrajabian, M.H., Sun, W. and Cheng, Q.,A survey of biological nitrogen fixation in adzuki beans, soybeans, and mung beans, three legumes in traditional Chinese medicine. In Functional Foods and Nutraceuticals in Metabolic and Non-Communicable Diseases. (2022) (pp. 301-316). Academic Press.

Shakya, A., Kumar, P., Verma, A.P., Raj, A. and Batham, P. Studies on population dynamics of sucking insect pests whitefly (Bemisia tabaci), jassid (Empoasca kerri) and thrips (Caliothrips indicus) on mungbean [Vigna radiata (L.) Wilczek]. 2019.

Singh, A.K., Meena, M.K., Bharati, R.C. and Gade, R.M., Effect of sulphur and zinc management on yield, nutrient uptake, changes in soil fertility and economics in rice (Oryza sativa)–lentil (Lens culinaris) cropping system. Indian J. Agril. Sci, (2013). 83(3), pp.344-348.

Singh, K., Gera, R., Sharma, R., Maithani, D., Chandra, D., Bhat, M.A., Kumar, R. and Bhatt, P. Mechanism and application of Sesbania root-nodulating bacteria: an alternative for chemical fertilizers and sustainable development. Archives of Microbiology. (2021). pp.1-12.

Sulieman, S. and Tran, L.S.P. Phosphorus homeostasis in legume nodules as an adaptive strategy to phosphorus deficiency. *Plant Science*. (2015). *239*, pp.36-43.

Tarafder, S., Rahman, M.A., Hossain, M.A. and Chowdhury, M.A.H. Yield of Vigna radiata L. And post-harvest soil fertility in response to integrated nutrient management. Agricultural and Biological Sciences Journal. (2020). 6(1), pp.32-43.

Teferie, B.B., Admasu, M.A. and Damessa, G.G. Assessment and characterization of mung bean (Vigna radiata) bacterial brown spot in Eastern Amhara, Ethiopia. African Journal of Agricultural Research. (2020). 16(5), pp.606-621

Thapa, S., Bhandari, A., Ghimire, R., Xue, Q., Kidwaro, F., Ghatrehsamani, S., Maharjan, B. and Goodwin, M. Managing Micronutrients for Improving Soil Fertility, Health, and Soybean Yield. Sustainability, (2021). 13(21), p.11766.

Ullah, S., Liang, H., Ali, I., Zhao, Q., Iqbal, A., Wei, S., Shah, T., Yan, B. and Jiang, L. Biochar coupled with contrasting nitrogen sources mediated changes in carbon and nitrogen pools, microbial and enzymatic activity in paddy soil. Journal of Saudi Chemical Society. (2020). 24(11), pp.835-849.

Umair Hassan, M., Aamer, M., Umer Chattha, M., Haiying, T., Shahzad, B., Barbanti, L., Nawaz, M., Rasheed, A., Afzal, A., Liu, Y. and Guoqin, H. The critical role of Zn in plants facing the drought stress. Agriculture. (2020). 10(9), p.396.

Wang, S., Ren, X., Huang, B., Wang, G., Zhou, P. and An, Y. Aluminium-induced reduction of plant growth in alfalfa (Medicago sativa) is mediated by interrupting auxin transport and accumulation in roots. Scientific reports. (2016). 6(1), pp.1-13.

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ORIGINAL RESEARCH

Impact of Nitrogen, Zinc and Humic Acid Application on Wheat Growth, Morphological Traits, Yield and Yield Components

Anas Iqbal[1]*, Haneef Raza[1], Maid Zaman[2], Rayyan Khan[1], Muhammad Adnan[1], Abdullah Khan[1], Syeda Wajeeha Gillani[3], Shad Khan Khalil[1]

[1]Department of Agronomy, The University of Agriculture Peshawar, Pakistan.
[2]Department of Entomology, University of Haripur, Pakistan.
[3]Deprtment of Biotechnology, National University of Sciences & Technology (NUST), Pakistan

Correspondence
Anas Iqbal
Corresponding Author:
anasiqbalagr@gmail.com

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ABSTRACT

To investigate the response of wheat to different levels of nitrogen (N), zinc (Zn) and humic acid (HA), an experiment was conducted at Agronomy Research Farm, the University of Agriculture, Peshawar, during 2014-15. The experiment was laid out in a randomized complete block design having three replications. Three levels of N (80, 120 and 160 kg ha^{-1}), Zn (6, 12 and18 kg ha^{-1}) and HA (5, 10 and 15 kg ha^{-1}) were used. Results showed that N application at the rate of 160 kg ha^{-1} manifested maximum days to physiological maturity (164 days), productive tillers m^{-2} (248), spikes m^{-2}(258), leaf area $tiller^{-1}$ (113.6 cm^2), spike length (10.4 cm), grains $spike^{-1}$ (52), 1000-grain weight (47.5 g), biological yield (9260 kg ha^{-1}), grain yield (3723 kg ha^{-1}) and harvest index (40%). Zn treated plots at the rate of 12 kg ha^{-1} showed maximum days to physiological maturity (162 days), productive tillers m^{-2} (241), spikes m^{-2} (252), grains $spike^{-1}$ (51), 1000-grain weight (45.2 g), biological yield (8843 kg ha^{-1}), grain yield (3375 kg ha^{-1}) and harvest index (39 %). Similarly, HA treated plots at the rate of 12 kg ha^{-1} revealed maximum days to physiological maturity (162 days), productive tillers m^{-2} (238), spikes m^{-2}(249), spike length (9.7 cm), 1000-grain weight (45.00 g), biological yield (8649 kg ha^{-1}), grain yield (3342 kg ha^{-1}) and harvest index (39%). The combined application of N, Zn, and HA had significantly affected wheat yield and yield components. It was concluded that N at the rate of 160 kg ha^{-1}, Zn 12 kg ha^{-1} and HA 10 kg ha^{-1}significantly increased yield and yield components of wheat.

KEYWORDS: Wheat, nitrogen, zinc, humic acid, Tillers, Physiological maturity

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1. INTRODUCTION

Wheat (*TriticumaestivumL.*) is an annual, long-day, self-pollinated plant grown in winter. It belongs to the family Poaceae (Gramineae). It is used as a staple food in Pakistan (Ali et al., 2019a). It dominates other agronomic crops in production (Eeswaran., 2021). Wheat is a rich source of carbohydrates and gluten, increasing its demand for baking products (Cappelli et al., 2020). Wheat straw is also used as fodder for livestock. It is estimated that 5-10% wheat grain is now being consumed as poultry and livestock feed (Tricase et al., 2018). Nitrogen is often the most deficient of all the plant nutrients. Nitrogen is the key element in achieving consistently high yields in cereals (Iqbal et al., 2019; Iqbal et al., 2020, Ali et al., 2020; Wu et al., 2021, Ali et al., 2022).

Zinc is an essential micronutrient for wheat. Zn plays very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome (Yuvaraj et al., 2020). Plant enzymes activated by Zn are involved in carbohydrate metabolism, maintenance of the integrity of cellular membranes, protein synthesis, and regulation of auxin synthesis and pollen formation (Gondalet al., 2021). Zn is a micronutrient that is required for plant growth relatively in a smaller amount. Zn plays a vital role in the physiological process of wheat plants such as cell elongation, cell maturation, sugar translocation, meristematic tissues development and protein synthesis (Mumivand et al., 2021).

Nitrogen and Zn together play a significant role in crop production. The importance of nutrients (micro and macro) for the normal growth of crop plants is universally recognized. Zn is an essential element present in plant enzymatic systems (Yuvaraj et al., 2020). Zn has vast numbers of functions in plant metabolism and consequently, Zn deficiency has many effects on plant growth (Umair et al., 2020). Zn deficiency is a worldwide nutritional constraint for crop production in many types of soil worldwide, particularly in cereals growing on calcareous soil (Rehman et al., 2020; Amanullah, 2020). In Pakistan, Zn deficiency is a general micronutrient disorder on calcareous soil and is considered the third most common deficient nutrient after N and phosphorous (Amanullah, 2020).

Humic acid is complex organic molecules that are formed by the breakdown of organic matter. HA influences soil fertility through its effect on the soil's water-holding capacity (Ali et al., 2019b; Sutton and Sposito, 2005). HA constitutes a stable fraction of carbon that improves soil characteristics such as improved water holding capacity, pH buffering, and thermal insulation (Izhar et al., 2020). It also increases N use efficiency and stimulates shoot and root growth (Leite et al., 2020; Lodhi*et al*., 2013). HAs contributed to soil stability and soil fertility, leading to exceptional plant growth and micronutrient uptake. HA is an organically charged bio-stimulant that significantly affects plant growth and development and increases crop yield. It has been extensively investigated that HA improves soils' physical, chemical, and biological properties (Zaremanesh et al., 2020; Karim et al., 2020; Skowrońska et al., 2020).

Nitrogen, Zinc and HumicAcid are essential nutrient which play a pivotal role in increasing fertility of the soil which in turn increases the yield of the crop. Keeping in view the above facts and figures, the present investigation was therefore conducted to determine the best level of N, Zn and HA for obtaining maximum yield and yield components of wheat.

2. MATERIALS AND METHODS

2.1 Field location and experiment management

The experiment on the response of wheat to different levels of N, Zn and HA on the yield and yield components of wheat was conducted at Agronomy Research Farm, The University of Agriculture, Peshawar, Pakistan, during winter 2014-15. The research comprised of three factors (i) N N kg ha^{-1} (N1 80 kg ha^{-1}, N2 120 kg ha^{-1} and N3 160 kg ha^{-1}) (ii) Zn kg ha^{-1} (Zn_1 6 kg ha^{-1}, Zn_2 12 kg ha^{-1}, Zn_3 18 kg ha^{-1}) and (iii) HA kg ha^{-1} (HA_1 5 kg ha^{-1}, HA_2 10 kg ha^{-1}, HA_3 15 kg ha^{-1})and one control plot for each treatment where no N, Zn and HA applied. All three factors were used in the following combinations. T_{1}: (Control), T_2: $N_1Zn_1HA_1$, T_3: N_1Zn_1 HA_2, T_4: $N_1Zn_1HA_3$, T_5: $N_1Zn_2HA_1$, T_6: $N1Zn_2HA_2$, T_7: $N_1Zn_2HA_3$, T_8: $N_1Zn_3HA_1$, T_9: $N_1Zn_3HA_2$,T_{10}: $N_1Zn_3HA_3$, T_{11}: $N_2Zn_1HA_1$, T_{12}: $N_2Zn_1HA_2$, T_{13}: $N_2Zn_1HA_3$, T_{14}: $N_2Zn_2HA_1$, T_{15}: $N_2Zn_2HA_2$, T_{16}: $N_2Zn_2HA_3$, T_{17}: $N_2Zn_3HA_1$, T_{18}: $N_2Zn_3HA_2$, T_{19}: $N_2Zn_3HA_3$, T_{20}: $N_3Zn_1HA_1$, T_{21}: $N_3Zn_1HA_2$, T_{22}: $N_3Zn_1HA_3$, T_{23}: $N_3Zn_2HA_1$, T_{24}: $N_3Zn_2HA_2$, T_{25}: $N_3Zn_2HA_3$, T_{26}: $N_3Zn_3HA_1$, T_{27}: $N_3Zn_3HA_2$, T_{28}: $N_3Zn_3HA_3$. The experiment was laid out in a randomized complete block design having three replications. Wheat variety 'Atta Habib-2010' was sown on 20^{th} November 2014 at the rate of 120 kg ha^{-1} in a plot size of 3 × 1.8 m having 6 rows 30 cm apart. At the time of sowing full dose of Zn, HA, and half dose of N was used while the rest of 50% N was applied at second irrigation. A basal dose of phosphorous (P_20_5) 100 kg ha^{-1} was applied to each plot. Urea, diammonium phosphate (DAP), Zn sulphate and HA was used as a source for N, P, Zn and HA respectively. Irrigation was done according to the need of crop. Weedicides was used for control of common weeds of wheat. The crop was harvested on May 15, 2015. Excluding the treatments, all the further agronomic practices were done normal and uniform.

2.2 Data collection and analysis

Data concerning days to emergence was recorded by counting the number of days from sowing to 80% of the seedlings emerged in each plot. Emergence m^{-2} data was recorded by measuring the number of plants emerged in one meter row length at three randomly selected rows in each plot and was converted to emergence m^{-2} using the following formula (1);

$$\text{Emergence } m^{-2} = \frac{\text{Total number of seedling emerged}}{\text{Row - row distance} \times \text{row length} \times \text{No. of rows}} \times 1m^{-2}$$

Data on days to anthesis was recorded when 80% of plants extruded the anthers in each treatment. Days were counted from the date of sowing till the date of anthers extrusion in each plot. Days to physiological maturity were recorded by measuring the days from date of sowing to the date when 80% plants get physiologically mature (Anderson *et al.*, 1985). Leaf area tillers^{-1} was calculated at the anthesis stage from the leaves of ten tillers in four central rows selected randomly at each plot. Leaf area tiller^{-1} was calculated by multiplying the average leaf length (cm), leaf width (cm), number of leaves, and correction factor then dividing by ten tillers.

Leaf area tiller^{-1}= No. leaves × Avg Leaf width (cm) ×Avg leaf length × CF/ No. tiller

Correction factor = 0.75 (Abbas *et al.*, 2014)

For spike length data, the length of ten randomly selected spikes was measured from the basal joint of the spike till the top of the spike and then averaged. Spikes m^{-2} data was recorded by counting the number of spikes in central four rows of each plot and then were converted into spikes m^{-2}. The data on the number of productive tillers m^{-2} was recorded by counting the tillers having a spike in place one meter selected at three time randomly in each plot and were converted into productive tillers m^{-2}. Ten randomly selected spikes for grains spike-1 of wheat were estimated and then averaged. Similarly, a thousand grains were randomly taken from each plot and the weight was calculated with the help of electronic balance. Whereas, the biological yield was recorded by harvesting four central rows in each plot; tied into bundles, sun-dried, weighed and then biological yield was calculated in kg ha^{-1} by using formula ;

$$\text{Biological yield (kg ha}^{-1}) = \frac{\text{Biological yield in four central rows}}{\text{Row} - \text{row distance x Row length x No. of rows}} \text{x10000}$$

The four central rows was harvested, dried, threshed, cleaned, weighed and then converted into kg ha^{-1}. For Harvest index was calculated by using the following formula; Harvest index (%) = $\frac{\text{Economic yield}}{\text{Biological yield}} \times 100$

2.3 Statistical Analysis

Analysis of variance procedure was followed for the statistical analysis of recorded data according to the design used. Means compared using least significant differences (LSD) test at $P \leq 0.05$ upon significant F-test (Steel, 1997).

3. RESULTS

3.1 Phenology and Growth traits

Analysis of the data on days to emergence and emergence m^{-2} showed that N, Zn, HA, control vs. rest and all the interaction between the nutrients were found non-significant (Figure. 1). Days to anthesis were significantly affected by N and control vs. rest had (Figure. 2). While Zn, HA and all the interaction were non-significant for days to anthesis. The treated plots took more days to anthesis (134) as compared to control (133). N's mean data revealed that minimum days to anthesis (133) were recorded at 80 kg N ha^{-1}. Days to anthesis increased with each increment of N and maximum days to anthesis (134) was observed at 160 kg N ha^{-1}. Similarly data on days to physiological maturity revealed that N, Zn, HA, control vs. rest and N × Zn interaction significantly affected days to physiological maturity, while all other interactions were non-significant (Figure 2B). The treated plots showed more days to physiological maturity (163) as compared to control (156). N's mean data exhibited that minimum days to physiological maturity (161) were noted at 80 kg N ha^{-1}. Days to physiological maturity increased with each increment of N and maximum days to physiological maturity (165) was noted at 160 kg N ha^{-1}. Minimum days to physiological maturity (162) were observed at 6 kg Zn ha^{-1}. Days to physiological maturity increased with each increment of Zn and full days to physiological maturity (163) was observed at 18 kg Zn ha^{-1}. Low level of HA (5 kg ha^{-1}) resulted in minimum days to physiological maturity (162). Days to maturity increased with each increment of HA The maximum days to physiological maturity (163) were revealed at 15 kg HA ha^{-1}. Interaction among N × Zn showed that days to physiological maturity increased with each increment of N up to 160 kg N ha^{-1} with 6 kg Zn ha^{-1}. Similar increase in days to physiological maturity was observed with each increment of N with 12 and 18 kg Zn ha^{-1}. Data regardingleaf area $tiller^{-1}$ revealed that N, Zn, HA, control vs. rest, N × Zn and N × Zn × HA interaction had significantly affected leaf area $tiller^{-1}$(Figure 3A), while all other interactions were non-significant for leaf area $tiller^{-1}$. The treated plots showed maximum leaf area $tiller^{-1}$ (111.01) as compared to control (99.00). Mean data for N showed that minimum leaf area $tiller^{-1}$ (108.84) was observed at 80 kg N ha^{-1}. Leaf area $tiller^{-1}$ increased with each increment of N and higher leaf area $tiller^{-1}$(113.66) was observed at 160 kg N ha^{-1}. Minimum leaf area $tiller^{-1}$ (108.88) was noted at 6 kg Zn ha^{-1}. Leaf area $tiller^{-1}$ increased with each increment of Zn and maximum leaf area $tiller^{-1}$ (112.63) was recorded at 18 kg Zn ha^{-1}. Lower level of HA (5 kg ha^{-1}) resulted in lowest leaf area $tiller^{-1}$ (107.88). Leaf area $tiller^{-1}$ increased with HA increment and higher leaf area $tiller^{-1}$ (112.44) was recorded at 15 kg HA ha^{-1}. Interaction between N × Zn revealed that leaf area $tiller^{-1}$ increased with each increment of N up to 160 kg N ha^{-1} with 6 kg Zn ha^{-1}. Similar increase in leaf area $tiller^{-1}$ was noted with each increment of N with 12 and 18 kg Zn ha^{-1}. In the case of Plant height lower level of HA (5 kg ha^{-1}) produced in smaller plant height (88.3), plant height increased with the increment of HA and taller plant height (92.6) was obtained at 15 kg HA ha^{-1} (Figure 3B). Interaction between N × Zn showed that plant height improved with each increment of N up to 160 kg N ha^{-1} with 6 kg Zn ha^{-1}. Alike increase in plant height was noted with each increment of N with 12 and 18 kg Zn ha^{-1}. Data on spike length of the data pertaining that N, Zn, HA, control vs. rest and N × Zn × HA interaction had significantly affected spike length (Figure 4A). While all other interactions were non-significant spike length. The treated plots resulted in greater spike length (9.7) than control (8.1). Mean data for N showed that smaller spike length (9.2) was recorded at 80 kg N ha^{-1}. Spike length enhanced with each

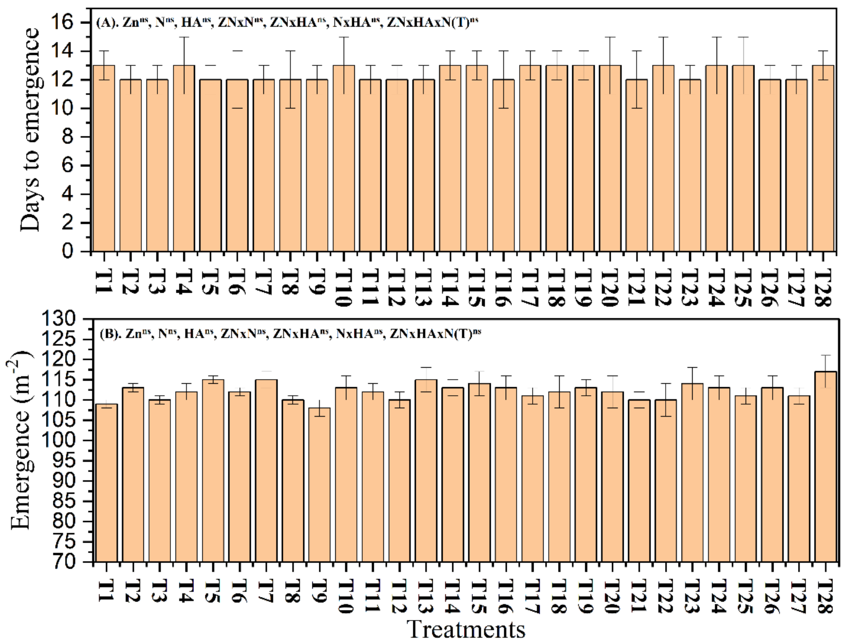

Figure 1. Changes in Days to emergence and emergence (m^{-2}) of wheat to different N, Zn and HA applications. T1 to T28 indicates different treatments, for detail see material and methods.

