Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10(2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

APPLICATION OF BIOCHAR IN MITIGATION OF NEGATIVE EFFECTS OF DROUGHT STRESS IN GLYCINE MAX L

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ABSTRACT

Crop yield, plant growth, development, and seed germination are all negatively impacted by drought. To mitigate this, the effects of poultry litter biochar (produced at 450°C via slow pyrolysis) on soybean (Glycine max L.) seedling development and seed germination under drought conditions were investigated the experimental materials were evaluated through three treatments i.e. drought stress, drought stress with biochar application, and control (well-watered) by using a Randomized Complete Block Design with three replicates. This study examined the impact of drought and biochar application on two soybean varieties such as SKP (South Korean Variety) and Malakand-96 with respect to growth, physiological, and biochemical parameters. Such stress significantly reduced plant height, shoot biomass, pod number, chlorophyll content, oil content, and protein levels in both varieties, while more severity. However, biochar supplementation alleviated the adverse effects and enhanced plant height, biomass, seed number, and photosynthetic pigments of both varieties, especially Malakand-96. Biochar supplementation enhanced the quality of seeds by upgrading oil and protein contents in both varieties. Improved quality of seeds was recorded after the application of biochar, but higher values were recorded in Malakand - 96. Therefore, the present study provides a way to enhance the drought tolerance of soybean with the

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assistance of biochar, especially by using drought - tolerant varieties like Malakand - 96. These results suggest the feasibility of enhancing soybean productivity in drought - affected regions through interaction of genotypes with drought tolerance and biochar. Further research will be needed to long-term impacts of biochar on crop and soil performance.

Key words: Biochar, Drought, soybean, Malakand-96.

INTRODUCTION

The three major abiotic stresses such as temperature, salt, and drought have an impact on the geographic distribution, productivity of plants, and food security at world level.[8] Abiotic stress has negative impact on legume crops especially on their yield. [7, 16] Drought stress is among the leading contributors to agricultural crop failure.[11] Droughts frequently have a negative effect on the local economy, the environment, and farming in the impacted areas [13] recognizing the importance of water for many essential processes of plant growth, it is evident that crop production is severely low during droughts.[12] Drought has adverse effects on the development and quality of plant. it resists the proper development and germination of seedling initially, as drought disturbs the elongation of cell, [4, 6, 26] mitosis, lowers the turgor pressure resulting to insufficient growth. Problems in growth of leaves, decreased photosynthesis and distribution changes are all associated with drought stress on production of crops.[6]

Glycine max L., commonly known as soybean belong to family Fabaceae and genus Glycine. Glycine max having 25 species growing in habitat having pH ranges from 6.5 – 7. [22, 25] Around the world, 57% of all oilseed production comes from soybean.[25] Soybean seeds contain 40% protein and 20% oil, therefore it is referred as wonder crop .[22] Given its nutritional value and wide range of uses, soybeans have been dubbed the "golden bean," "queen of pulses," "wonder crop," "farmer's friend," "agriculture's Cinderella," "meat of the field," and "meat without bones".[17, 23] Raw soybean seeds have a food energy content of 1,866 kilojoules (446 kcal) per 100 grams. They also contain 36% protein, 30% carbs, 20% total fat, and 9% water.[1] The majority of soybeans are grown in rain-fed, drought-prone conditions. The most detrimental environmental stress is drought stress, which can reduce soybean output by up to 40%.[19] Raw soybean seeds have a food energy content of 1,866 kilojoules (446 kcal) per 100 grams. They also contain 36% protein, 30% carbs, 20% total fat, and 9% water. As an example, a 100-gram raw dose of soybeans contains high levels of the Daily Value (DV) for many nutrients, including iron (121%), manganese (120%), phosphorus (101%), protein (36% DV), dietary fiber (37%) and numerous B vitamins, including folate (94%). Magnesium, zinc, potassium, and vitamin K also have high amounts.[1]

Biochar is a carbon-rich porous material synthesized by pyrolysis of organic biomass, applied to improve soil, filtration of water [2, 18] and available nutrients results in increased fertility and higher agricultural output.[15] Its use in acidic soils, may have unfavorable consequences including lowering pH levels and adding toxic chemicals that may have a micro-level effect on soil health.[14] The feedstock used in producing biochar effects the properties of biochar. Several categories in which these properties are classified include Chemical making up, Element analysis, Acidity/basicity and

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porous structure and surface area.[5] Biochar has shown notable benefits for soil health in tropical areas, resulting in higher fertility and resistance to disease. Similarly, biochar has been shown to improve soil fertility and disease resilience in West European soils, making it a valuable tool for sustainable farming and soil health maintenance.[9] poultry litter is often applied to different crops as a fertilizer, it is however better to make biochar out of it. chicken litter biochar production has many advantages converting this waste stream into a productive end product with many environmental benefits. Even though pyrolysis causes some nitrogen loss, poultry litter biochar has improved nutrient richness, especially in nitrogen and phosphorus.[27]
Producing biochar on-site from poultry litter has several potential benefits for farmers. Farmers can make money by selling of biochar to distributers and also in markets as a replacement of trading poultry litter for unprofitable cleanup services. Converting poultry litter into biochar can help reduce pollution risks and promote sustainable farming practices by mitigating the environmental liabilities associated with long-term retention.[15] Re-cycling organic waste such as chicken litter into biochar is an effective waste management practice coupled with generating useful byproducts in the process. Animal using a biochar made from biological waste like chicken and bird droppings is said to have a relatively high amount of organic nutrients.[10]

Methodology

The research was carried out with utilization of a Randomized Complete Block Design (RCBD) in laboratory conditions at Centre for Plant Sciences and Biodiversity University of Swat. Plastic pots made of polyethylene pipe with a length of 25 cm and a diameter of 20 cm were utilised in experiment. Every pot was filled up with 4.5 kg air-dried soil. For the treatments of biochar, (2 % w/w) biochar was added to each pot and mixed evenly with the soil. Due to the addition of biochar, the soil surface in the amended pots rose by 4–7 cm above that of the control pots. For biochar production granular poultry litter were collected in raw form and dried naturally in the sunshine, and then crushed into tiny fragments. Poultry litter were slowly pyrolyzed in pyrolyzer at more than 450°C for three hours under semi- anaerobic condition to create biochar. For the creation of Biochar, a batch pyrolysis temperature-controlled device at 450°C For the purpose of the experiment, the prepared BC was kept in sanitized plastic bags. A thermometer was used to periodically check the temperature, which ranged from 0°C to 500°C.The seeds were submerged in water for six to eight hours prior for planting. Ten seeds were sown in each pot at a depth of one centimeter in the ground.[24] Normal tap water was used to irrigate the pots in order to maintain the soil's moisture content. Three treatments of drought stress and biochar application were applied to soybean varieties at different growth stages. The treatments include normal watered conditions without drought and biochar (T1), drought without biochar (T2), drought with 2% BC (T3), during different growth stage each with 3 replications. Data on plant morphological characters such as plant height, root and shoot length, fresh and dry weight, relative water, leaf area, number of branches and seeds per plant, chlorophyll a, chlorophyll b, carotenoids, total chlorophyll contents in leaf, pod per plant, seed per pod, weight and yield of seed per plant were recorded. For the statistical analysis of data Microsoft Word, Excel and SPSS version 22 was used, and the ANOVA test was

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Volume 3, Issue 10(2025)

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used for comparison of treatment means. [20]

RESULTS AND DISCUSSIONS

Morphological

The findings of figure.1 indicated that under SKP Drought minimized growth and biomass, while biochar alleviated slightly shoot characteristics but not root characteristics or water content whereas in Malakand-96 Drought lowered shoot characteristics but increased root length; biochar enhanced water content and root fresh weight despite shorter shoot length. This result aligns with the findings of (Batool et al., 2022) and (Majeed et al., 2021). In SKP, drought decreased shoot length by 35.3% (from 34 cm to 22 cm), root length by 15.4%, and fresh weight of shoots by 36.8%, whereas water content fell from 91% to 78% (14.3% reduction). When biochar was used under drought, shoot length was increased by 13.6% above drought alone but was still 26.5% shorter than the control. Root length reduced even further with biochar by 18.2% than drought, while shoot fresh weight declined by 22% than drought, reflecting poor recovery. Water content was still low at 77%, which is almost the same as drought without biochar. Malakand 96 exhibited improved tolerance to it. Shoot length reduced by 22.4%, leaves number by 33.3%, but surprisingly root length enhanced by 69.8% (from 10.6 cm to 18 cm) presumably as drought avoidance mechanism, similar findings were made by (Feng et al., 2021). Biochar treatment under drought decreased shoot growth by 27% versus drought only, but enhanced root fresh mass by 189.5% (from 0.19 g to 0.55 g), indicating increased water and nutrient uptake. Additionally, biochar enhanced water content by 9.1% (from 77% to 84%), far surpassing the control (80%), indicating its beneficial effect in retaining water under stress. As a whole, drought adversely influenced most of the growth characteristics, but biochar application reduced some of the negative impacts, especially in Malakand-96, increasing the root biomass and water content, thus suggesting its potential use in enhancing drought tolerance in soybean. [Fig.1-11, Table 1]