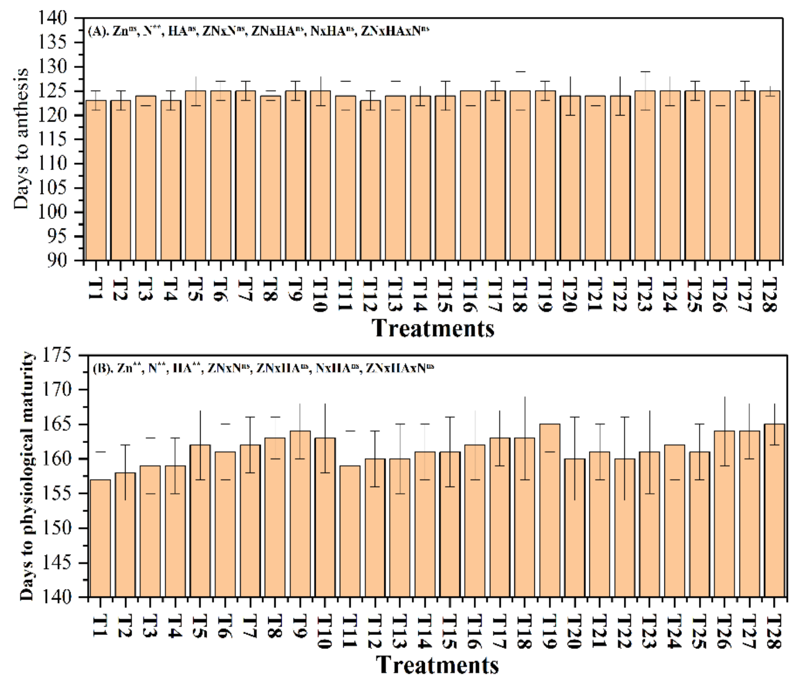

Figure 2. Changes in Days to anthesis and Daysof wheat to different N, Zn and HA applications. T1 to T28 indicates different treatments, for detail see material and methods.

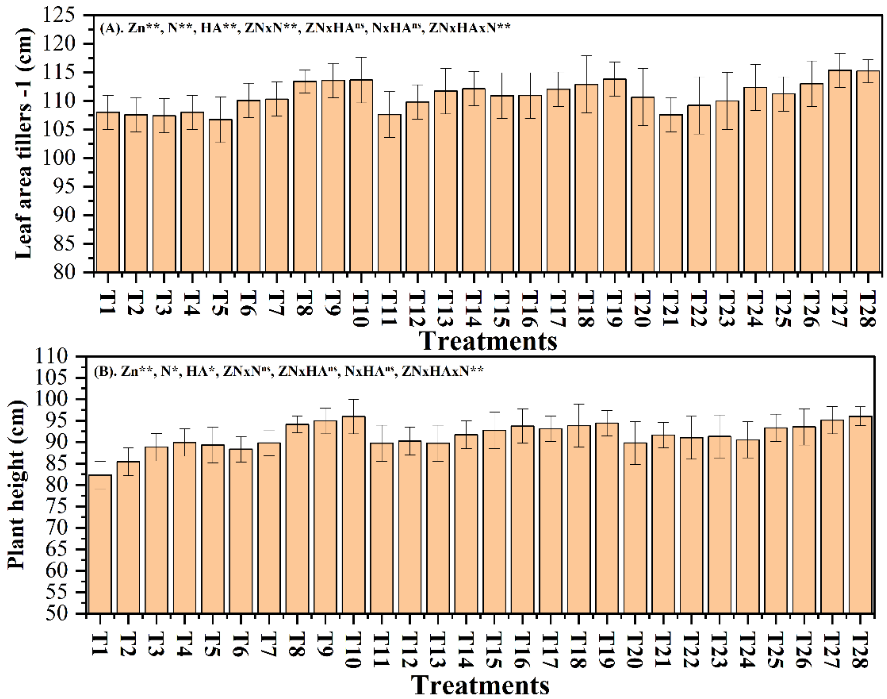

Figure 3. Changes in leaf area tiller^{-1} and plant height of wheat to different N, Zn and HA applications.T1 to T28 indicates different treatments, for detail see material and methods.

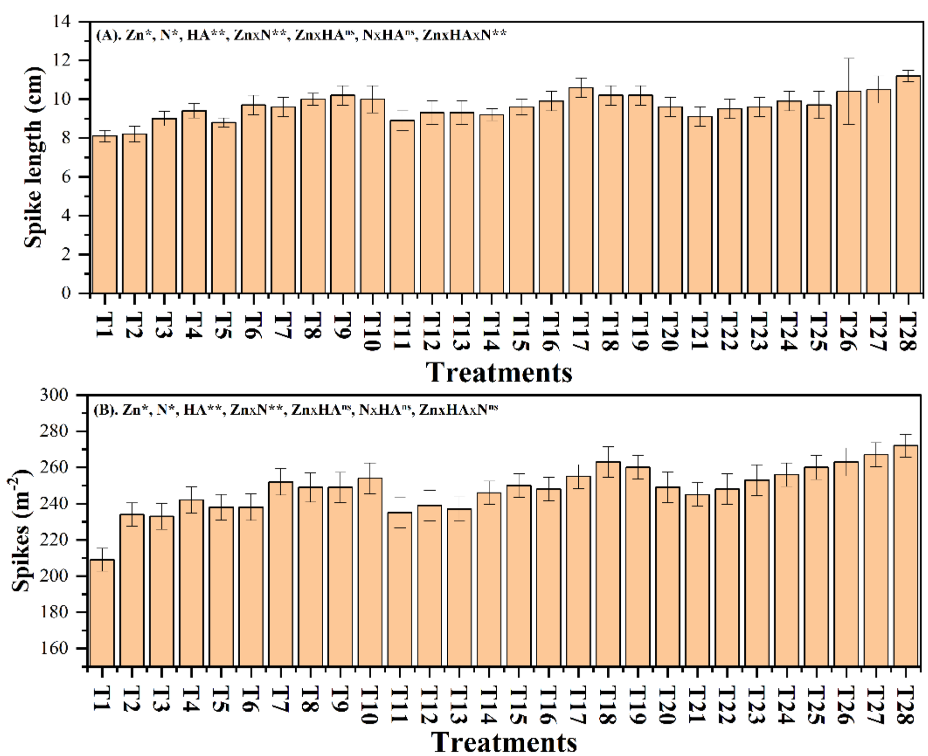

Figure 4. Changes in leaf area tiller-1 and wheat plant height to different N, Zn and HA applications.T1 to T28 indicates different treatments, for detail see material and methods.

increment of N and greater spike length (11) was observed at 160 kg N ha^{-1}. Minimum spike length (9.2) was recorded at 6 kg Zn ha^{-1}. Spike length enhanced with each increment of Zn and maximum spike length (10) was obtained at 18 kg Zn ha^{-1}. The lower level of HA (5 kg ha^{-1}) resulted minimum spike length (9.1). Spike length increased with each increment of HA and maximum spike length (9.9) was recorded at 15 kg HA ha^{-1}.

The application of N, Zn, HA, control vs. rest and Zn × HA interaction significantly affected spikes m^{-2} of wheat (Figure 4B), while all other interactions were non-significant. The treated plots showed maximum spikes m^{-2} (249) as compared to control (209). N's mean data revealed that minimum spikes m-2 (239) were noted at 80 kg N ha^{-1}. Spikes m^{-2} improved with each increment of N and maximum spikes m^{-2} (258) was recorded at 160 kg N ha^{-1}. Minimum spikes m^{-2} (238) was recorded at 6 kg Zn ha^{-1}. Spikes m^{-2} improved with each addition of Zn and maximum spikes m^{-2} (253) was obtained at 18 kg Zn ha^{-1}. Lower level of HA (5 kg ha^{-1}) produced minimum spikes m^{-2} (238). Spikes m^{-2} increased with each increment of HA and more spikes m^{-2} (253) was noted at 15 kg HA ha^{-1}. Interaction between Zn × HA showed that spikes m^{-2} enhanced with each increment of Zn up to 18 kg N ha^{-1} with 5 kg HA ha^{-1}. Alike increase in spikes m^{-2} was noted with each increment of Zn with 10 and 15 kg HA ha^{-1}.

3.2 Yield Component and Yield Traits

The addition of N, Zn and HA significantly affected productive tillers m^{-2}(Table 9), whereas the interaction among Zn, N and HA were found non-significant. The treated plots exhibited maximum productive tillers m^{-2} (239) as compared to control (187). Mean data for N exhibited that minimum productive tiller m^{-2} (230) was recorded at 80 kg N ha^{-1}. Productive tillers m^{-2} increased with each increment of N and maximum productive tillers m^{-2} (248) was noted at 160 kg N ha^{-1}. Minimum productive tillers m^{-2} (232) was obtained at 6 kg Zn ha^{-1}. Productive tillers m^{-2} improved with each increment of Zn and higher productive tillers m^{-2} (243) was recorded at 18 kg Zn ha^{-1}. Lower level of HA (5 kg ha^{-1}) resulted lower productive tillers m^{-2} (232). Productive tillers m^{-2} increased with each increment of HA and maximum productive tillers m^{-2} (242) was noted at 15 kg HA ha^{-1}. Interaction between Zn × HA revealed that productive tillers m^{-2} increased with each increment of Zn up to 18 kg N ha^{-1} with 5 kg HA ha^{-1}. A similar increase in productive tillers m^{-2} was recorded with each increment of Zn with 10 and 15 kg HA ha^{-1}.

Analysis of the data showed that N, Zn, HA, control vs. rest and N × HA interaction had significantly affected number of grains $spike^{-1}$ (Table 11). At the same time, all other interaction were non-significant. The treated plots revealed more grains $spike^{-1}$ (51) as compared to control (36). Mean data for N manifested that minimum grains $spike^{-1}$ (48) was noted at 80 kg N ha^{-1}. Grains $spike^{-1}$ enhanced with each addition of N and maximum grains $spike^{-1}$ (52) was

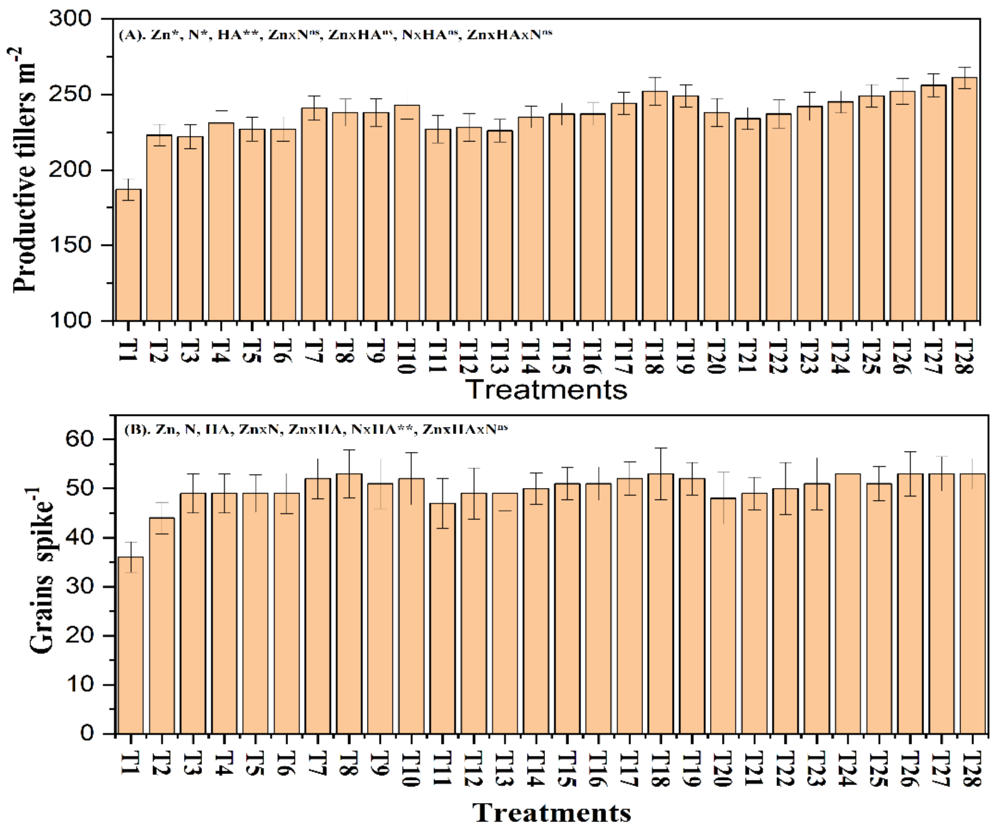

Figure 5. Changes in productive tillers $m^{-2}$and grains $spike^{-1}$leaf area tiller-1 and plant height of wheat to different N, Zn and HA applications.T1 to T28 indicates different treatments, for detail see material and methods.

recorded at 160 kg N ha^{-1}. Lesser grains $spike^{-1}$ (47) was observed at 6 kg Zn ha^{-1}.

Grains $spike^{-1}$ improved with each increment of Zn and more grains $spike^{-1}$ (51) was obtained at 18 kg Zn ha^{-1}. Lower level of HA (5 kg ha^{-1}) produced in lesser grains $spike^{-1}$ (47). Grains $spike^{-1}$ increased with each increment of HA and more grains $spike^{-1}$ (51) was obtained at 15 kg HA ha^{-1}. Interaction between N × HA showed that grains $spike^{-1}$ improved with each increment of N up to 160 kg N ha^{-1} with 5 kg Zn ha^{-1}. Alike increase in grains $spike^{-1}$ was recorded with each increment of N with 10 and 15 kg Zn ha^{-1}. Data on 1000 grains weight revealed that N, Zn, HA, control vs. rest, N × Zn, Zn × HA and N × Zn × HA interaction had significantly affected 1000-grains weight (Table 1). While other interaction were non-significant. The treated plots showed more 1000-grains weight (45) as compared to control (36). Mean data for N showed that less 1000-grains weight (41.3) was obtained 1000-grains weight improved with each increment of N and higher 1000-grains weight (47.5) was noted at 160 kg N ha^{-1}. Minimum 1000-grains weight (42.4) was recorded at 6 kg Zn ha^{-1}. 1000-grains weight increased with each increment of Zn and maximum 1000-grains weight (45.7) was recorded at 18 kg Zn ha^{-1}. Lower level of HA (5 kg ha^{-1}) resulted in minimum 1000-grains weight (43.0). 1000-grains weight increased with the increment of HA and 1000-grains weight maximum 1000-grains weight (45.5) was noted at 15 kg HA ha^{-1}. Interaction between N × Zn manifested that 1000-grains weight increased with each increment of N up to 160 kg N ha^{-1} with 6 kg Zn ha^{-1}. Similar improved 1000-grains weightwas noted with each increment of N with 12 and 18 kg Zn ha^{-1}. Interaction among Zn × HA showed that 1000-grains weight increased with each increment of Zn up to 18 kg N ha^{-1} with 5 kg HA ha^{-1}. Alike increase in 1000-grains weight was observed with each increment of Zn with 10 and 15 kg HA ha^{-1}.

Analysis of the data revealed that N, Zn, HA, control vs. rest, N × Zn and N × Zn × HA interaction had significantly affected biological yield (Table 2). While all other interaction were non-significant. The treated plots showed maximum biological yield (8805 kg ha^{-1}) as compared to control (6127 kg ha^{-1}). Mean data for N revealed that lower biological yield (7450 kg ha^{-1}) was noted at 80 kg N ha^{-1}. Biological yield increased with each increment of N and maximum biological yield (9260 kg ha^{-1}) was observed at 160 kg N ha^{-1}. Minimum biological yield (7843 kg ha^{-1}) was noted at 6 kg Zn ha^{-1}. Biological yield increased with each increment of Zn and maximum biological yield (8973 kg ha^{-1}) was noted at 18 kg Zn ha^{-1}. The lower level of HA (5 kg ha^{-1}) resulted in lowest biological yield (7997 kg ha^{-1}). Biological yield increased with increment of HA and higher biological yield (8900 kg ha^{-1}) was observed at 15 kg HA ha^{-1}. Interaction between N × Zn revealed that biological yield increased with each increment of N up to 160 kg N ha^{-1} with 6 kg Zn ha^{-1}. Similar increase in biological yield was noted with each increment of N with 12 and 18 kg Zn ha^{-1}.

The data analysis showed that N, Zn, HA, control vs. rest, N × Zn and N × Zn × HA interaction had significantly affected grain yield (Table 3). While all other interaction were non-significant. The treated plots showed maximum grain yield (3420 kg ha^{-1}) as compared to control (2360 kg ha^{-1}). N's mean data revealed that lower grain yield (2862 kg ha^{-1}) was observed at 80 kg N ha^{-1}. Grain yield increased with each increment of N and maximum grain yield (3723 kg ha^{-1}) was observed at 160 kg N ha^{-1}. Minimum grain yield (3080 kg ha^{-1}) was noted at 6 kg Zn ha^{-1}. Grain yield increased with each increment of Zn and maximum grain yield (3544 kg ha^{-1}) was noted at 18 kg Zn ha^{-1}. Lower level of HA (5 kg ha^{-1}) resulted in lowest grain yield (3187 kg ha^{-1}). Grain yield increased with the increment of HA and higher grain yield (3493 kg ha^{-1}) was observed at 15 kg HA ha^{-1}. Interaction between N × Zn revealed that grain yield increased with each increment of N up to 160 kg N ha^{-1} with 6 kg Zn ha^{-1}. Similar increase in grain yield was noted with each increment of N with 12 and 18 kg Zn ha^{-1}.Analysis of the data showed that N, Zn, HA, control vs. rest, N × Zn and N × Zn × HA interaction had significantly affected

Table 1. **1000-grain weight (g) of wheat as affected by nitrogen (N), Zinc(Zn) andhumic acid(HA).**

Zn (kg ha^{-1})	HA (kg ha^{-1})	N (kg ha^{-1})			Mean
		80	**120**	**160**	
		N x Zn x HA			**Zn x HA**
6	5	39.9	40.3	42.3	41.5
	10	41.6	42.8	42.8	41.8
	15	42.2	41.5	42.5	42.1
12	5	44.5	45.9	46.4	45.6
	10	46.3	45.4	45.1	45.6
	15	46.9	44.6	46.9	46.1
18	5	44.3	47.1	48.7	46.7
	10	45.3	47.8	49.1	47.6
	15	47.3	48.6	49.6	48.3
		N x HA			
	5	40.0	43.3	45.6	43.0 b
	10	41.8	45.6	47.6	45.0 a
	15	42.1	46.1	48.3	45.5 a
		N x Zn			
6		39.8	43.0	44.4	42.4 b
12		42.2	45.5	47.9	45.2 a
18		41.9	46.0	49.1	45.7 a
		41.3 c	45.8 b	47.5 a	
Planned Mean Comparison					
	Control	36			
	Rest	45			

Note: Means of same category followed by different letters are significantly different at ($P \leq 0.05$) using LSD test.

Table 2. Biological yield (kg ha^{-1}) of wheat as affected by nitrogen (N), Zinc(Zn) andhumic acid(HA).

Zn (kg ha^{-1})	HA (kg ha^{-1})	N (kg ha^{-1})			Mean
		80	120	160	
		N x Zn x HA			Zn x HA
6	5	7610	7724	7738	7691
	10	7634	7631	8150	7805
	15	7492	7818	8200	7837
12	5	8432	8850	9520	8934
	10	8915	9282	9394	9197
	15	9011	9152	9671	9278
18	5	9099	9604	9739	9481
	10	9259	9730	9866	9618
	15	9562	9864	9786	9737
		N x HA			
	5	7230	7880	8880	7997 b
	10	7636	8660	9650	8649 a
	15	7685	9278	9737	8900 a
		N x Zn			
6		7120	8160	8250	7843 b
12		7620	9178	9733	8843 a
18		7609	9528	9797	8978 a
		7450 c	8955 b	9260 a	
Planned Mean Comparison					
	Control	6127			
	Rest	8805			

Means of same category followed by different letters are significantly different at ($P \leq 0.05$) using LSD test.

harvest index (Table 4). While all other interaction were non-significant. The treated plots showed a maximum harvest index (39 %) compared to control (37 %). Mean data for N revealed that lower harvest index (38 %) was observed at 80 kg N ha^{-1}.

Harvest index increased with each increment of N and maximum harvest index (40 %) was observed at 160 kg N ha^{-1}. Minimum harvest index (38 %) was noted at 6 kg Zn ha^{-1}. Harvest index increased with each increment of Zn and maximum harvest index (39 %) was noted at 18 kg Zn ha^{-1}. Lower level of HA (5 kg ha^{-1}) resulted in lowest harvest index (38 %).Harvest index increased with increment of HA and higher grain yield (39 %) was observed at 15 kg HA ha^{-1}. Interaction between N × Zn revealed that harvest index increased with each increment of N up to 160 kg N ha^{-1} with 6 kg Zn ha^{-1}. Similar increase in harvest index was noted with each increment of N with 12 and 18 kg Zn ha^{-1}.

4. DISCUSSION

Our results showed that days to emergence and emergence m^{-2} of wheat to N, Zn, HA, control vs. rest and all the interactions among the nutrients were non-significantly affected. The possible reason may be that the consumption of its own stored food in the seed as an enough source for seedling to emerge. Our results are similar to (Waraich et al., 2007) who reported that N and HA did not affect emergence. Similar results are also reported by Leghari et al. (2016). It may be because fertilizer response is not so quick and observed immediately after two weeks. Similarly, Leghari et al. (2016) reported that emergence m^{-2} was not affected by N. The reason may be that seed used its own endosperm food for germination and plants did not use nutrients from outside source (Shah et al., 2012). The results also conform to Shah et al. (2009) who stated that fertilizer fertilization had no influence on the emergence m^{-2}.

N, Zn, HA, control vs. rest, and N × Zn, wheat's days to anthesis and maturity were significantly affected, while the early anthesis was recorded inin control plots. The delayed anthesis is due to the nutrients available in an adequate amount, which enlarged the growing time period of cereal crop (Arif et al., 2006). N fertilization increased vegetation, leaf area and light use efficiency (Zeidan et al., 2010). Similarly, N application delayed days to tasseling, silking and maturity in maize (Amanullah et al., 2008&Arif et al., 2006). However, the application of Zn and HA have no impact on days to anthesis (Nawab et al., 2011). Physiological maturity delayed with increased levels of N, Alike results were stated by Sivasankar et al. (1993) who stated that N is very imperative for crop development and growth. Fertilization of N promotes lived green foliage duration (Frederick &Camberato, 1995). Increase in N levels significantly delayed days to maturity Ayoub et al. (1994). The delayed in days to maturity may be due to enough nutrient accessibility, which finally enhanced the growing time period of cereal crops Ullah et al. (2021). Alike results were reported by Zeidan et al. (2010) who reported that N fertilizer improves vegetative growth, increased light use efficiency and grain filling period. Higher levels of Zn and HA increased the time of maturity of wheat crop. Comparable results were reported by Rajput et al. (2004) who stated that Zn and HA application with higher rates late maturity of the crop which due to easily availability of

Table 3. Grain yield (kg ha^{-1}) of wheat as affected by nitrogen (N), Zinc(Zn) and humic acid(HA).

Zn (kg ha^{-1})	HA (kg ha^{-1})	N (kg ha^{-1})			Mean
		N 1	N 2	N 3	
		N x Zn x HA			**Zn x HA**
6	5	2933	3060	3143	3045
	10	2886	2930	2970	2929
	15	2792	3153	3233	3059
12	5	3167	3503	3624	3431
	10	3415	3485	3498	3466
	15	3511	3355	3775	3547
18	5	3337	3797	3860	3665
	10	3497	3923	3969	3796
	15	3800	4057	4049	3969
		N x HA			
	5	2710	3340	3510	3187 b
	10	2890	3425	3710	3342 a
	15	2956	3547	3975	3493 a
		N x Zn			
6		2732	3214	3295	3080 b
12		2934	3395	3795	3375 a
18		2919	3632	4080	3544 a
		2862 c	3414 b	3723 a	
Planned Mean Comparison					
	Control	2360			
	Rest	3420			

Means of same category followed by different letters are significantly different at (P≤ 0.05) using LSD test.

Table 4. Harvest index (%) of wheat as affected by N, Zn and HA.

Zn (kg ha^{-1})	HA (kg ha^{-1})	N (kg ha^{-1}) 80	120	160	Mean
		N × Zn × HA			**Zn x HA**
6	5	38	37	39	38
	10	38	38	39	38
	15	37	39	39	38
12	5	38	38	38	38
	10	38	38	37	38
	15	39	37	39	38
18	5	37	40	41	39
	10	38	40	42	40
	15	40	41	42	41
		N × HA			
	5	38	38	39	38 b
	10	38	38	40	39 a
	15	38	38	41	39 a
		N × Zn			
6		38	38	38	38 b
12		39	38	40	39 a
18		38	38	42	39 a
		38 c	38 b	40 a	
Planned Mean Comparison					
	Control	37			
	Rest	39			

Means of same category followed by different letters are significantly different at ($P \leq 0.05$) using LSD test.

essential nutrients thus enhanced vegetative growth of crop which delayed maturity. Leaf area tiller^{-1} and plant height had significantly affected by N, Zn, HA, control vs. rest, N× Zn and N× Zn × HA interactions. Whereas, all further interaction were non-significant. Leaf area tiller^{-1} enhanced with each accumulation of N and maximum leaf area tiller^{-1} was observed at 160 kg N ha^{-1}. While minimum leaf area tiller^{-1} was noted in control plots. Our results conform with Vogeler et al. (2020) who stated that N fertilizer increased leaf area of wheat. This may be because Zn and HA fertilization increased leaf area due to increased vegetative growth. Our results are in contract with Arif et al. (2006) and Iqbal et al. (2020) who stated that significant increase was found in the height of crop with fertilization of N. Increased plant height due to enough amount of N may be credited to additional vegetative growth and improvement as a result in intermodal extension. The soil application of Zn increased plant height linearly. Plant height increased 18.7 % over control by fertilization of Zn (Ali et al., 2013).

Additionally, spikes m^{-2}, tillers m^{-2}, spike length, grains spike^{-1} and 1000-grains weight revealed that N, Zn, HA, control vs. rest and Zn × HA interaction had significantly affected. Whereas all other interactions were non-significant. The nutrients treated plots manifested more spikes m^{-2} as compared to control. Spikes m^{-2} increased with each increment of N and maximum spikes m^{-2} was noted at 160 kg N ha^{-1}. The reason may be that the availability of nitrogen in enough amounts facilitates the plant's tillering ability, which is directly related to spikes m^{-2}. Our results are in line with Jan et al. (2000) who find that spikes population and number of grains spike^{-1} had a significantly increased with increasing levels of N fertilizer. Alike results were published by Shahab et al. (2016). Spikes m^{-2} increased with each addition of Zn and maximum spike m^{-2} was observed at 18 kg Zn ha^{-1}. Our results are in conformity with Keram et al. (2014) who postulated that Zn at the rate of 20 kg ha^{-1} along with a recommended dose of NPK improved yield and yield-related components of wheat. Spikes m^{-2} increased with each incensement of HA and maximum spikes m^{-2} was noted at 15 kg HA ha^{-1}. The reason might be that HA stimulates microbial movement in soil to improve the physical structure and biological environment of soil, which enhances plant growth and development (Zancani et al., 2009). The possible reason increase in spike length might be due to that N fertilization increased the vegetative and reproductive growth of plant (Shahab et al., 2016). Our results are in agreement with Leghari et al. (2016). Zn fertilization also increased spike length linearly with each increment of Zn and maximum spike length was noted at 18 kg ha^{-1} Zn. Alloway et al. (2004) stated that the combine resulting from the effects of N application helps enhance plant growth, change the pH of the root environment, and found significant interactions increasing dose of Zn and N. Zn plays a vital role in protein degradation and protein-protein interactions. This way, it takes part in plant metabolism, growth, and development (Marschner et al., 1995). Spike length increased with each increment of HA and maximum spike length was noted at 15 kg ha^{-1} HA. Grains spike^{-1} might be improved because Zn's application makes readily available nutrients uptake from the soil to plant and enhances their effectiveness greatly in the grains creation time and resulted maximum grains spike^{-1} (Zeidan et al., 2010). HA application promotes plant growth by providing unavailable nutrients and buffering pH of soil (Julie and Bugbee, 2006). Improvement in 1000-grains weight may be due to that plant protein synthesis, production of plant hormones, pollination and fruit setting may be subjected to changes due to Zn (Alloway et al., 2004). Our results conform with Khan et al. (2008) and Alam et al. (2000) who stated that the application of Zn enhances the 1000-grain weight of wheat significantly. The application of HA also increased 1000-grains weight and maximum 1000-grains weight was observed at 15 Kg HA ha^{-1}. The probable reason may be that HA increased the fertility of the soil which contain some elements which have a pivotal role in enhancing the fertility of the soil to a greater extent, thus enhancing the growth of plant and yield components of the crop (Khan et al., 2006). The increase in yield and yield components of wheat due to Zn application might be the fact that Zn played a vital role in the biosynthesis of the IAA and initiation of primodia for reproductive parts and a result of the favorable effect of Zn on the metabolic reactions within the plants Singh et al. (2005).

Biological yield grain yield and harvest index were significantly affected by N, Zn, HA control vs. rest, N × Zn and N × Zn × HA. The treated plots showed maximum biological yield as compared to control. Biological yield, grain yield and harvest index were enhanced with each augmentation of N and maximum biological yield was noted at 160 kg N ha^{-1}. These results are in contract with Khan et al. (2008) who stated that N fertilization improved biomass and yield of the wheat. Zeidan and Amany (2006) gave the same results, who stated that N application enhances vegetative, reproductive growth and grain yield. Similar results are stated by Jan et al. (2000). Zn fertilization improved biological yield linearly with each increment of N and maximum biological yield was noted at 12 kg Zn ha^{-1}. The possible reason may be that Zn plays a vital part in plant growth and metabolism processes and is needed for enzyme activation in plants (Alloway et al., 2004). Our results are in line with Keram et al. (2014) who stated that Zn at the rate of 20 kg ha^{-1} combination with N improved equally grain and biological yield of wheat crop. The combined fertilization of N and Zn increased biological yield significantly. Similar results are reported by Asif et al. (2011) and Amanullah et al. (2009). Biological yield increased with HA increment and maximum biological yield was recorded at10 kg HA ha^{-1}. The possible reason may be that HA provides good soil form and enhances the accessibility of immobile nutrient uptake. These results agree with Ali et al. (2019) who reported that application of HA material has significantly influenced crop production, enhanced the fertility of soil, and minimized the requirement of NPK fertilizer application.

5. CONCLUSION

Based on the results, it is concluded that N fertilization at the rate of 160 kg ha^{-1} produced higher grain yield and yield components of wheat as compared to other levels. Zn fertilization significantly improved yield and yield contributing components. However, application of Zn above 12 kg ha^{-1} up to 18 kg ha^{-1} was found statistically the same. HA significantly increased yield and yield components at 10 kg HA ha^{-1}. Application of N at 160 kg ha^{-1}, Zn at 12 kg ha^{-1} and HA at 10 kg ha^{-1} is recommended for obtaining higher grain yield and yield components of wheat.

Authors Contributions:

A.I and S.K.K conceived the main idea of research, A.I wrote the manuscript. H.R, M.Z and R.K revised the manuscript and provided suggestions. In addition M.A, S.W.K and AK assessed and analyzed the data, and performed data collection. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability statements: The data presented in this study are available on request from the corresponding author.

REFERENCES

Waraich, E.A., Ahmad, R., Ali, A. and Ullah, S., Irrigation and nitrogen effects on grain development and yield in wheat (Triticumaestivum L.). Pak. J. Bot. (2007). 39(5), pp.1663-1672.

Leghari, S. J., Wahocho, N. A., Laghari, G. M., HafeezLaghari, A., MustafaBhabhan, G., HussainTalpur, K., ... &Lashari, A. A. Role of nitrogen for plant growth and development: A review. Advances in Environmental Biology. (2016).10(9), 209-219

Amanullah, Khattak, R.A.,Khalil, S.K. Effects of plant density and N on phenology and yield of cereal. J. Plant Nutrition. (2008). 27: 146-150.

Anderson, P.M., Oelke, E.A., Simmons, S.R. Growth and Development Guide for Spring Barley. University of Minnesota Agricultural Extension Folder AG-FO-(1985). 2548.2.

Arif, M., Chohan, M. A., Ali, S., Gul, R., Khan, S. Response of wheat to foliar application of nutrients. (2006). J. Agric. Biol. Sci. 1(4): 30-34.

Ali, I., Khan, A.A., Munsif, F., He, L., Khan, A., Ullah, S., Saeed, W., Iqbal, A., Adnan, M. and Ligeng, J. Optimizing rates and application time of potassium fertilizer for improving growth, grain nutrients content and yield of wheat crop. Open Agriculture. (2019a). 4(1), pp.500-508.

Eeswaran, R., Nejadhashemi, A.P. and Miller, S.R. Evaluating the climate resilience in terms of profitability and risk for a long-term corn-soybean-wheat rotation under different treatment systems. Climate Risk Management. (2021)32, p.100284.

Cappelli, A., Oliva, N., Bonaccorsi, G., Lorini, C. and Cini, E. Assessment of the rheological properties and bread characteristics obtained by innovative protein sources (Cicerarietinum, Achetadomesticus, Tenebriomolitor): Novel food or potential improvers for wheat flour?. Lwt, (2020). 118, p.108867.