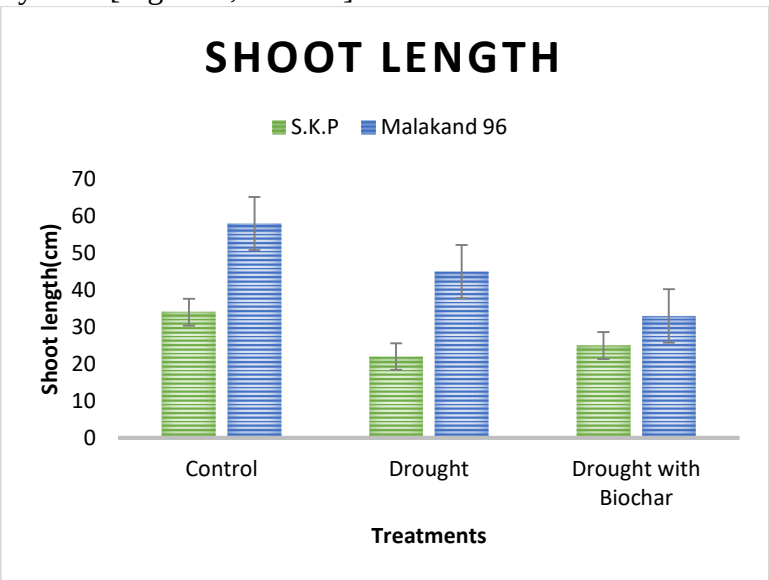


Figure 1: Impact of dry spell on the growth of shoots (measured in centimeters) when

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Volume 3, Issue 10(2025)

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treated with 2% biochar. The results represent the mean of measurements taken from three replicas.

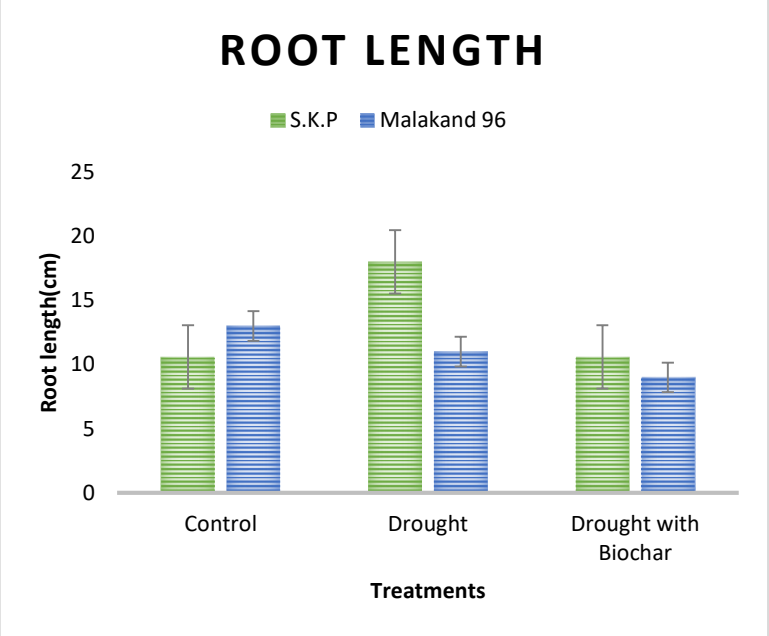


Figure 2: Effect of drought on Root Length (cm) by applying 2% biochar. Data are the average of 3 replicas.

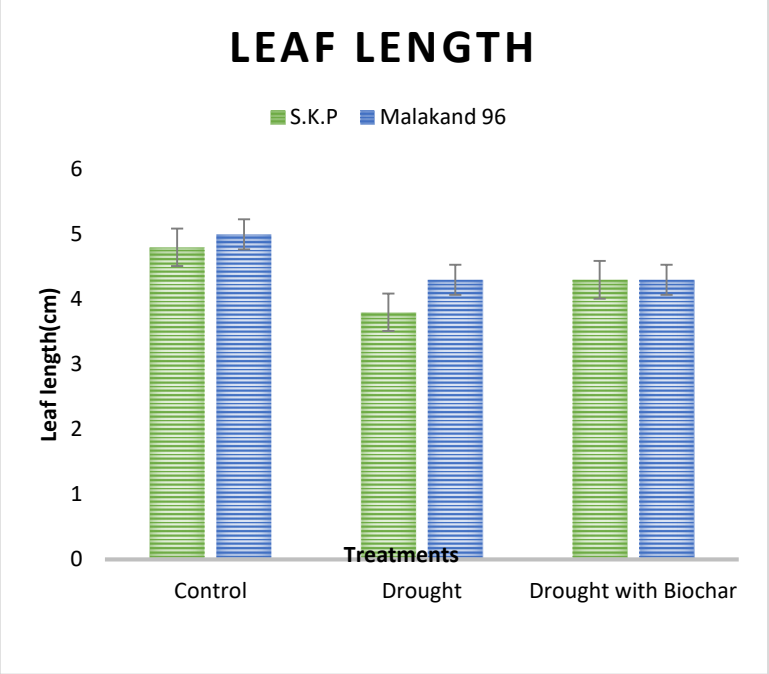


Figure 3: Effect of drought on Leaf length (cm) with application of 2% biochar. Data are the average of 3 replicas.