Tricase, C., Amicarelli, V., Lamonaca, E. and Rana, R.L. Economic analysis of the barley market and related uses. Grasses as food and feed. (2018). 10.

Iqbal, A., He, L., Khan, A., Wei, S., Akhtar, K., Ali, I., Ullah, S., Munsif, F., Zhao, Q. and Jiang, L. Organic manure coupled with inorganic fertilizer: An approach for the sustainable production of rice by improving soil properties and nitrogen use efficiency. Agronomy. (2019). 9(10), p.651.

Ali, I., He, L., Ullah, S., Quan, Z., Wei, S., Iqbal, A., Munsif, F., Shah, T., Xuan, Y., Luo, Y. and Tianyuan, L., 2020. Biochar addition coupled with nitrogen fertilization impacts on soil quality, crop productivity, and nitrogen uptake under double-cropping system. Food and Energy Security. (2020). 9(3), p.e208.

Iqbal, A., He, L., Ali, I., Ullah, S., Khan, A., Khan, A., Akhtar, K., Wei, S., Zhao, Q., Zhang, J. and Jiang, L. Manure combined with chemical fertilizer increases rice productivity by improving soil health, post-anthesis biomass yield, and nitrogen metabolism. Plos one (2020). 15(10), p.e0238934.

Yuvaraj, M. and Subramanian, K.S. Significance of Zinc in Plant Nutrition. Biotica Research Today. (2020). 2(8), pp.823-825.

Gondal, A.H., Zafar, A., Zainab, D., Toor, M.D., Sohail, S., Ameen, S., Ijaz, A.B., Ch, B.I., Hussain, I., Haider, S. and Ahmad, I.A. A detailed review study of zinc involvement in animal, plant and human nutrition. Indian Journal of Pure & Applied Biosciences (2021). 9(2), pp.262-271.

Mumivand, H., Khanizadeh, P., Morshedloo, M.R., Sierka, E., Żuk-Gołaszewska, K., Horaczek, T. and Kalaji, H.M. Improvement of Growth, Yield, Seed Production and Phytochemical Properties of SaturejakhuzistanicaJamzad by Foliar Application of Boron and Zinc. Plants. (2021). 10(11), p.2469.

Bhantana, P., Rana, M.S., Sun, X.C., Moussa, M.G., Saleem, M.H., Syaifudin, M., Shah, A., Poudel, A., Pun, A.B., Bhat, M.A. and Mandal, D.L. Arbuscularmycorrhizal fungi and its major role in plant growth, zinc nutrition, phosphorous regulation and phytoremediation. Symbiosis. (2021). 84(1), pp.19-37.

Umair Hassan, M., Aamer, M., UmerChattha, M., Haiying, T., Shahzad, B., Barbanti, L., Nawaz, M., Rasheed, A., Afzal, A., Liu, Y. and Guoqin, H., 2020. The critical role of zinc in plants facing the drought stress. Agriculture. (2020). 10(9), p.396.

Rehman, A., Farooq, M., Ullah, A., Nadeem, F., Im, S.Y., Park, S.K. and Lee, D.J., 2020. Agronomic biofortification of zinc in Pakistan: Status, benefits, and constraints. Frontiers in Sustainable Food Systems. (2020). p.276.

Amanullah, Alwahibi, M.S., Elshikh, M.S., Alkahtani, J., Muhammad, A., Khalid, S., Ahmad, M., Khan, N., Ullah, S. and Ali, I. Phosphorus and Zinc Fertilization Improve Zinc Biofortification in Grains and Straw of Coarse vs. Fine Rice Genotypes. Agronomy. (2020) 10(8), p.1155.

Ali, I., Khan, A.A., Khan, A., Asim, M., Ali, I., Zib, B., Khan, I., Rab, A., Sadiq, G., Ahmad, N. and Iqbal, B.,.Humic acid and nitrogen levels optimizing productivity of green gram (Vigna radiate L.). Russian Agricultural Sciences, (2019b). 45(1), pp.43-47.

Izhar Shafi, M., Adnan, M., Fahad, S., Wahid, F., Khan, A., Yue, Z., Danish, S., Zafar-ul-Hye, M., Brtnicky, M. and Datta, R. Application of single superphosphate with humic acid improves the growth, yield and phosphorus uptake of wheat (Triticumaestivum L.) in calcareous soil. Agronomy. (2020). 10(9), p.1224.

Leite, J.M., Arachchige, P.S.P., Ciampitti, I.A., Hettiarachchi, G.M., Maurmann, L., Trivelin, P.C., Prasad, P.V. and Sunoj, S.J. Co-addition of humic substances and humic acids with urea enhances foliar nitrogen use efficiency in sugarcane (Saccharumofficinarum L.). Heliyon. (2020). 6(10), p.e05100.

Zaremanesh, H., Akbari, N., Eisvand, H.R., Ismaili, A. and Feizian, M. The Effect of Humic Acid on Soil Physicochemical and Biological Properties under Salinity Stress Conditions in Pot Culture of SaturejakhuzistanicaJamzad. ECOPERSIA. (2020). 8(3), pp.147-154.

Karimi, E., Shirmardi, M., DehestaniArdakani, M., Gholamnezhad, J. and Zarebanadkouki, M. The Effect of Humic Acid and Biochar on Growth and Nutrients Uptake of Calendula (Calendula officinalis L.). Communications in Soil Science and Plant Analysis. (2020). 51(12), pp.1658-1669.

Skowrońska, M., Bielińska, E.J., Szymański, K., Futa, B., Antonkiewicz, J. and Kołodziej, B. An integrated assessment of the long-term impact of municipal sewage sludge on the chemical and biological properties of soil. Catena. (2020). 189, p.104484.

Steel, R.G.D., Torrie, J.H. and Dicky, D.A. Principles and procedures of statistics a biometrical approach. McGraw Hill Book International Co. (1997).

Abbas, S.H., Sohail, M., Hussain, I., Saleem, M., Qamar, M., Aslam, M. Grain yield of newly developed wheat cultivar (narc 2011) as enhanced by foliar application of humic acid under rainfed conditions. Sarhad J Agric. 2014. 30, pp.173-178.

Ahmed, N. and Rashid, M. Fertilizer use in Pakistan. National Fertilizer Development Center, Planning and Development Division, Islamabad. 2003.

Manaf, A., Raheel, M., Sher, A., Sattar, A., Ul-Allah, S., Qayyum, A. and Hussain, Q. Interactive effect of zinc fertilization and cultivar on yield and nutritional attributes of canola (Brassica napus L.). Journal of Soil Science and Plant Nutrition. (2019). 19(3), pp.671-677.

Alam, S., Khalil, S., Ayub, N., Rashid, M. In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. Intl. J. Agric. Biol. 2002. 4:454-458.

Alam, E., Rashid, A., Ryan, J., Bhatti, A.U. Zn deficiency in rain fed wheat in Pakistan: Magnitude, spatial variability, management and plant analysis diagnostic norms. Comms. Soil Sci. Plant Anal. (2000). 37: 144-150.

Ali, Y., Farhatullah, Rahman, H., Nasim, A., Azam, S.M., Khan, A., Heritability and correlation analysis for morphological and biochemical traits in brassica carinata. Sarhad J. Agric. (2013). 29(3): 359-369

Wu, K., Ali, I., Xie, H., Ullah, S., Iqbal, A., Wei, S., He, L., Huang, Q., Wu, X., Cheng, F. and Jiang, L. Impact of fertilization with reducing in nitrogen and phosphorous application on growth, yield and biomass accumulation of rice (Oryza sativa L.) under a dual cropping system. PeerJ. (2021). 9, p.e11668.

Ali, I., Adnan, M., Ullah, S., Zhao, Q., Iqbal, A., He, L& Jiang, L. Biochar combined with nitrogen fertilizer: a practical approach for increasing the biomass digestibility and yield of rice and promoting food and energy security. *Biofuels, Bioproducts and Biorefining.* (2022). https://doi.org/10.1002/bbb.2334

Alloway, B. 2004.In Zn in Soil and Crop a Mediterranean climates. Nutrition. International Zn Association. Brussel. Agron. J., 86: 221-226.

Ayoub M, Guertin S, Lussier S, Smith DL. Timing and levels of N fertility effects on spring wheat. Crop Science. (1994). 34: 748–750.

Khan, M.A., Jan, N., Sultana, Q., Ahmad, S.R. and Rahman, A. Effect of different organic materials and chemical fertilizers on the yield of wheat and physical properties of soil. Sarhad Journal of Agriculture. (2006) 22(3), p.437.

Frederick, J.R. and Camberato, J.J., Water and nitrogen effects on winter wheat in the southeastern Coastal Plain: I. Grain yield and kernel traits. Agronomy Journal. (1995). 87(3), pp.521-526.

Jan, M.T., Khan, S. 2000. Response of wheat yield components to type of N fertilizer their levels and application time. Pak. J. Bio. Sc. 3(8): 1227-1230.

Keram, K.S., Sharma, B.L., Sharma, G.D. and Thakur, R.K. Impact of zinc application on its translocation into various plant parts of wheat and its effect on chemical composition and quality of grain. Scientific Research and Essays. (2013). 8(45), pp.2218-2226.

Khan, M., Fuller, M. and Baloch, F. Effect of soil applied zinc sulphate on wheat (Triticumaestivum L.) grown on a calcareous soil in Pakistan. Cereal Research Communications. (2008) 36(4), pp.571-582..

Lodhi, A. M., Khanzada, M. A., Shahzad, S., Ghaffar, A., & Lévesque, C. A. Prevalence of Pythiumaphanidermatum in agro-ecosystem of Sindh province of Pakistan. Pak. J. Bot. (2013). 45(2), 635-642.

Marschner, H. Mineral Nutrition of Higher Plants 2nd Ed. pp.: 99-101 Academic press. (1995).

Vogeler, I., Hansen, E. M., Nielsen, S., Labouriau, R., Cichota, R., Olesen, J. E., & Thomsen, I. K. Nitrate leaching from suction cup data: Influence of method of drainage calculation and concentration interpolation . 2020. 49(2).440-449.

Nawab, N. N., Saeed, A., Tariq, M. S., Nadeem, K., Mahmood, K., Ul-Hassan, M., Khan, A. A. Inheritance of okra leaf type in different genetic backgrounds and its effects on fibre and agronomic traits in cotton. African Journal of Biotechnology. (2011b). 10(73), 16484-16490.

Shahab, Q., Afzal, M., Hussain, B., Abbas, N., Hussain, S. W., Zehra, Q., Abbas, Y. Effect of different methods of zinc application on maize (Zea mays L.). International Journal of Agronomy and Agricultural Research. (2016). 9(3), 66-75.

Singh, R.P., Huerta-Espino J., William H.M. Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. Turkish Journal of Agriculture and Forestry. (2005). 29: 121–127.

Sivasankar, K. Bansal, C., Abrol, Y. P. N in relation to leaf area development and photosynthesis. Proc. Indian natn. Sci Acad. 3 (4). (1993).pp 235-244.

Sutton, R., Sposito, G. Molecular structure in soil humic substance: the new view Environ. Sci. Technol. (2005). 39: 9009-9011

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ORIGINAL RESEARCH

Rosmarinus officinalis Might be Exploited as a Natural Antifouling Agent: A Potentially Promising Strategy for Curbing Membrane Biofouling

Abdulkadir Rabiu Salisu[1*] , Shamsuddeen Umar[2], Abdullahi Hassan Kawo[2], Cosa Sekelwa[3]

[1]Department of Environmental Science, Kaduna Polytechnic, Kaduna, PMB 2021.
[2]Department of Microbiology, Bayero University, Kano, PMB 2011.
[3]Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Private bag X20, Hatfield, 0028, South Africa.

Corresponding author:
ar.salisu@kadunapolytechnic.edu.ng
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ABSTRACT
Membrane biofouling is the coverage of membrane surfaces due to undesirable development of biofilms causing a decrease and subsequent loss of productivity in water treatment settings. Continuous use of synthetic chemicals against biofouling is inept as it leads to the emergence of multi-antibiotic resistance. Application of natural products such as plants can be apt in curbing biofouling while checking the resistance challenge. This study aimedto evaluate the potential of *Rosmarinus officinalis* in the control of membrane biofouling. Bacteria from biofouling environments were subjected to a biofilm confirmation test and identified at cultural, morphological, biochemical and molecular levels. Leaves of *R.officinalis* were extracted in solvents of varying polarity and activities. These extracts were evaluated against bacterial biofilm formation via minimum biofilm inhibitory concentration (MBIC), minimum biofilm eradication concentration (MBEC) and mesocosm bioassays. Biofilm formation was confirmed in 68% of the isolates identified as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The methanol and ethyl acetate extracts of *R.officinalis* indicated the least MICs (0.313mg/L and 1.25mg/L) against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. Both extracts recorded the highest MBIC (50.00%) against *Pseudomonas aeruginosa*. The peak MBEC (57.88%) was obtained from the methanol extract against *Staphylococcus aureus* and this same extract inhibited 56.23% density of bacterial biofilms on glass slides. The methanol and ethyl acetate crude extracts of *R. officinalis* appreciably reduced bacterial biofilms; hence, this plant can be exploited as a natural antifouling agent, with reduced toxicity and low risk of resistance.

KEYWORDS: Bacterial biofilms, natural product, quorum sensing, rosemary, water treatment.

1. Introduction

Membrane Bioreactor (MBR) is an efficient, state-of-the-art, high-quality water treatment technology that consists of bioreactors modified with membrane filtration units for biomass retention (Meng et al., 2017; Waheed et al., 2017). Recent technological innovations and significant footprint reduction further made MBR an established water treatment system (Oh and Lee, 2018). Despite its advantages, MBR is characterized with challenges such as: pre-treatment huddles, lack of long-term performance and, to a larger extent, membrane biofouling. Membrane biofouling is the coverage of membrane surfaces due to undesirable development of biofilms (sessile slimy multicellular microbial communities) (Whiteley et al., 2017). This phenomenon hinders the effective use of MBR in water treatment settings (Biofilms, 2019).

Strategies employed to control membrane biofouling mostly spin around physical cleansing of biofilms, modification of the membranes and incorporation of antimicrobial substances such as peptides and nitrofurazones (Hook et al., 2012). In addition to being pricy, the use of these chemicals is associated with resistance, environmental pollution and non-specificity (Lade et al., 2014; Alghamdi and Quijada, 2019). Addressing biofoulingusing natural products (such as plants) as an alternative can be apt since the life of the bacteria must not be the primary target but their ability to form and express biofilms (Paluch et al., 2020). Thus, it would be imperative to develop a strategy for mitigating membrane biofouling using natural products; which can be safe, efficient, readily available, cost-effective and eco-friendly. In this study, the leaves (commonly used part) (Meziane-Assane et al., 2013) of rosemary (*Rosmarinus officinalis*) were exploited based on its perceived ethno medicinal advantages and wide applications as herb and spice (Kalamartzis et al., 2020). The objectives of the research were to: (1) Isolate and purify bacteria from visibly biofouled environments, (2) screen and confirm the ability of the isolates to form biofilms and identify the biofilm-forming bacteria (3), extract the leaves of *R.officinalis*using organic solvents of different polarity and (4) investigate the antifouling activities of the extracts against the biofilm-forming bacteria.

2. MATERIALS AND METHODS

2.1. Description of Sampling Locations

The sample sites (biofouled environments) were selected based on their likelihood to harbor biofilm-forming bacteria. These environments include parts of a biofouling model, locally fabricated at the Department of Environmental Science, Kaduna Polytechnic, Kaduna, Nigeria (10º 29' 20.79"N, 07º 25' 21.35"E); which consist of membrane filter (BMm), glass (BMg) and plastic (BMp) substrates. Other sampling points include surfaces of solid objects (metals, plastics and wooden materials) from River Kaduna (RK), Kaduna, Nigeria (10º 29' 47.13"N, 07º 25' 19.95"E) as well as walls and floors of water reservoirs and chambers (IB) in Kaduna State Water Treatment Plant, Malali, Kaduna, Nigeria (10º 33' 26.80"N, 07º 29' 01.11"E).

2.2 Sample Collection and Transportation

A total of 117 slimy scrapings were collected using a simple random sampling technique using a sterile scoop over the duration of 6 months (January-June, 2020). Each sample was immediately transferred to a tightly capped Bijou bottle containing 10mL of peptone water (Digel et al., 2018). The samples were transported in a cold condition to the microbiology laboratory, Department of Environmental Science, Kaduna Polytechnic, Kaduna, Nigeria for microbiological analyses.

2.3 Isolation of Bacteria from the Biofouled Environments

The bacterial isolation was conducted using standard plate technique as described by Wilson et al. (2018). The biofouled scrapings in peptone water were vortexed (Digel et al., 2018) and serially diluted to 10^{-6} using sterile normal saline as the diluent. A volume (100µL) from each dilution was inoculated (using spread plate technique) onto correspondingly labeled Nutrient Agar (gL^{-1} of peptone: 5.0, NaCl: 5.0, beef extract: 1.5, yeast extract: 1.5 and agar 15; pH: 7.2) as well as MacConkey agar (gL^{-1} of peptone: 17, proteose: 3g, lactose monohydrate: 10g, bile salt: 1.5, NaCl: 5, neutral red: 0.03, crystal violate: 0.001 and agar: 13.5; pH: 7.1) plates (Aryal, 2019; Julistiono et al., 2018). The inoculated Petri dishes were incubated at 28ºC for 24 hours, following when randomly selected isolates were sub-cultured and purified using the same media, under similar culture conditions.

2.4 Confirmation of Bacterial Biofilm Formation

Ability of the bacterial isolates to form biofilms was verified using a tube method (TM) as described by Kırmusaoğlu (2019). Freshly-grown bacterial culture was inoculated into replicate test tubes, each of which contained 5mL of prepared Tryptic Soy Broth (TSB) (gL^{-1} of tryptone: 17.0, soytone: 3.0, glucose: 2.5, NaCl: 5.0 and dipotassium phosphate: 2.5; pH 7.3 ± 0.2) and incubated for 24 hours at 37°C. Following this incubation, the tubes were carefully emptied and the planktonic cells were discharged by rinsing twice with phosphate-buffered saline (PBS, pH 7.2). The sessile isolates of biofilms formed on the test tubes were stained with safranin for 1 hour. The safranin-stained tubes were rinsed twice with PBS to discharge the excess stain. After air drying, appearance of a visible film lining the walls and bottom of the tubes indicated biofilm production. The same volume (5mL) of a sterile uninoculated TSB was used as control under similar culture conditions.