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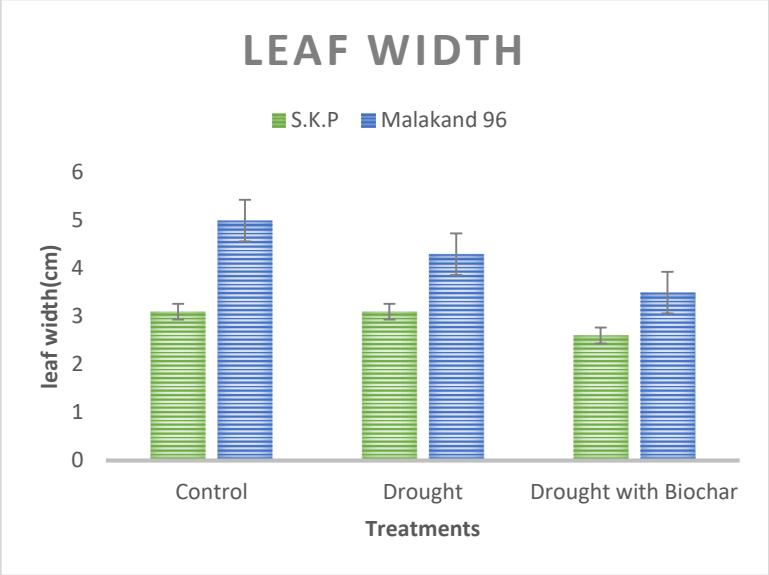


Figure 4: Effect of drought on Leaf width (cm) with application of 2% biochar. Data are the average of 3 replicas.

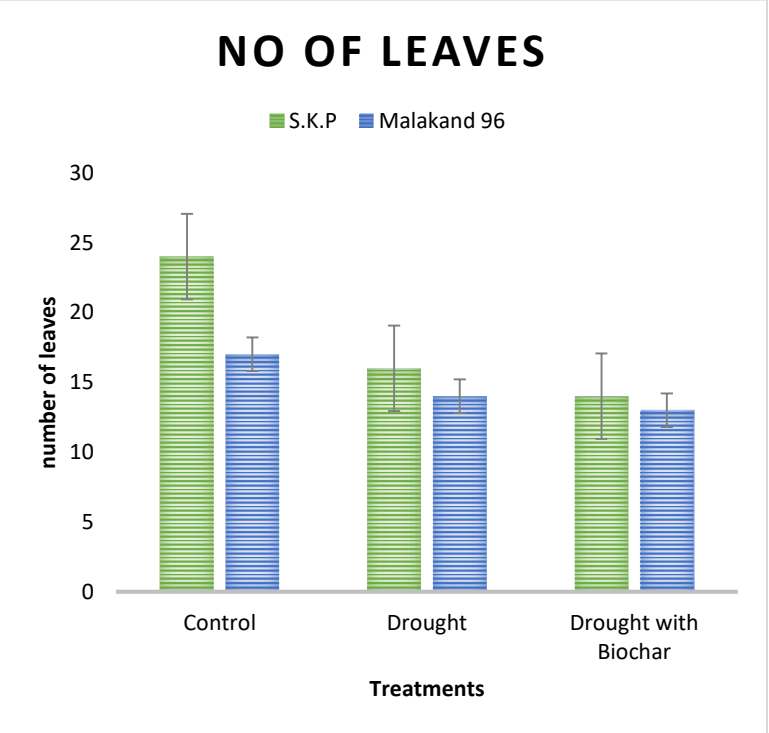


Figure 5: drought's impact on no. of leaf using a 2% biochar application. Three replicates' average is used as the data.

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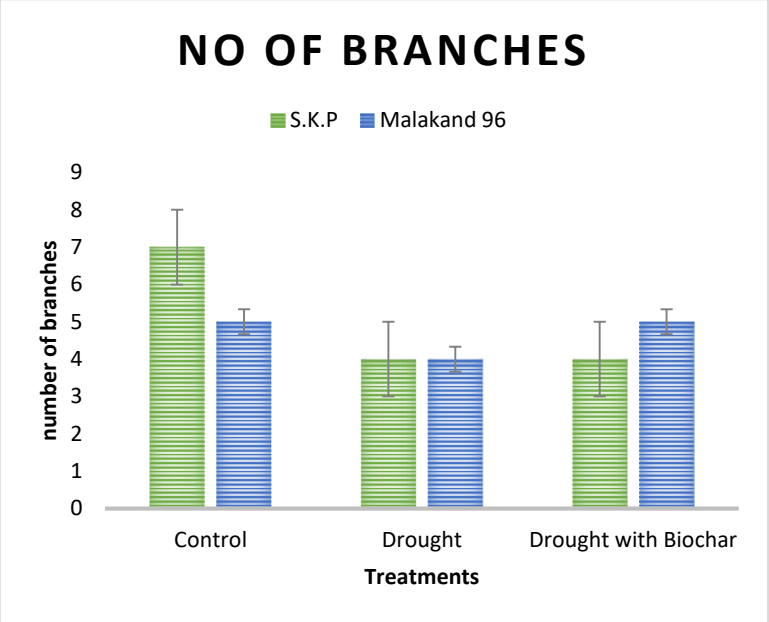


Figure 6: Effect of drought on number of branches with application of 2% biochar. Data are the average of 3 replicas.

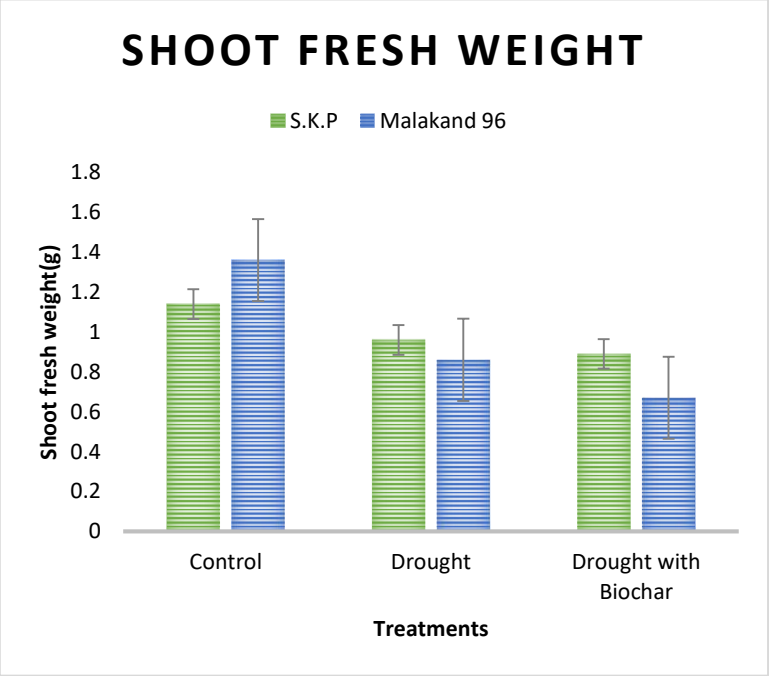


Figure 7: Effect of drought on shoot fresh weight (mg) with application of 2% biochar. Data are the average of 3 replicas.

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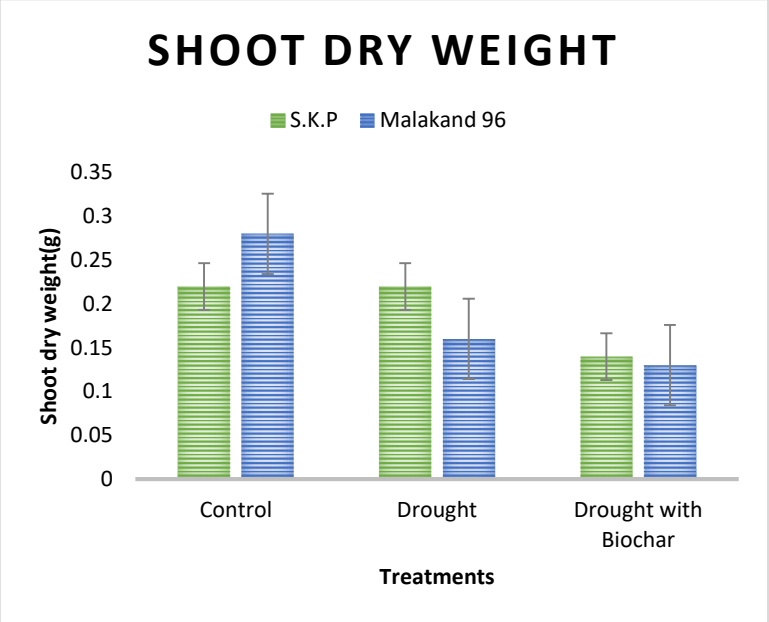


Figure 8: Effect of drought on shoot dry weight (mg) with application of 2% biochar. Data are the average of 3 replicas.

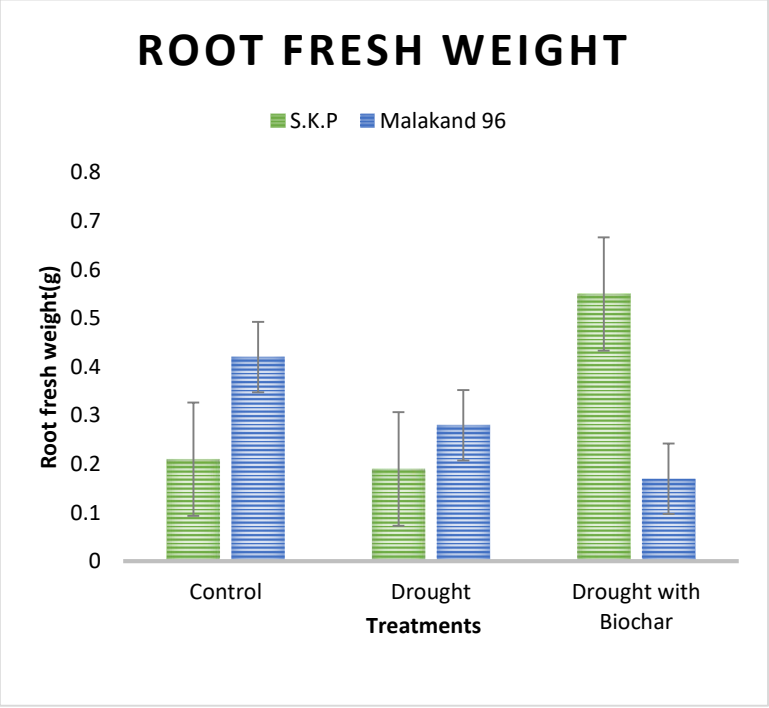


Figure 9: Effect of drought on Root fresh weight (mg) with application of 2% biochar. Data are the average of 3 replicas.