2.5 Identification of the Biofilm-Forming Bacteria

The biofilm producing bacterial isolates were characterized based on their morphological properties (Gram's stain reaction) and subjected to a series of biochemical (oxidase, catalase, indole, methyl red, Voges-Proskauer, triple sugar iron, citrate, urease, motility as well as H_2S production) tests (Farinde et al., 2014; Gohet al., 2014; UK Standards for Microbiology Investigations, 2014; Cappuccino and Sherman, 2013; MacFaddin, 2000). Identities of the various bacterial species were confirmed using 16S rRNA gene sequencing (Julistiono et al., 2018) via Genomic DNA Extraction, Polymerase Chain Reaction, Gel Electrophoresis and Visualization of the PCR Products, DNA Band Cutting, Gel Extraction, Purification of the PCR Fragments and Sequencing (Wang et al., 2011).

2.6 Collection and Identification of the Plant Sample

Apparently healthy whole plant of *R.officinalis* was identified using standard keys and descriptions (Myers, 2019) and harvested from its cultivated site in Malali

Plant Gardens, Kaduna, Nigeria (10°32'8.7"N, 09°27'37.2" E) in May, 2020. A taxonomist authenticated the plant's identity at the herbarium section of the Department of Plant Biology, Bayero University, Kano, Nigeria. Voucher specimens of the authenticated plant was pressed in-between clean sheets of paper, dried (Bulugahapitiya, 2013) and deposited at the herbarium for reference purpose.

2.7 Preparation of Plant Material

Leaves of the freshly collected *R.officinalis* were detached, pre-washed with clean water to remove extraneous materials (Lohaet al., 2019), rinsed with distilled water and distributed evenly to air-dry at room temperature (Gahlot et al., 2018). The dried leaves were excised and pulverized to fine powder using laboratory mortar and pestle. The ground powder was sieved through a 0.5mm mesh gauze to standardize the particle size (Teresa-May, 2018) and stored at room temperature in an air-tight dry container until needed for analyses (Ibrahim et al., 2017).

2.8 Extraction of the Plant Material

The leaf powder was extracted using cold maceration method (De Oliveira et al., 2019) in n-hexane, acetone, ethyl acetate, methanol and distilled water, based on the phases of non, less, medium, high and comparably high polar solvents, respectively (Bulugahapitiya, 2013). According to a procedure described by Sagbo et al. (2020), 100g of the leaf powder was macerated in 1000mL of the solvent and the set-up was allowed to stand for 72 hours at room temperature with intermittent agitation (De Oliveira et al., 2019; Loha et al., 2019). The damp plant material was passed through a cheese cloth, allowed to settle and re-filtered via a Whatman grade 1 filter paper (11μm) (Loha et al., 2019). The residue (marc) was re-extracted in similar solvent to recover as much occluded solution as possible. The extracts were concentrated using rotary evaporator at 40°C *in vacuo* and air dried in a fume hood (Teresa-May, 2018). The dried fractions of the crude extracts were stored in air tight glass containers at 4°C under refrigeration until required for further analyses (Sagbo et al., 2020).

2.9 Preparation of the Plant Extracts

Exactly 0.1g of the dried crude extract was dissolved in 10mL of 1% Dimethyl Sulfoxide (DMSO) to achieve 10mg/mL (Famuyide et al., 2019). This (stock) was diluted serially to prepare subsequent test concentrations (5.000, 2.500, 1.250, 0.625, 0.313, 0.156 and 0.078mg/mL). Sterility of the extracts was verified by inoculation (via streaking) on freshly prepared NA, which was incubated at 37°C for 24 hours.

2.10 Minimum Inhibitory Concentration (MIC) Assay

Five milliliters (5mL) of standardized (0.5 OD_{595nm}) bacterial inoculums (Lade et al., 2014) from an overnight culture was inoculated into 95mL of freshly prepared LB broth (Lade et al., 2014) and incubated at 37°C for 48 hours (Julistiono et al., 2018). Wells of a flat bottom polystyrene microtiter plate were conditioned by introducing 200μL of (plain) LB broth to each and allowed to stand for 1 hour at room temperature (Lade et al., 2014). The wells were emptied and 20µL of the activated culture, followed by 180µL of the plant extract (0.078–10.000mg/mL)were added to each (Taufik et al., 2018). The plate was covered and incubated under static condition at 28°C for 48 hours (Biswa and Doble, 2013). Plain (uninoculated) LB broth was used as negative control while ciprofloxacin (5µg/mL) was applied as positive control. Bacterial MIC was determined with the use of a micro plate reader (AMR-100, China) at OD_{595} as the minimum concentration where absorbance of the treatment was less or equal to that of the negative control (Da Rosa et al., 2016).

2.11 Minimum Biofilm Inhibitory Concentration (MBIC) Assay

Inhibition of the bacterial biofilm formation was determined using a crystal violet method in microtiter plate (Julistiono et al., 2018). Equal volumes (100µL each) of the activated culture and the extract (0.078–1.250mg/mL) were introduced to each well following conditioning. The plate was incubated in static condition at 28ºC for 48 hours. Supernatants from the wells were carefully aspirated out without disrupting the biofilms on the base and the wells were washed thrice with PBS to remove any unattached bacterial cells. The plate was incubated at 37°C for 15minutes. Formed biofilms were fixed with 200µL of 99% methanol for 20 minutes and stained with 100µL of 0.2% (w/v) crystal violet solution for 15 minutes at room temperature. Excess stain was removed from the wells by rinsing four times with PBS, after which 100µL of 95% ethanol was introduced to extract the crystal violet in solution from the biofilms (Hossain et al., 2017). Absorbance of the dissolved crystal violet (which corresponds to a measure of bacterial cells that formed the biofilms) was determined at 595nm (Taufik et al., 2018) using the micro plate Reader. Inoculated LB broth (without the extract) was used as control. The MBIC was determined as the minimum concentration where absorbance of the treatments was less than or equal to that of the control (Da Rosa et al., 2016). Percentage inhibition of biofilm was calculated using the equation below (Julistiono et al., 2018):

$$\% \text{ biofilm inhibition } (595\text{nm}) = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

2.12 Minimum Biofilm Eradicating Concentration (MBEC) Assay

Following conditioning of the micro plate wells, 100µL of the activated culture was inoculated into each well. The set-up was incubated under static condition at 28ºC for 48 hours (Taufik et al., 2018). The liquid culture was aspirated out and the wells were washed with PBS. Wells with successfully induced biofilms were filled with 200µL of the extracts at 0.078–1.250mg/land the plates were incubated for 6 hours at 37°C. The biofilms were fixed with 200µL of 99% methanol for 20 minutes and stained with 100µL of 0.2% (w/v) crystal violet solution for 15 minutes at room temperature. Excess stains were removed by rinsing four times with PBS, which preceded the addition of 100µL of 95% ethanol (to each well) to extract the crystal violet in solution from the biofilm (Hossain et al., 2017). Absorbance (595nm) of the dissolved crystal violet was determined with the use of micro plate reader as a measure of cells that formed the biofilms (Taufik et al., 2018). Inoculated LB broth (without the extract) was used as control. The MBEC was calculated as the minimum concentration where absorbance of treatment was at least 50% less than that of the control, indicating up to 50% eradication of the formed biofilms (Da Rosa et al., 2016).

2.13 Mesocosm Experiment

To further confirm their antifouling activities, the crude plant extracts were subjected to mesocosm experiment in accordance with a procedure described by Dobretsov et al. (2011). Specifically, 2 sets of 2L capacity transparent plastic containers were filled with 1L of the compound solution. This solution was prepared by dissolving the extract in standard unfiltered seawater, sampled from the Eleko beach, Lagos, Nigeria (06º 26' 17.23"N, 03º 51' 06.51"E) using a standard procedure (Cheesbrough, 2006). Final concentrations of 0.313mg/mL and 0.156mg/mL extract in seawater were used. Replicates of sterile microscope glass slides (25 × 75mm) were immersed horizontally into each container. Microscope slides were also dipped into a similar container filled with an equal volume of the unfiltered seawater (control). Containers with the slides were kept under illumination at room temperature (25°C±2°C) for 5 days (Wilson et al., 2018). The slides were brought out and fouling was fixed with 1% formaldehyde in seawater. These slides were then stained with DNA-binding fluorochrome 4,6-diamidino-2-phenylindole solution (0.5µgmL^{-1}) and air dried. Bacteria in 10 randomly selected fields of view were enumerated under an epifluorescence microscope (Wild M20, Switzerland) using direct count at a total magnification of ×2000.

$$\text{Percentage biofilm inhibition} = \frac{\text{control count} - \text{test count}}{\text{control count}} \times 100$$

2.14 Statistical Analyses

Data were presented as mean ± standard deviation (SD) of replicate assays. The mean and standard deviation of bioassays were computed using Microsoft Excel (version 2016). Values of inhibitory activities were appraised by one-way analysis of variance (ANOVA) with the use of GraphPad Instat (version 3.10); in comparison with controls. All P-values <0.05 were regarded as statistically significant, which were illustrated with different superscript alphabets, while those >0.05 were considered insignificant and denoted by similar superscripts.

3. RESULTS AND DISCUSSION

In this study, biofilm production among bacteria isolated from the various biofouled environments was rated ‘strong’ where there was a visibly high biofilm adherence to both the wall and bottom of the test tubes. It was regarded as ‘moderate’ in case of less adherence to the tubes and ‘weak’ where only a trace of biofilms manifests in the walls or bottom of the test tube(s). The result indicates that the trait of biofilm formation was confirmed in the majority (63.16%) of the screened isolates. This implies that most of the sampled environments harboured the typical biofilm-forming bacteria, which can easily be isolated using protocols adopted in this research. Among the biofilm formers, isolates from the membrane filter, plastic and glass substrates (M_1, M_2 and M_3, respectively) of the biofouling model excelled with up to 25% 'strong' formation. Since all the three isolates originated from the (controlled) biofouling model, this signifies that biofilm formation could depend strongly on environmental conditions such as availability of nutrients, moisture and temperature. The result of Zuberi and Nadeem (2017) corroborates the finding of this study as they similarly reported as high as 63.64% biofilm formation in bacteria isolated from contact lenses and their accessories in Karachi, Pakistan.

4.1 Identity of the Biofilm-Producing Bacteria

The isolates with confirmed biofilm formation ability were identified as *Staphylococcus aureus* RBSB2_C1, *Pseudomonas aeruginosa* NT 10038 and *Klebsiella pneumoniae* C2244. This confirmed the inherent biofilm formation property of these bacterial species. Awoke et al. (2019) support this finding as they similarly identified the capability of biofilm production in *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella* sp. in Southwest Ethiopia. Although, *Staphylococcusaureus* itself has been reported (Paluch et al., 2020) to control biofilm production in some other bacteria.

4.2Minimum Inhibitory Concentration of the Plant Extracts

The MICs of the extracts were determined as 0.31 to 1.25mg/mL against *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus aureus*. Majorly, lower MICs were recorded from the methanol and ethyl acetate extracts. This might owe to the fact that many phytochemicals are polar in nature; hence, significant biological activities concentrate mostly in the polar regions (Bogavaca et al., 2017). The least MIC (0.31mg/mL) was obtained from the methanol extract against *Pseudomonas aeruginosa*, followed by 0.63mg/mL from the same extract against *Klebsiella pneumonia*. This implies that the biofilm producing bacteria were most susceptible to the methanol extract. The study of Van-Vuuren (2008) corroborates this as MICs ≤1.25mg/mL of some South African plants were reported and regarded as strong values. In the result of Bogavaca et al. (2017), much higher (50mg/mL) MIC value of *R. officinalis* (but essential oil)was identified against *Pseudomonas aeruginosa*. Similar to our finding, Jarrar et al. (2010) recorded activities of the ethanol extract of *R. officinalis*, collected

Table 1: Confirmed Biofilm Production of Bacterial Isolates from Slimy Surfaces of the Membrane Filter, Plastic and Glass Substrates of the Biofouling Model, Water Reservoirs from Kaduna State Water Treatment Plant and Solid Objects from River Kaduna

Bacterial Isolates	I_1	I_2	I_3	I_4	I_5	I_6	I_7	M_1	M_2	M_3	M_4	M_5	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Extent of Biofilm Production	-	++	++	+	-	+	++	+++	+++	+++	-	++	+	-	-	+	+	-	-

Percentage Biofilm Formation: N (%)	19(63.16)
Strong: n (%)	12(25.00)
Moderate: n (%)	12(33.33)
Weak: n (%)	12(41.67)

Key: +, ++ and +++ = Weak, moderate and strong biofilm production respectively; - = No visible biofilm production.
M_{1-5} = Isolates from plastic, glass and membrane filter substrates of the biofouling model, R_{1-7} = Isolates from biofouled substrates from River Kaduna and I_{1-7} = Isolates from surfaces of water reservoirs from Kaduna state water treatment plant.

Table 2: Cultural, Morphological and Biochemical Identities of the Biofilm-Forming Bacteria

Colonial Characteristics on NA	on MacConkey	Gram's Stain Reaction	Ox	Cat	Ind	MR	VP	Gl	Lc	Su	H_2S	Cit	Ur	Mot	Inference
Blue-green	Yellow-green	Gram - bacilli	++	+++	-	-	-	+	+	-	-	+	-	+	*Pseudomonas aeruginosa* **
Mucoid milky	Pink mucoid	Gram - bacilli	-	+	-	-	+	+	+	+	-	+	+	-	*Klebsiella*sp.
Large smooth circular	NG	Gram + cocci	-	+	-	+	+	+	+	+	-	+	+	-	*Staphylococcus aureus*

Key: - = negative, + = positive, Ox = oxidase, Cat = catalase, Ind = indole, MR = methyl red, VP = VogesProskauer, Gl = glucose, Lc = lactose, Su = sucrose, Cit = citrate (Simon's), Ur = urease, Mot = motility, ** = grown at an elevated temperature (42°C), NG = No growth.

Table 3: Minimum Inhibitory Concentration (mg/mL) of *Rosmarinus officinalis* Leaf Extracts against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*

	Pseudomonas aeruginosa					*Klebsiella pneumoniae*					*Staphylococcus aureus*				
Extracts	Aq	M	E	A	H	Aq	M	E	A	H	Aq	M	E	A	H
R.officinalis	2.500	0.313*	1.250	-	-	-	0.625*	-	-	2.500	-	-	1.250	-	-
Ciprofloxacin	< 0.078					< 0.078					< 0.078				
Plain LB	-					-					-				

Key: Aq = Aqueous, M = Methanol, E = Ethyl acetate, A = Acetone and H = N-hexane. Results ≤1.250mg/mL (especially those indicated with*) were considered strong MIC values. - = MIC values >5.000mg/mL.Ciprofloxacin and Plain LB = positive and negative controls respectively.

Table 4: Inhibitory Potential of the Methanol and Ethyl Acetate Leaf Extracts of *Rosmarinus officinalis* Against the Biofilms of *Pseudomonas aeruginosa*,*Klebsiella pneumonia* and *Staphylococcus aureus*

Inhibition of Biofilm Development (%)			
	Bacterial species		
Extracts (0.078–1.250mg/mL)	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Staphylococcus aureus*
Methanol	50.00±0.66(b)^{g}	43.10±3.84(c)^{i}	37.69±5.48(c)^{i}
Ethyl Acetate	NA	42.41±2.24(a)^{j}	48.8±1.00(b)^{j}
Ciprofloxacin (0.078mg/mL)	51.90±12.61^{h}	51.38±3.51^{f}	53.80±4.82^{k}

Values are mean (±SD) percentage biofilm inhibition.
Values with different superscripts across the same column are significantly different (P<0.05).
Key: NA = not active against biofilms at all tested concentrations.
a, b, c, d and e = 0.078, 0.156, 0.313, 0.625 and 1.250mg/mL respectively.

Table 5: Eradication Potential of *Rosmarinus officinalis*Leaf Extractsagainstthe Biofilms of *Pseudomonas aeruginosa*,*Klebsiella pneumonia* and *Staphylococcus aureus*

Eradication of formed Biofilms (%)			
	Bacterial species		
Extracts (0.078–1.250mg/mL)	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Methanol	48.67±6.14(c)^{m}	**51.17±2.24(c)^{m}	**57.88±4.76(c)^{m}
Ethyl Acetate	**52.35±6.79(c)^{n}	41.92±4.09(d)^{n}	48.22±3.84(c)^{n}
Ciprofloxacin (0.078mg/mL)	**64.21±3.02^{p}	**61.55±3.38^{p}	**56.39±1.58^{p}

Values are mean (±SD) of percentage biofilm eradication.
Values with different superscripts across the same column are significantly different (P<0.05).
Key: - = no biofilm eradication recorded, ** = active eradication of biofilms (≥50%)
a, b, c, d and e = 0.078, 0.156, 0.313, 0.625 and 1.250mg/mL respectively.

Table 6: Density of Biofilm-Forming Bacteria on Glass Slides Exposed to the Extracts of *Rosmarinus officinalis*in Unfiltered Seawater

		Count (cells/mm^2)	
Plant Extracts	Control	0.156mg/mL	0.313mg/mL
Methanol	127.93±4.65^q	102.47±2.96^r (19.90%)	56.00±2.76^s (56.23%)
Ethyl Acetate	125.67±4.19^q	103.17±3.59^r (17.90%)	73.00±4.16^s (41.91%)

Values are mean (±SD) bacterial density with percentage inhibition in parenthesis.
Mean values with different superscripts across the same row are significantly different (P<0.05).

from the Northern Palestine against *Staphylococcus aureus,* at an MIC of 0.39mg/mL. It was established (Abkhoo et al., 2010) that ethanol extracts of *R. officinalis* from Tehran, Iran, significantly inhibited the growth of *Pseudomonas aeruginosa* at the MIC of 0.1mg/mL.