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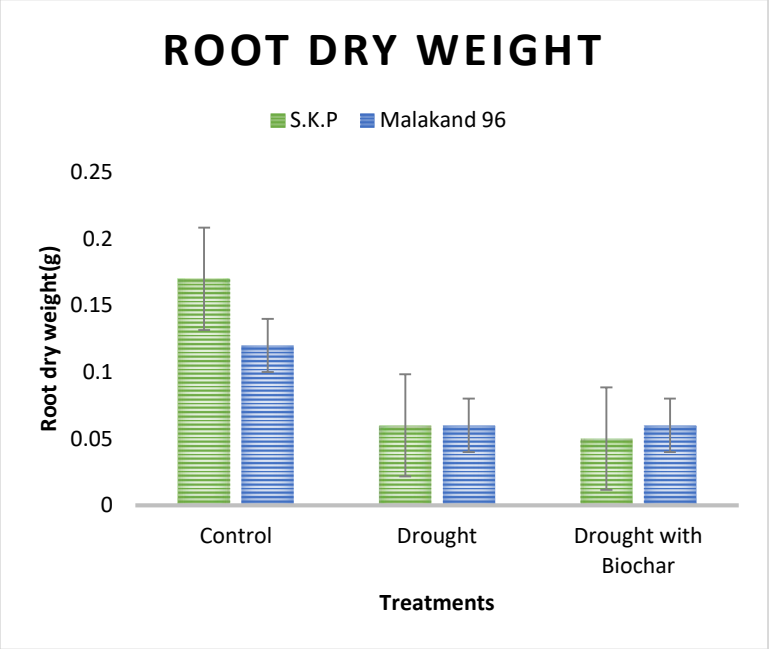


Figure 10: Effect of drought on Root dry weight (mg) with application of 2% biochar. Data are the average of 3 replicas.

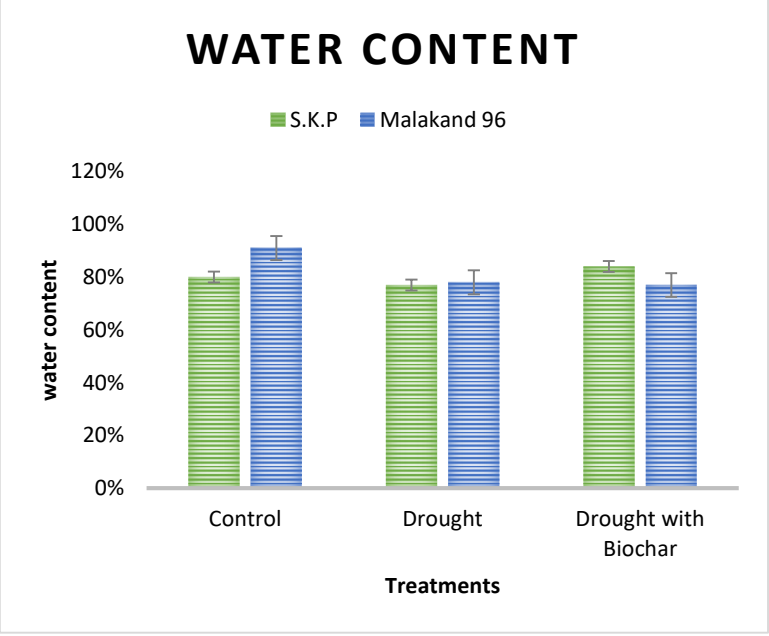


Figure 11: Effect of drought on water content with application of 2% biochar. Data are the average of 3 replicas.

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Table 1: Effects of biochar amendments made from poultry litter on the length of the roots, shoots, and number of branches per plant, leaf area, leaf numbers, fresh and dry weight and water content of SKP variety and Malakand-96 variety under drought conditions.