Generally, variation in MIC values was observed across the three (3) bacterial isolates in the present study. This can be due to the presence of different intrinsic levels of tolerance to the tested plant compounds, as similarly observed by Ahmad and Aqil (2007).

4.3 Biofilm Inhibitory Potential of the Plant Extracts

Almost all the extracts inhibited the formation and development of preformed biofilms at appreciable limits. The extracts were able to hinder the development of biofilms formed by *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus aureus* at the range of 24.89 to 50.00%. The methanol extract displayed the highest (50.00%) potential of biofilm inhibition against *Pseudomonas aeruginosa* at 0.16mg/mL. This unveils the puissance of *R. officinalis* as a potential antifouling agent. The probable mechanism of this biofilm inhibition may be reduction in the production of extra polymeric substances (EPS), which is an important component of bacterial biofilms, crucial to the maintenance of the spatial structure of the consortium, as established by Paluchet al. (2020). Exactly 50.00% of biofilm formed by *Pseudomonas aeruginosa* was similarly inhibited but at 7.80mg/mL of *R. officinalis* in the study of Yazdeliet al. (2021). Ziemichód and Skotarczak (2017) further established the capability of plant products to inhibit both formed and preformed biofilms of *Staphylococcus aureus.* Studies conducted by Endo et al. (2018) were in agreement with ours as they equally revealed as high as 50.00% activity against the preformed biofilm of *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 30.00 to 250.00µg/mL of the leaf extracts of *R. officinalis.* Likewise, Ceylan et al. (2014) recorded 39.49% and 51.30% inhibition capacity of the preformed biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus,* respectively using *R. officinalis* essential oil at 10.00 to

0.08µg/mL. Up to 57.00% of biofilm formed by *Staphylococcus Epidermidis* was inhibited by the essential oil of *R. officinalis obtained from Tunisia, as reported* in the work of Jardak et al. (2017).

4.4 Biofilm Eradication Potential of the Plant Extracts

Results of the eradication assay revealed that biofilms formed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were appreciably reduced by the extracts, indicating an activity range of 33.95 to 57.88%. The highest (57.88%) activity was obtained from the methanol extract of *R. officinalis* at 0.31mg/mL against the biofilm formed by *Staphylococcus aureus.* Jardak et al. (2017) also established that the biofilm formed by *Staphylococcus epidermidis* was eradicated by up to 67.53% when exposed to *R. officinalis* essential oil at the concentration of 50.00mg/mL. In this study, *R. officinalis* might have reduced bacterial biofilms by producing and releasing compounds capable of affecting bacterial molecular signals and inhibiting behaviors under the control of quorum sensing (QS) (Yazdeli et al., 2021).

4.5 Effect of the Extracts on the Density of Biofilm-Forming Bacteria

From the mesocosm experiment, both methanol and ethyl acetate extracts of *R. officinalis* inhibited the formation of microbial communities on the glass slides at both tested concentrations. The methanol extract significantly ($P<0.05$) decreased bacterial densities (56.23% and 19.90%) at 0.313mg/mL and 0.156mg/mL respectively; in relation to the control. Activities recorded from this extract (especially at 0.33mg/mL) might indicate probable inhibition of QS among the exposed bacteria. This might have led to low attachment, hindering the subsequent biofilm formation and development. This idea was supported by Dobretsovet al. (2007), who established that QS inhibitors affect microbial composition and densities. According to Kjelleberg et al. (2001), anti-quorum sensing (AQS) agents may alter bacterial composition in biofilm formation, leading to a shift in the microbial communities from being dominated by Gram-negative to Gram-positive bacteria. However, this does not exclude the possibility that the biofilm inhibition (recorded in this study) might have also arisen from the obstruction (by the extracts) of other regulatory cascades that may govern the process of biofilm formation, as highlighted in the study of Dobretsovet al. (2011).

5. CONCLUSION

The biofilm formation trait was confirmed in the majority of the bacteria isolated from the biofouled environments. These bacteria were identified as *Staphylococcus aureus* RBSB2_C1, *Pseudomonas aeruginosa* NT10038 and *Klebsiella pneumoniae*C2244. Evaluation of the antibiofilm activities of *R. officinalis* revealed that this plant (especially its methanol extract) possessed some features of inhibitory activities against bacterial biofilms. This indicates that the methanol, followed by ethyl acetate extracts of this plant, might contain many phytoconstituents at varying concentrations, which can be responsible for their antifouling effects. Therefore, this study has identified the potential of *R. officinalis* as, a source of active antifouling compounds that can be cheap, eco-friendly and readily available.

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Conflicts of Interest: The authors declare no conflict of interest

Data Availability statements: The data presented in this study are available on request from the corresponding author.

REFERENCES

Abkhoo, J., &Jahani, S. Antibacterial effects of aqueous and ethanolic extracts of medicinal plants against pathogenic strains. International Journal of Infection. (2017). 4(2), 1–4.

Ahmad, I., &Aqil, F. In-vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiological Research. (2007). 162(3), 264–275.

Alghamdi, S. A. The impact of biofouling on marine environment: A qualitative review of the current antifouling technologies. World Maritime University, Malmö, Sweden (2019)..

Aryal, S. Hydrogen sulphide test: Principle, Procedure, uses and interpretation. (2019). Accessed from www.*microbiologyinfo.com*.

Awoke, N., Kassa, T., & Teshager, L. The magnitude of biofilm formation and antimicrobial resistance pattern of bacteria isolated from urinary catheterized inpatients of Jimma University Medical Centre, Southwest Ethiopia. International Journal of Microbiology, (2019). 1–9.

Biofilms: the hypertextbook. Accessed from https://www.cs.montana.edu/webworks/projects/stevesbook/contents/chapters/chapter002/section002/blue/page001.html. (2019).

Biswa, P., &Doble, M. Production of acylated homoserine lactone by Gram-positive bacteria isolated from marine water. Microbiology Letters. (2013). 343, 34–41.

Bogavaca, M. A., Karamanb, M. A., Suđic, J. J., Radovanovićd, B. B., Janjuševićb, L. N., Ćetkovića, N. B., &Tešanović, K. D. Antimicrobial potential of *Rosmarinus officinalis*commercial essential oil in the treatment of vaginal infections in pregnant women. Natural Product Communications. (2017).12(1), 127–130.

Bulugahapitiya, V. P. Plants Based Natural Products Extraction, Isolation and Phytochemical Screening Methods. Indika Graphics. (2013). pp 1–93.

Cappuccino, J., & Sherman, N. Microbiology-A laboratory manual 10/E. Pearson. (2013). Pp 1 59.

Ceylan, O., Uğur, A., Saraç, N., Ozcan, F., &Baygar, T. The in vitro antibiofilm activity of *Rosmarinus officinalis* L. essential oil against multiple antibiotic resistant *Pseudomonas* sp. and *Staphylococcus* sp. Journal of Food, Agriculture and Environment. (2014). 12(3 and 4), 82–86.

Cheesbrough, M..Laboratory Practice in Tropical countries. Cambridge University Press. (2006). pp. 180.

Da Rosa, J. P., Tiburcio, S. R. G., Marques, J. M., Seldin, L., & Coelho, R. R. R. *Streptomyces lunalinharesii* 235 prevents the formation of a sulfate-reducing bacterial biofilm. Brazilian Journal of Microbiology. (2016). 47, 603–609.

De Oliveira, J. R., Camargo, S. E. A., & De Oliveira, L. D. *Rosmarinus officinalis* L. (rosemary) as therapeutic and prophylactic agent. Journal of Biomedical Science, 26(5). (2019). 1–22.

Digel, I., Akimbekov, N., Kistaubayeva, A., &Zhubanova, A. Microbial sampling from dry surfaces: Current challenges and solutions.*Biological*, Physical and Technical Basics of Cell Engineering. (2018). 421–456.

Dobretsov, S., Dahms, H. U., Yili, H., Wahl, M., &Qian, P. Y. The effect of quorum-sensing blockers on the formation of marine microbial communities and larval attachment. FEMS Microbiology Ecology, .(2007). 60, 177–188.

Dobretsov, S., Teplitski, M., Bayer, M., Gunasekera, S., Proksch, P., & Paul, V. J. Inhibition of marine biofouling by bacterial quorum sensing inhibitors. Biofouling. (2011). 27(8), 893–905.

Endo, E. H., Costa, G. M., Makimori, R. Y., Ueda-Nakamura, T., Nakamura, C. V., &Filho, B. P. D. Anti-biofilm activity of *Rosmarinus officinalis*, *Punicagranatum* and *Tetradeniariparia* against methicillin-resistant *Staphylococcus aureus* (MRSA) and synergic interaction with penicillin. Journal of Herbal Medicine. (2018).14: 48–54.

Famuyide, I. M., Aro, A. O., Fasina, F. O., Eloff, J. N., & McGaw, L. J. Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated South African *Eugenia* and *Syzygium* (Myrtaceae) species and their selectivity indices. BMC Complementary and alternative Medicine. (2019). 19, 141–154.

Farinde, E. O., Adeniran, H. A., &Abiose, S. H. Comparative microbial assessment of fermented Lima bean (*Phaseoluslunatus*) and locust bean (*Parkiabiglobossa*) in production of *Daddawa.British* Microbiology Research Journal. (2014).4(7), 772–784.

Gahlot, M., Bhatt, P., & Joshi, J. Study on yield of plant extracts using different solvents and methods. *Bulletin of Environment, Pharmacology and Life Science*. (2018). 7(6), 65–67.

Goh, T. K., Wong, S. Y., Ismail, N. I. M., &Teo, K. C. Isolation and characterization of *Chromobacteriumviolaceum* from disused Tin mining lake in Malaysia. African Journal of Microbiology Research. (2014). 8(35), 3255–3264.

Hook, A. L., Chang, C. Y., & Yang, J. Combinatorial discovery of polymers resistant to bacterial attachment. Nature Biotechnology. (2012).30, 868–875.

Hossain, M. A., Lee, S., Park, N., Mechesso, A. F., Birhanu, B. T., Kang, J., Reza, M. A., Suh, J., &Park, S. Impact of phenolic compounds in the acyl homoserine lactone-mediated quorum-sensing regulatory pathways. Scientific Reports. (2017). 7, 2045–2322.

Ibrahim, M. A., Isah, M. B., Yunusa, I., Hamza, S. A., Isah, S., Yahya, K. A., Tajuddeen, N., Salisu, A. R., &Abdullahi, H. L. Aqueous fruit extracts of *Cassia fistula* and *Vitexdoniana*improve maltose and sucrose tolerance in rats. Nigerian Journal of Pharmaceutical Sciences. (2017). 16(1), 17–23.

Jardak, M., Elloumi-Mseddi, J., Aifa, S., &Mnif, S.. Chemical composition, antibiofilm activity and potential cytotoxic effect on cancer cells of *Rosmarinus officinalis* L. essential oil from Tunisia. Lipids in Health and Disease. (2017). 16(190), 1–10.

Jarrar, N., Abu-Hijleh, A., &Adwan, K. Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin-resistant *Staphylococcus aureus*. Asian Pacific Journal of Tropical Medicine. (2010). 121–123.

Julistiono, H., Hidayati, Y., Yuslaini, N., Nditasari, A., Dinoto, A., Nuraini, L., Priyotomo, G., &Gunawan, H. (2018). Identification of biofilm forming bacteria from steel panels exposed in sea waters of Jakarta Bay and Madura Strait. *AIP Conference Proceedings*,pp. 2002.

Kalamartzis, L., Dordas, C., Georgiou, P., &Menexes, G. The use of appropriate cultivar of basil (*Ocimumbasilicum*) can increase water use efficiency under water stress. Agronomy. (2020). 10(70), 1–16.

Kırmusaoğlu, S. The methods for detection of biofilm and screening antibiofilm activity of agents. Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies and Activity Methods. London. (2019). pp. 1–52.

Kjelleberg, S., Steinberg, P., Givskov, M., Gram, L., Manefield, M., & De Nys, R. Do marine natural products interfere with prokaryotic AHL regulatory systems? Aquatic Microbial Ecology. (2001). 13, 85–93.

Lade, H., Paul, D., &Kweon, J. H. Quorum-quenching mediated approaches for control of membrane biofouling. International Journal of Biological Sciences. (2014). 10(5), 550–565.

Loha, M., Mulu, A., Abay, S. M., Ergete, W., & Geleta, B. Acute and subacute toxicity of methanol extract of *Syzygiumguineense* leaves on the histology of the liver and kidney and biochemical compositions of blood in rats. Evidence Based Complementary *and* Alternative Medicine. (2019). 1–15.

MacFaddin, J. F. Biochemical tests for identification of medical bacteria. Lippincott Williams & Wilkins, Philadelphia. (2000). pp. 23–50.

Meng, F., Zhang, S., Oh, Y., Zhou, Z., Shin, H., &Chae, S..Fouling in membrane bioreactors: An updated review. Water Research. (2017) 114, 151–180.

Meziane-Assane, D., Tomao, V., Ruiz, K., Meklati, B. Y., &Chemat, F. Geographic differentiation of Rosemary based on GC/MS and fast HPLC analyses. Food Analytical Methods. (2013). 6, 282–288.

Myers, V. R. Types of leaf arrangement, tree and shrub basics. (2019). The Spruce.Accessed from https://www.thespruce.com/types-of-leafarrangements-3269814.

Oh, H., & Lee, C. Origin and evolution of quorum-quenching technology for biofouling control in MBRs for wastewater treatment. Journal of Membrane Science. (2018).554, 331–345.

Paluch, E., Rewak-Soroczyńska, J., Jędrusik, I., Mazurkiewicz, E., &Jermakow, E. Prevention of biofilm formation by quorum-quenching. Applied Microbiology and Biotechnology. (2020). 104, 1871–1881.

Sagbo, I. J., Orock, A. E., Kola, E., & Otang-Mbeng, W. Phytochemical screening and gas chromatography-mass spectrometry analysis of ethanol extract of *Scambiosacolumbabria* L. Pharmacognosy Research. (2020). 12(1), 35–39.

Taufik, I.,Astuti,D. I., Purwasena, A. I., & Rodiana,W. N. Effect of Lemon grass (*Cymbopogoncitratus*) essential oil on biofilm-biocorrossion in formation water environment. Asian Journal of Scientific Research. (2018).11(3), 329–336.

Teresa-May, B. B.. Extraction and quantitative phytochemical screening of medicinal plants: A brief summary. International Journal of Pharmacy. (2018) 8(1), 137–143.

UK Standards for Microbiology Investigations (2014).Introduction to the preliminary identification of medically important bacteria. Bacteriology Identification, 16, 3–20.

Van-Vuuren, S. F. Antimicrobial activity of South African medicinal plants. Journal of Ethnopharmacology. (2008). 119, 462–472.

Waheed, H., Xiao, Y., Hashmi, I., Stuckey, D., & Zhou, Y. Insights into quorum-quenching mechanisms to control membrane biofouling under changing organic loading rates. Chemosphere. (2017). 182, 40–47.

Wang, L., Liu, J., Li, X., Shi, J., Hu, J., Cui, R., Zang, Z. L., Pang, D. W., & Chen, Y. Growth propagation of yeasts in linear arrays of microfluidic chambers over many generations. Biomicrofluidics. (2011). 5(4), 44118–441189.

Whiteley, M., Diggle, S. P., & Greenberg, E. P. Progress in and promise of bacterial quorum-sensing research. Nature. (2017).551, 313–320.

Wilson, C., Lukowicz, R., Merchant, S., Valquier-Flynn, H., Caballero, J., Sandoval, J., Okuom, M., Huber, C., Brooks, T. D., Wilson, E., Clement, B., Wentworth, C. D., & Holmes, A. E. Quantitative and qualitative assessment methods for biofilm growth: A mini-review. Research Review Journal of Engineering Technology. (2018). 6(4), 1–42.

Yazdeli, M. Z., Ghazaei, C., Ebrahimi, S. A. S., Arfaatabar, M., Alipanah, H., &Noori, M. Evaluation of antimicrobial activity and anti-quorum sensing of *Rosmarinus* methanol extract on *Pseudomonas aeruginosa*. International Journal of Infection. (2021). 8(1), 1-7.

Ziemichód, A., &Skotarczak, B. Quorum-sensing systems communication of gram-positive bacterial cells. ActaBiologica. (2017). 24, 51–56.

Zuberi, S. N., &Nadeem, S. G. Detection of biofilm forming ability of bacterial isolates from contact lenses and their accessories. Journal of Bacteriology and Parasitology. (2017). 8, 321.

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ORIGINAL RESEARCH

Productivity and the Qualitative Response of Sorghum to Different Planting Patterns and Various Cultivars

Muhammad Asim [1*], Muhmmad Israr Khan[2], Abdul Rab[2]

[1]Department of Agronomy, University of Agriculture Faisalabad, Pakistan

[2]The University of Agriculture, Peshawar Pakistan

Corresponding author: masim@aup.edu.pk
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ABSTRACT

Planting patterns and different cultivars play a significant role in forage crops quality and productivity. Therefore, we conducted a field experiment under different planting patterns and cultivars to evaluate sorghum crop yield, yield components, and quality at Agronomic Research Farm, Department of Agronomy, University of Agriculture Faisalabad, Pakistan, in 2015. The experiment consists of three sorghum cultivars (Jawar 2002, Sorghum-2011, and JS-2002) with a seed rate of 75 kg ha^{-1} at different planting patterns (P1=60 cm × 20 cm, P2=50cm × 24 cm, and P3=340 cm × 30 cm). Results showed that sorghum 2011 resulted in higher growth and qualitative attributes than other cultivars. For example, increase in plant height (237.11 cm), dry weight $plant^{-1}$ (40.61 g), forage yield (57.66 ton ha^{-1}), crude protein contents (6.12 %), fiber contents (32.12 %) and ash contents (8.73%) was observed in sorghum 2011 as compared to other cultivars. Whereas, among planting pattern P_3 (40 x 30 cm) produced maximum plant height (236.33 cm), leaves $plant^{-1}$(13.66), stem diameter (1.09 cm), forage yield (55.52 ton ha^{-1}), dry matter yield (18.53 ton ha^{-1}) and crude protein contents (6.06 %) as compared to P1 and P2. This study suggested that the cultivar sorghum 2011 with a planting pattern of 40 x 30 cm is a promising option to improve yield, yield components and quality of sorghum crop.

Keywords: Sorghum, Planting pattern, Cultivars, Crude Protein, Fiber content

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1. INTRODUCTION

Agriculture is the most important sector of Pakistan and greatly influences economic growth. It accounts for 21.4% of GDP and 45% of employment is engaged with agriculture (Gecho, 2017, Iqbal et al., 2015). Development in the agriculture sector stimulates growth in the agro-industry, especially the textile sector. Livestock is an important agriculture sector and has a central role in our country's rural economy. Its accounts for 54% of agricultural GDP and 11.9% of total GDP (Mehmood et al., 2020, Jahnke and Jahnke, 1982). Sorghum (*Sorghum bicolor* L.) is a member of the Poaceae family is locally called Jawar and is commonly used as fresh, silage or hay form. Sorghum is a dual-purpose (i.e., grown for grain and fodder yield) summer season crop

(Hedayetullah and Zaman, 2018; Sarfraz et al., 2012). It requires fewer resources and gives a high yield being the most drought-tolerant crop of the universe (Ringo et al., 2014) and can be grown in tropical and sub-tropical countries of the world. Sorghum has more potential to fulfill the future demands of livestock, grain food and beverages. Its syrup extracted from sweet sorghum is mainly used in ethanol production (Klasson et al., 2021, Rao et al., 2016; Kirouani et al., 2021). Sorghum is the fourth cereal crop of Kharif season and an important forage crop in many regions of the world, including Pakistan (Ghani et al., 2015; Rana et al., 2014). It exhibits rapid growth, is relatively resistant to dryness, high productivity and high percentage of protein. In short period sorghum produced a large amount of seed and fodder (Djanaguiraman et al., 2020). Its fodder comprises 11 % protein, 71 % carbohydrates, 2% crude fiber, mineral and nitrogen-free extract (Hussain et al., 2020). Sorghum accounts for half of the forage in the rainfed area. In Pakistan sorghum was cultivated on 198thousand hectares land with the production of 123 thousand tones and an average grain yield of 621 kg ha^{-1}(Hussain et al., 2015).

Fodder crops play a crucial role in the agricultural economy of developing countries by providing the cheapest source of feed for livestock (Herrero et al., 2013, Upton, 2004; Ramana, 2022). Livestock is a vital part of farming plays an important role in the economic development of the rural community of Pakistan. Livestock accounts for 55 % of agricultural GDP and 12 % of total GDP (Hussain et al., 2015). Lower fodder production and less accessibility to feed are the main factors of decreasing livestock in Pakistan. Furthermore, providing quality animal feed in a suitable amount can increase livestock production. Fodder production fulfills 30 to 50 % of the total fodder consumption in Pakistan. While, low quality of animal feed caused low meat and milk production (Nouman et al., 2014).

The available fodder contributes 1/3 less than that needed fodder and its deficiency is further increased due to a decline in area under fodder crops by 2% after each decade (Nadeem et al., 2017, Herrero et al., 2013). Amongst the Kharif forage crops, sorghum is an important one that possesses a wide range of ecological flexibility. The extension growers largely sow it for feed and fodder in rainfed and irrigated regions of the country. Almost sorghum is fed to every class of livestock (Kumar and Upadhyay, 2008). The performance of dairy animals depends on the regular availability of quality fodder insufficient amount. The vital limitation on profitable animal production in developing countries is the unavailability of quality forage (Kumar and Upadhyay, 2008, Quddus, 2012). Considerable, differences have been reported among the sorghum cultivars for yield, quality traits (Rao et al., 2013), and response to planting densities (Moosavi and Sciences, 2012).

Keeping in view the importance of fodder crop, the present study was conducted to find out the most suitable sorghum variety under suitable planting pattern for higher yield and fodder in terms of quality and quantity in the agro-ecological climate of Faisalabad.

2. MATERIAL AND METHODS

2.1 Experimental Site and Design

The experiment was performed at Agronomic Research Area, University of Agriculture Faisalabad, Pakistan in 2015. The experimental site lies between 30.35-41.47°N latitude and 72.08-73.40°E longitude at an elevation of 184.4 m above sea level. The experimental site's maximum and minimum temperature, rainfall, sunshine hours, relative humidity, wind speed, and evapo-transpiration were recorded at local meteorological station. The weekly maximum and minimum values of temperature, rainfall, sunshine hours, and wind speed are given below in figure 1.

The experiment was laid out in randomized complete block design with split plot arrangement using three replications and a net plot size of 6 m × 7.2 m. All the varieties were sown with a seed rate of 75 kg ha^{-1}. Fertilizers are applied at the rates of 58:58:0 (N: P: K). The experiment wasconsisted of three Sorghum cultivars (Jawar 2002, Sorghum-2011, and JS-2002) and three treatments of planting patterns (P1= 60 cm × 20 cm, P2 = 50 cm × 24 cm and P3= 40 cm × 30 cm). All other agronomic practices were kept the same for all the treatments.

2.2. Measurement and Analysis

2.2.1. Agronomic and Yield-related Parameters

Sorghum plants were counted in the one-meter length of three randomly selected rows in each plot and then averaged per square meter. For plant height (cm), ten sorghum plants were selected and their height was measured from the base to the tip of the longest leaf with measuring tape and then averaged. The total number of leaves from ten plants was counted and then average leaves per plant were calculated. Furthermore, to determine leaf area per plant (cm^2), at each harvest, leaves were removed from ten randomly selected plants and passed through the leaf area meter model LI-3000 and readings were noted. For the measurement of stem diameter (cm), the diameter of ten randomly selected plants from each plot was measured with the help of Vernier Caliper from the base, middle and top portions of the stem and then averaged.

Moreover, for the determination of the weight per plant (g), ten plants were randomly selected from each plot at each harvest with the help of sickle. Each plant was weighed and averages of these plants weights were calculated to get the fresh weight of each plant in grams. Fresh weight per plant (g) was observed by selecting five plants randomly and taken from each plot then weighted to determine the mean fresh weight per plant. While to evaluate dry weight per plant (g), fresh samples were dried at 60°C for 48 hours in a fan-assisted oven until a constant weight was reached and weighted to obtain the mean dry weight per plant. For the determination of forage yield (t ha^{-1}), all the crop plants in each net plot reserved for recording yield at final harvest and weighed separately with the help of a spring balance and converted into t ha^{-1}.

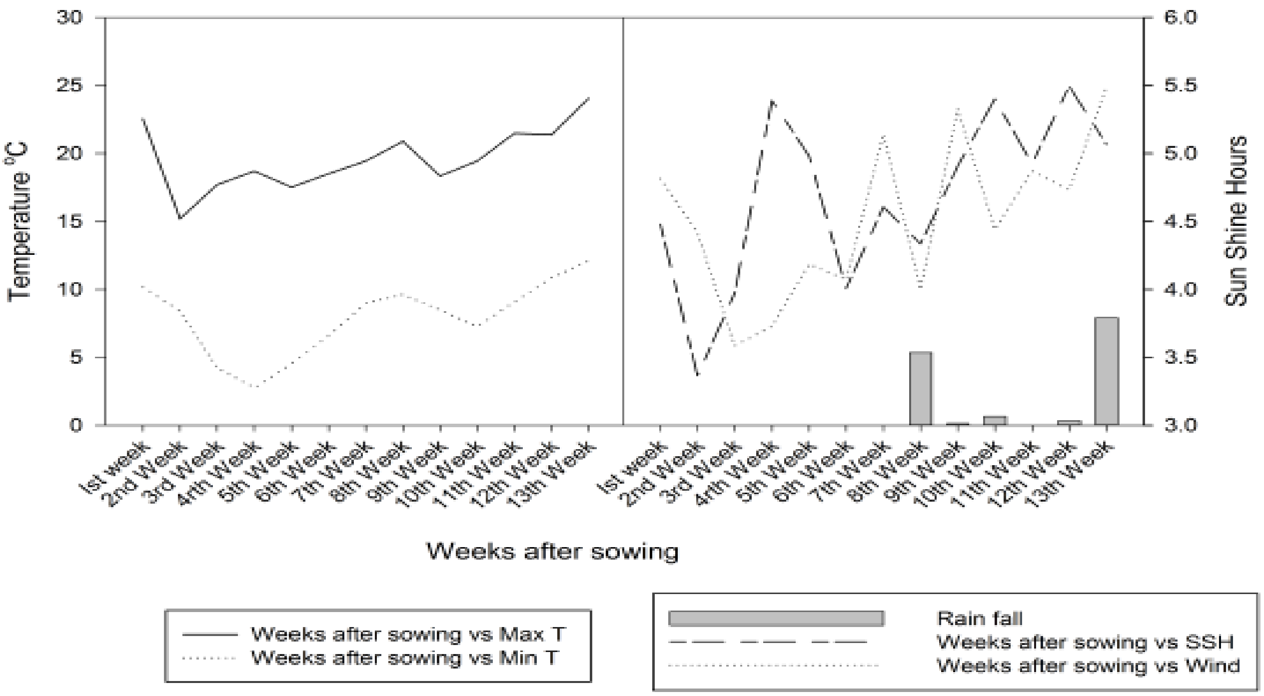

Figure 1. Weekly maximum and minimum values of temperature, rainfall, sunshine hours, and wind speed of the experimental site

The d**ry** matter yield (t ha^{-1}) were assessed by selecting randomly ten plants at harvest from each plot and chopped with the help of a forage cutter and then thoroughly mixed. The fresh weight of the sample was recorded and a sample of 500g was taken from each plot and dried in an oven at 70°C to a constant dry weight. These plants were selected from the plot area used for green forage yield and their weight was added in each respective plot. Dry matter percentage calculated was used to convert green forage yield to dry matter yield. Furthermore, to record dry matter (%), **a** **c**hopped known weight of forage from each plot was taken and then dried at 80 ^{0}C in an electric oven to a constant weight. The dry matter percentage for each plot was calculated following the formula below.

Dry matter (%) = Dry weight/fresh weight × 100

2.2.2. Quality Parameters

3.2.1. Crude Protein (%)

Initially, the samples were grinded with a locally made grinder until the sample was almost converted into a powdered form and no sieve was used. The powdered sample (1g) was added to KJeldahl digestion flask along with 30ml concentrated H_2SO_4 and 10g digestion mixture. The powdered sample (1g) was added to KJeldahl digestion flask along with 30ml concentrated H_2SO_4

and 10g digestion mixture. After keeping for half an hour it was heated slowly in the beginning and then on full heat until a transparent green liquid material resulted. On cooling transferred to a 250 ml volumetric flask and volume was made up to the mark. Aliquot of 10 ml from this material was taken in the micro kjeldahl apparatus using 15 ml 40% NaOH for each sample. It involves digestion of the plant material dried at 70°C with concentrated sulphuric acid and digestion mixture, comprising K_2SO_4, $CuSO_4$ and $FeSO_4$ in the ratio of 10: 0.5:1.

Nitrogen evolved as ammonia was collected in a receiver containing boric acid (4%) solution and mixed indicator of bromocresol green and methyl. The distillation was titrated against N/10 sulphuric acid till the original color of methyl red was restored. Blank was run to eliminate the percentage of nitrogen present in other chemicals used to digest the sample. From the quantity of acid used in titration, the percentage of element nitrogen was calculated by using the formulas. The reading obtained was multiplied by 6.25 to get crude protein percentage. The crude protein percentage was determined by using the standard procedure as recommended by (Salo-väänänen and Koivistoinen, 1996).

N (%)=A-B×100×100×0.0014/ Volume of digested sample used.

Where A= quantity of acid (N/10 H_2SO_4) used.

B= Blank reading (N/10 H_2SO_4 used in blank reading), 100= volume made after digestion, 100 for percentage (Which is equal to grams of N in 1ml of N),

0.0014= Factor (Which is equal to grams of N in 1ml of N/ 10H_2SO_4

2.2.3. Crude Fiber (%)

Two grams of oven-dried sample were digested in 200 mL of 1.25 % H2SO4 in 500 ml beaker for 30 minutes to determine crude fiber. Then contents were filtered by linen cloth and residues were washed and digested again with 200 ml 1.25% NaOH for 30 minutes and after that, it was again filtered and washed. The residues were put in a weighed china dish and dried in an oven for 24 hours at 105°C. After recording, the dry weight samples were placed in a muffle furnace at 600°C until grey or white ash was obtained. The weight of the ash was recorded. Crude fiber (%)= {(Wt. of dried residues – Wt. of ash)/Wt. of the dried sample)} ×100

The crude fiber percentage was determined by using the standard procedure as recommended by (Salo-väänänen and Koivistoinen, 1996).

2.2.4. Ash Contents (%)

A 5g of oven-dried sample was placed in a clean previously weighed china dish (W1) to determine ash content. The samples were placed in a muffle furnace at (550-650°C) until white or grey ash was obtained. After that, residues were cooled in a desiccator and recorded the weight (W2), and the percentage was calculated as follows:

Total ash % = [(W2 – W1) ÷ (Weight of the sample)] × 100

Total ash percentage was determined using the standard procedure proposed by AOAC (1990).

2.3. Statistical Analysis

Data collected on all parameters were analyzed statistically using MSTAT-C software (Crop and Soil Sciences Department of Michigan University of the United States). The least significance difference (LSD) test at the 5% probability level was applied to compare the treatment's means (Shrestha, 2019).

3. RESULTS

3.1 Growth and Yield Traits

Our results showed that growth and yield attributes were significantly affected by different planting patterns, different cultivars and their interactions (Table 1). Higher planting density (40.67 m^{-2}), plant height (237.11 cm), leaves per plant (13.67), leaf area per plant (2520.20 cm^2), stem diameter (1.11cm), weight per plant (280.94 g) were recorded in Sorghum 2011cultivar as compared to other cultivars. In the case of planting patterns, P_3 (40 × 30) resulted in higher planting density (40.77 m^{-2}), plant height (236.33 cm), number of leaves (13.66), stem diameter (1.09 cm), weight per plant (136.3 g) and dry weight per plant (40.34g) as compared to P_1 and P_2.The lowest growth and yield attributes were recorded in P_1(60 × 20 cm).

Data on forage yield showed significant difference among cultivars and planting patterns (Figure 2). The cultivar Sorghum-2011 produced maximum forage yield (57.6t ha^{-1}) followed by Jawar-2002 (52.5 t ha^{-1}) and cultivar JS-2002 produced a minimum forage yield (45.5 t ha^{-1}). In case of planting patterns, forage yield were found significant and it ranged from 47.39-55.5 t ha^{-1}. The maximum forage yield (55.52 t ha^{-1}) was found in plots where sorghum was sown using narrow row spacing P_3 (40 × 30). The minimum forage yield (47.39 t ha^{-1}) was recorded in P_1 followed by P_2.

Table 1. Response of Sorghum planting density (m^{-2}), plant height (cm), leaves plant^{-1}, leaf area plant^{-1}stem diameter (cm), weight plant (g), fresh weight per plant (g) and dry weight per plant (g) to different varieties and planting patterns.

Planting Pattern	Varieties	Planting density (m^{-2})	PH (cm)	Leaves plant^{-1}	LA plant^{-1}	Stem diameter (cm)	Weight plant (g)	Fresh weight per plant (g)	Dry weight per plant (g)
60 × 20 cm	Jawar 2002	39.33b	229.67cd	12.00b	2224.80e	1.04ab	241.45d	132.74c	38.00bc
60 × 20 cm	Sorghum 2011	39.00b	234.00c	12.33b	2406.50c	1.06ab	251.23c	135.03bc	38.89bc
60 × 20 cm	JS -2002	37.33c	223.67d	11.00c	2206.00c	1.08ab	235.36d	138.22b	35.51c
50 × 24 cm	Jawar 2002	38.33b	232.67c	13.00ab	2406.50c	1.09ab	261.23b	135.03bc	38.89bc
50 × 24 cm	Sorghum 2011	41.00a	236.33b	13.67ab	2513.40b	1.10a	265.14b	138.68	40.40ab
50 × 24 cm	JS -2002	38.33b	229.33cd	11.66c	2240.40e	1.13a	252.23c	141.59a	42.53a
40 × 30 cm	Jawar 2002	41.00a	235.00b	14.00a	2470.90c	1.02ab	283.45ab	138.22b	40.08ab
40 × 30 cm	Sorghum 2011	42.00a	241.00a	15.00a	2640.70a	1.02ab	294.14a	141.59a	42.53a
40 × 30 cm	JS -2002	39.33b	233.00c	12.00b	2340.30d	1.04a	265.24b	129.08c	38.40bc
ANOVA (F values)									
Varieties (V)		19.33**	8.23**	9.97**	27.24**	28.91**	42.83**	48.23**	13.62**
Planting Pattern (P)		18.45**	6.00*	7.65*	16.63**	6.74*	6.57*	9.52*	8.36*
V × P		2.95^{NS}	0.22^{NS}	0.51^{NS}	0.53^{NS}	0.27^{NS}	0.16^{NS}	0.08^{NS}	0.23^{NS}

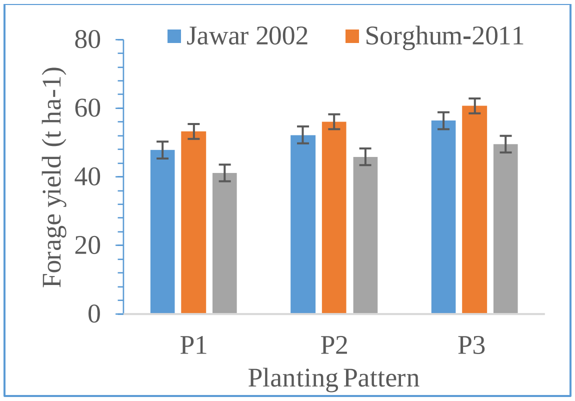

Figure 2. Changes in forage yield to different varieties and planting patterns. Note. P1, P2 and P3 represent different planting patterns.