Plant part	Normal Control (T1) (SKP)	Normal Control (T1) (Malakand-96)	Drought (T2) (SKP)	Drought (T2) (Malakand-96)	Drought with biochar (T3) (SKP)	Drought with biochar (T3) (Malakand-96)
Shoot length /cm	□□□□□ □□□□□ □□	□□□□□ □□□□□ □□	□□□□□ □□□□□ □□	□□□□□ □□□□□ □□	□□□□□ □□□□	□□□□□ □□□□
Root length /cm	□□□□□ □□□□□ □□	□□□□□ □□□□□ □□	□□□□□ □□□□□ □□	□□□□□ □□□□	□□□□□ □□□	□□□□□ □□□□□ □□
Leaf length /cm	□□□□□ □□□□	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □
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No. of leaves/ plant	□□□□□ □□□□	□□□□□ □□□□□	□□□□□ □□□□□ □□	□□□□□ □□□□□ □□	□□□□□ □□□□	□□□□□ □□□□□
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Shoot fresh weight /mg	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □
Shoot dry weight /mg	□□□□□ □□□□□ □□	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □	□□□□□ □□□□□ □
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Water content/%	□□□□□ □□□□	□□□□□ □□□□	□□□□□ □□□□	□□□□□ □□□□	□□□□□ □□□□	□□□□□ □□□□

Data represent mean values ± standard error (SE) of triplicate measurements, as determined by analysis of variance (ANOVA)

Post-harvest Morphological characters

Fig.2 indicated that SKP had better performance under control conditions, whereas Malakand-96 was more resilient under drought and had the most appreciable improvement with the use of biochar, which reflects its better adaptability and response to biochar over SKP. Similar findings are documented by (Majeed et al., (2021) and (Jahan et al., 2020). Under drought stress in SKP, 21.7% of the pod length, 23.1% of the pod thickness, 29.1% of the plant height, and 30.6% of the shoot biomass was less under control compared to drought stress. Under drought and biochar, additional decreases were 25% in the number of pods, 34.8% in pod length, 25.5% in plant height, and 49.2% in shoot biomass, indicating that the performance of this variety was not enhanced by biochar. Conversely, Malakand-96 (Variety 2) was hardy as drought moderately improved length of pods (+4.8%), plant growth (+2.6%), and biomass of shoots (+34%). Surprisingly, biochar addition further increased growth in Malakand-96: pod thickness grew by 9.1%, plant height by 37.9% and shoot biomass by 125.4% over control, pointing out that Malakand-96 was highly responsive and positive to biochar application compared to SKP. [Fig.12-15, Table 2]

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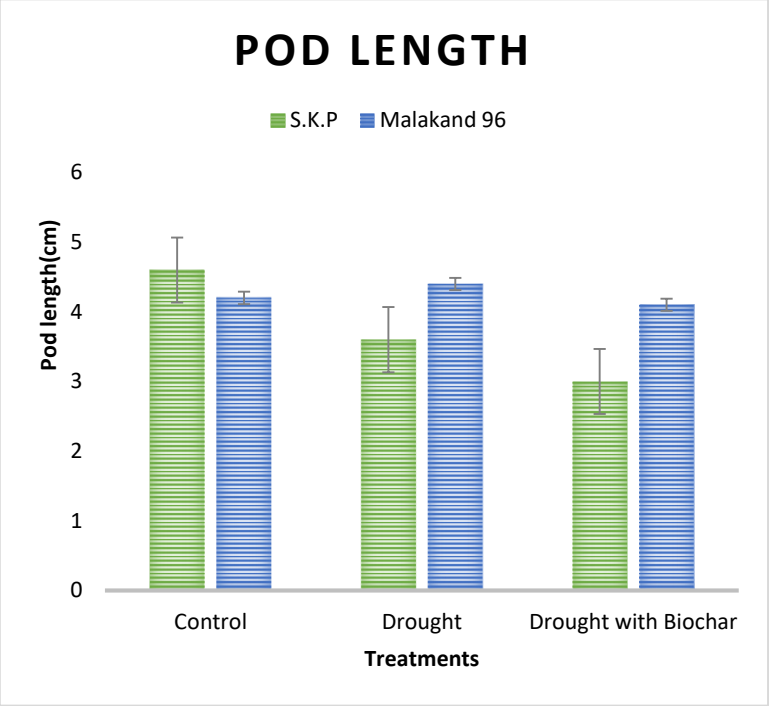


Figure 12: Effect of drought on pod length (cm) with application of 2% biochar. Data are the average of 3 replicas.