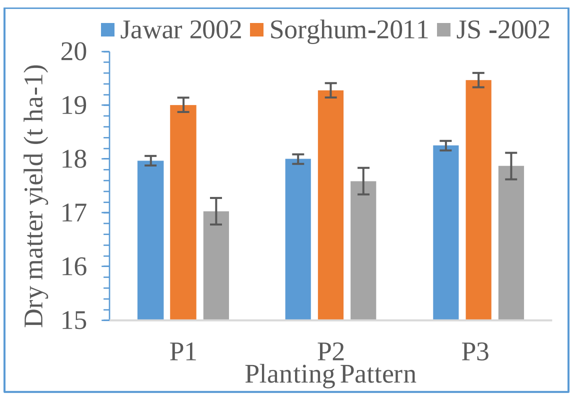

Figure 3. Changes in dry matter in response to different varieties and planting patterns. Note. P1, P2 and P3 represent different planting patterns.

Dry matter production of sorghum was significantly affected by cultivars and planting patterns (Figure. 3). Among cultivars, the cultivar Sorghum-2011 produced highest dry matter of 19.25t ha^{-1} followed by Jawar 2002, while cultivar JS-2002 (17.5t ha^{-1}) produced less dry matter. In case of planting pattern, the highest yield of dry matter (18.53 t ha^{-1}) was recorded in P_3 (40 × 30 cm). The minimum dry matter yield (18.0 t ha^{-1}) was observed in P_1 followed by P_2.

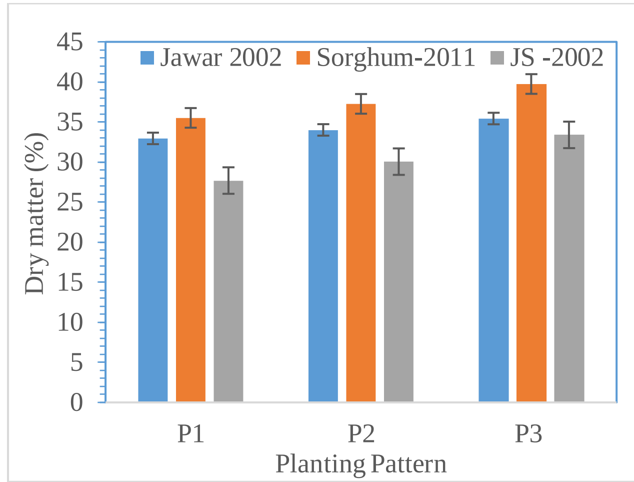

Figure 4. Changes in dry percent in response to different varieties and planting patterns. Note. P1, P2 and P3 represent different planting patterns.

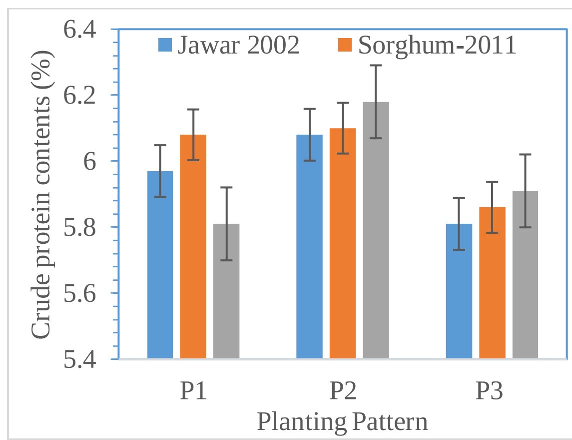

Figure 5. Changes in crude protein (%) in response to different varieties and planting patterns. Note. P1, P2 and P3 represent different planting patterns.

The data regarding dry matter (%) of three sorghum cultivars affected by planting patterns is presented in figure 4. The maximum dry matter (%) was recorded in cultivar Jawar-2002 (34.13%), followed by Sorghum-2011, while minimum dry matter percentage (30.38 %) was observed in JS-

2002. Among different planting patterns, dry matter percentage was found significant, and it ranged from 32.05-36.19 %. The maximum dry matter percentage (36.19%) was recorded in the treatment in which sorghum was sown by using a P_3 (40 × 30 cm) planting pattern. The minimum dry matter percentage (32.05 %) was recorded in the treatment P_1 followed by P_2.

3.2 Qualitative Attributes

The crude protein content of different cultivars as affected by various planting patterns is presented in figure 5. Maximum crude proteins (%) were observed in Sorghum-2011 (6.12%) followed by Jawar-2002 (6.03%), while minimum crude protein (%) was observed in JS-2002 (5.85%). In the case of planting patterns, crude protein percentages were found significant, and it ranged from 5.90-6.06% the highest crude protein (6.06%) was recorded in the treatment in P_3 (40 × 30 cm) planting pattern. The minimum crude protein percentage (5.90%) was recorded in the treatment P_1(60 × 20 cm) followed by P_2.

Analysis of variance indicates that the percentage of crude fiber was considerably diverse in all varieties of sorghum (Figure 6). Maximum crude fiber (32.12%) was observed in Sorghum-2011, and it was followed by Jawar-2002 (30.38 %), while minimum crude fiber (28.33 %) was observed in JS-2002. The effect of planting pattern on crude fiber percentage was found significant, ranging from 28.9-32.8%.

The maximum crude fiber percentage (32.88%) was recorded in treatment P_3. The minimum crude fiber percentage (28.91%) was recorded in the treatment P_1, followed by P_2.

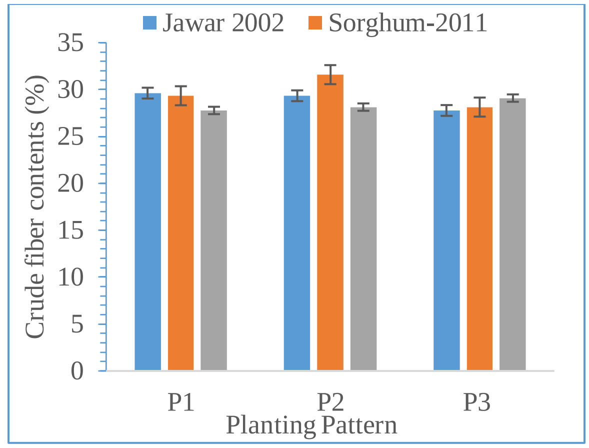

Figure 6. Changes in crude fiber content (%) in response to different varieties and planting patterns. Note. P1, P2 and P3 represent different planting patterns.

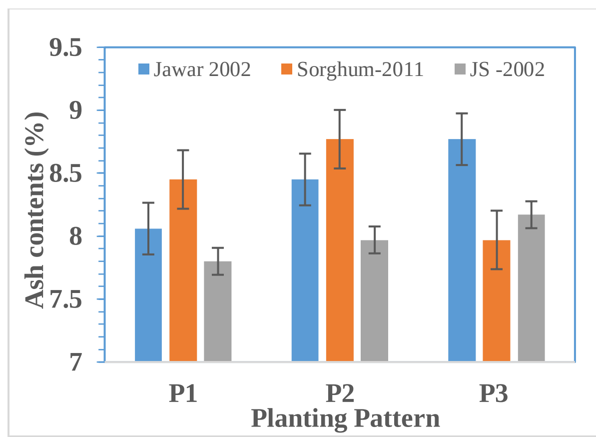

Figure 7. Changes in crude protein (%) in response to different varieties and planting patterns. Note. P1, P2 and P3 represent different planting patterns.

The data regarding ash percentage shows significant variation among sorghum cultivars (Figure 7). Ash percentage was maximum (8.73%) in cultivar Sorghum-2011 which was followed by Jawar-2002 (8.43%) and were minimum (7.98 %) in JS-2002. Among planting patterns, ash contents were found significant and ranged from 8.10-8.64 %. The maximum ash percentage (8.64 %) was recorded in P_3(40 × 30 cm) planting pattern. The minimum ash percentage (8.10 %) was recorded in the treatment P_1 (60 × 20 cm) planting patterns followed by P_2.

4. DISCUSSION

Selection of cultivar and planting pattern is one of the most important method to get higher yield and quality of sorghum crop. Therefore, we conducted a field experiment to evaluate the best responsive planting pattern and cultivar to improve sorghum yield and quality under sustainable agriculture.

Our results showed that growth and yield parameters including planting density (m^{-2}), plant height (cm), leaves plant^{-1}, leaf area plant^{-1}, stem diameter (cm), weight plant^{-1} (g) fresh weight plant^{-1} (g) and dry weight plant^{-1} (g) were significantly increased in the planting pattern of P3 (40 × 30 cm) under sorghum 2011 cultivar. These increments might be due to differentiation in the genetic makeup of cultivars and the adaptability of these varieties to different environmental conditions (Blum, 2004). Yousif et al. (2012) also found that different sorghum cultivars vary in plant height. Similarly Zulfiqar et al.(2009) documented that the number of leaves per plant were influenced by different planting pattern and cultivars. Present results contradict Miranda et al.(2013), who found non-significant dissimilarity in leaves number of various sorghum varieties. These contradictory results might have been due to differences in environmental conditions and the genetic potential of the varieties. An increase in weight per plant of Sorgum-2011 was due to greater plant height, leaf area, and stem diameter. Similarly, Gondal et al.(2017) showed significant differences among sorghum cultivars regarding weight per plant. Furthermore, Ayub et al.(2010) found non-significant differences in plant dry weight among six sorghum varieties, while Afzal et al. (2012) compared different sorghum cultivars. They found a significant difference in weight per plant of different forage sorghum cultivars.

Protein contents are a major parameter affecting forage crops' nutritional value and quality. It’s the mixture of true protein and non-protein nitrogen and the fodder with high protein contents is considered a good quality fodder. While crude fiber percentage is one of the most important parameters that influences forage crops' quality. Fodders with low crude fiber contents are considered good quality forage because low fiber contents increase digestibility and palatability and improve intake. In addition, ash contents are described as the mineral contents in dry matter feedstuff and are mostly measured on a % basis. In the present study, protein contents, crude fiber percentage and ash content were improved in the planting pattern of P_3 (40 × 30 cm) under sorghum 2011 cultivar. These significant differences may be due to the difference of growth stage at harvest. Another reason for these increments might be the more nutrient availability to plant in P_3 patterns. Panwar et al. (2000) also showed significant differentiation in crude fiber contents among varieties of sorghum. Ayub et al. (2010) studied two sorghum cultivars viz. JS -263 and Hegari for crude fiber percent and ash percentage revealed Hegari produced higher protein and ash contents than JS-263.

5. CONCLUSION

Based on our results, we observed that among different cultivars, sorghum 2011 resulted in higher yield, yield components and quality traits as compared to other cultivars. Similarly, Sorghum-2011 also performed better at the Planting pattern of P_3 (40 x 30 cm) compared to other planting patterns. Therefore, we concluded that the cultivar Sorghum-2011 performed better than the other two cultivars because it produced a higher yield with quality at Planting pattern P_3 (40 x 30 cm). It can be recommended for cultivation under Faisalabad conditions.

Authors Contributions:
M.A conceived the main idea of research, M.A wrote the manuscript. M.I.K and A.R revised the manuscript and provided suggestions. In addition M.A and AR assessed and analyzed the data, and performed data collection. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

Afzal, M., Ahmad, A. & Ahmad, A. H. J. C. A. Î. M. 2012. Effect of nitrogen on growth and yield of sorghum forage (Sorghum bicolor (L.) Moenchcv.) under three cuttings system. 45, 57-64.

Ayub, M., Nadeem, M. A., Tahir, M., Ghafoor, A., Ahmed, Z. &Naeem, M. J. P. J. L. S. S. 2010. Comparative studies on the growth, forage yield and quality of sorghum (Sorghum bicolor L.) varieties under irrigated conditions of Faisalabad. 8, 94-97.

Blum, A. J. P. & Biotechnology Integration For Plant Breeding, C. P., Boca Raton, Fl, Usa 2004. Sorghum Physiology. 141-224.

Djanaguiraman, M., Prasad, P., Ciampitti, I. &Talwar, H. S. 2020. Impacts of Abiotic Stresses on Sorghum Physiology. Sorghum in the 21st Century: Food–Fodder–Feed–Fuel for a Rapidly Changing World. Springer.

Gecho, Y. J. S. S. 2017. Rural farm households' income diversification: The case of Wolaita Zone, Southern Ethiopia. 6, 45-56.

Ghani, A., Saeed, M., Hussain, D., Arshad, M., Shafique, M. & Shah, S. A. S. J. S. L. 2015. Evaluation of different sorghum (Sorghum bicolor L. moench) varieties for grain yield and related characteristics. 3, 72-74.

Gondal, M., Hussain, A., Yasin, S., Musa, M. &Rehman, H. J. S. J. O. A. 2017. Effect of seed rate and row spacing on grain yield of sorghum. 15, 81-91.

Hedayetullah, M. &Zaman, P. 2018. Forage Crops of the World, Volume I: Major Forage Crops, CRC Press.

Herrero, M., Grace, D., Njuki, J., Johnson, N., Enahoro, D., Silvestri, S. &Rufino, M. C. J. A. 2013. The roles of livestock in developing countries. 7, 3-18.

Hussain, J., Rabbani, I., Aslam, S. & Ahmad, H. J. W. S. P. S. J. 2015. An overview of poultry industry in Pakistan. 71, 689-700.

Hussain, M. I., Farooq, M., Muscolo, A., Rehman, A. J. E. S. & Research, P. 2020. Crop diversification and saline water irrigation as potential strategies to save freshwater resources and reclamation of marginal soils—A review. 27, 28695-28729.

Iqbal, M. A., Iqbal, A., Afzal, S., Akbar, N., Abbas, R. N., Khan, H. Z. J. A.-E. J. O. A. & Sciences, E. 2015. In Pakistan, agricultural mechanization status and future prospects. 15, 122-128.

Jahnke, H. E. &Jahnke, H. E. 1982. Livestock production systems and livestock development in tropical Africa, KielerWissenschaftsverlagVauk Kiel.

Kirouani, A., Laouar, M., Nadjemi, B. &Abdelguerfi, A. J. G. 2021. Genetic diversity of Algerian sorghum (Sorghum bicolor (L.) Moench) landraces by using agro-morphological markers. 5, 1-11.

Klasson, K. T., Boone, S. A. J. I. C. & Products 2021. Bioethanol fermentation of clarified sweet sorghum (Sorghum bicolor (L.) Moench) syrups sealed and stored under vegetable oil. 163, 113330.

Kumar, S. &Upadhyay, A. J. I. J. O. A. E. 2008. Goat Based Integrated Farming System: Farmers' Coping Strategy for Sustainable Livelihood Security in Arid Rajasthan. 63.

Mehmood, N., Arshad, M., Ahmed, H., Simsek, S. &Muqaddas, H. J. T. K. J. O. P. 2020. Comprehensive account on prevalence and characteristics of hydatid cysts in livestock from Pakistan. 58, 121.

Miranda, R. D. S., Alvarez-Pizarro, J. C., Araújo, C. M. S., Prisco, J. T. & Gomes-Filho, E. J. A. P. P. 2013. Influence of inorganic nitrogen sources on K+/Na+ homeostasis and salt tolerance in sorghum plants. 35, 841-852.

Moosavi, S. J. I. J. O. A. & Sciences, C. 2012. Effect of density plant and planting pattern on yield, yield components and morphological traits of forage sorghum in the second cultivation. 4, 28-32.

Nadeem, A., Nadeem, J., Sarwar, M. H., Sarwar, M. J. A. J. O. F. S. & Health 2017. Making the decision to breastfeed the baby and its advantages for the women's health'. 3, 88-94.

Nouman, W., Basra, S. M. A., Siddiqui, M. T., Yasmeen, A., Gull, T., Alcayde, M. A. C. J. T. J. O. A. & Forestry 2014. Potential of Moringaoleifera L. as livestock fodder crop: a review. 38, 1-14.

Panwar, V., Tewatia, B. &Lodhi, G. J. I. J. O. A. N. 2000. Performance and value of different sorghum fodder varieties. 17, 67-69.

Quddus, M. J. B. J. O. A. S. 2012. Adoption of dairy farming technologies by small farm holders: practices and constraints. 41, 124-135.

Ramana, D. 2022. Livestock Based Production Systems for Climate Adaptation in Dryland Areas. Climate Change Adaptations in Dryland Agriculture in Semi-Arid Areas. Springer.

Rana, A., Ahmad, A., Saleem, N., Nawaz, A., Hussian, T. &Saad, M. J. J. G. I. A. S. S. 2014. Differential response of sorghum cultivars for fodder yield and quality. 2, 6-10.

Rao, P. S., Vinutha, K., Kumar, G. A., Chiranjeevi, T., Uma, A., Lal, P., Prakasham, R., Singh, H., Rao, R. S., Chopra, S. J. S. S. O. T. A. & Perspectives, F. 2016. Sorghum: A multipurpose bioenergy crop.

Rao, S., Patil, J., Umakanth, A., Mishra, J., Ratnavathi, C., Prasad, G. S. & Rao, B. D. J. S. T. 2013. Comparative performance of sweet sorghum hybrids and open pollinated varieties for millable stalk yield, biomass, sugar quality traits, grain yield and bioethanol production in tropical Indian condition. 15, 250-257.

Ringo, J., Onkware, A., Were, B. A. I., Too, E. &Gudu, S. 2014. Diversity of Sorghum (Sorghum bicolor L. Moench) Germplasm from Tanzania.

Salo-Väänänen, P. P. &Koivistoinen, P. E. J. F. C. 1996. Determination of protein in foods: comparison of net protein and crude protein (N× 6.25) values. 57, 27-31.

Sarfraz, M., Ahmed, N., Farooq, U., Ali, A. &Hussain, K. J. J. O. A. R. 2012. Evaluation of sorghum varieties/lines for hydrocyanic acid and crude protein contents. 50, 39-47.

Shrestha, J. P-Value: A true test of significance in agricultural research.2019.

Upton, M. 2004. The role of livestock in economic development and poverty reduction.

Yousif, A., Nhepera, D. & Johnson, S. J. F. C. 2012. Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability. 134, 880-887.

Zulfiqar, B., Abbasi, N. A., Ahmad, T. & Hafiz, I. A. J. P. J. B. 2009. Effect of explant sources and different concentrations of plant growth regulators on in vitro shoot proliferation and rooting of avocado (Perseaamericana Mill.) cv.“Fuerte”. 41, 2333-2346.

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