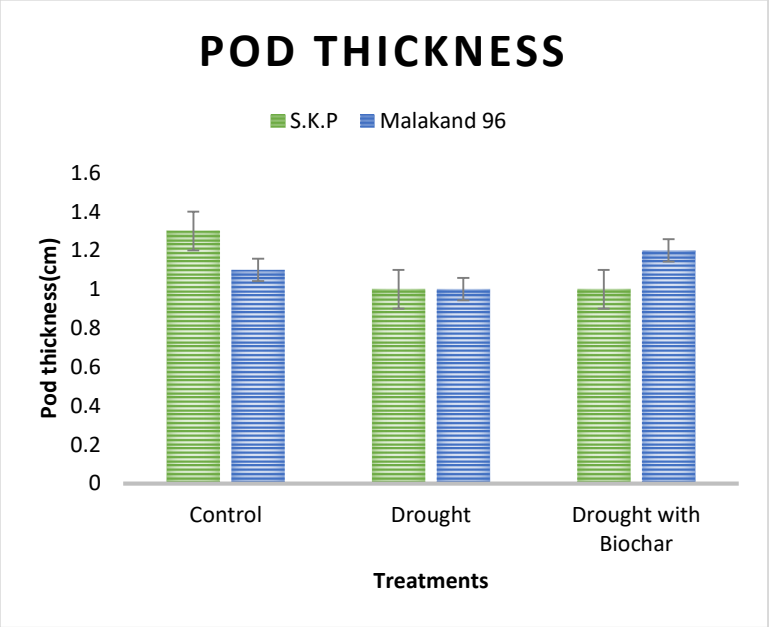


Figure 13: Effect of drought on pod width (cm) with application of 2% biochar. Data are the average of 3 replicas.

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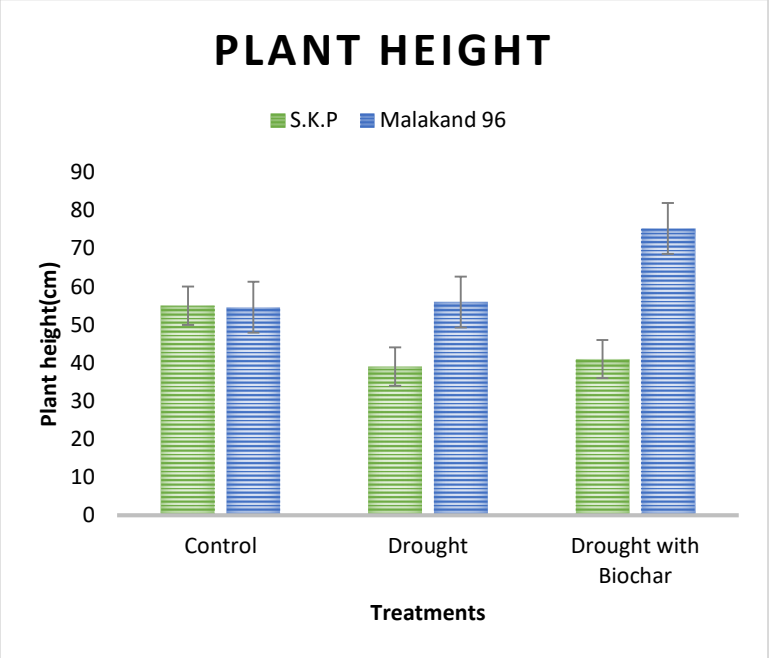


Figure 14: Effect of drought on plant height (cm) with application of 2% biochar. Data are the average of 3 replicas.

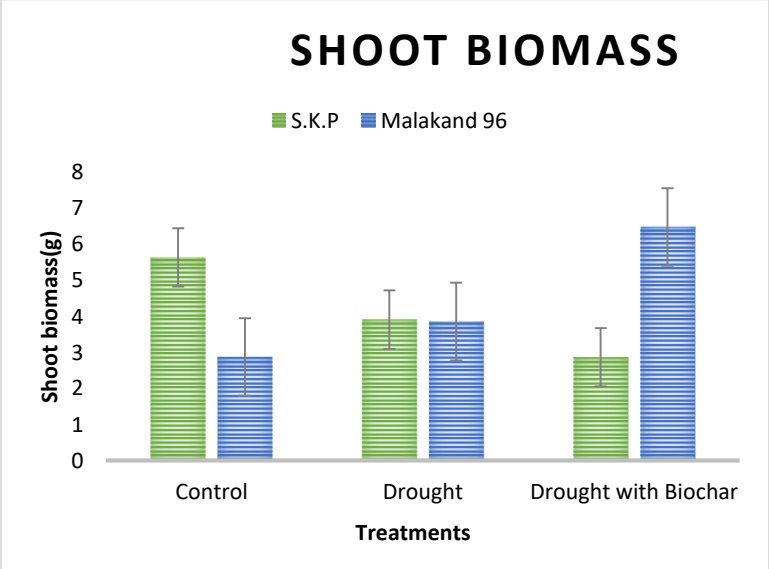


Figure 15: Impact of drought on shoot biomass (mg) when 2% biochar is applied. Three replicas are averaged to create the data.

Table 2: Effect of poultry litter biochar amendments on pod area, plant height and shoot biomass of SKP variety and Malakand-96 variety under drought conditions.

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Environmental condition	Pod length/cm (SKP)	Pod length/cm (Malakand-96)	Pod thickness/cm (SKP)	Pod thickness/cm (Malakand-96)	plant height/cm (SKP)	plant height/cm (Malakand-96)	shoot biomass/mg (SKP)	shoot biomass/mg (Malakand-96)
control (T1)	4.66±0.31	4.26±0.16	1.3±0.040	1.1±0.04	55±6.32	54.7±2.49	5.64±0.26	2.87±0.48
Drought (T2)	3.66±0.31	4.4±0.81	1±0	1.6±0.04	39±1.87	50±7.3	3.91±0.69	3.85±0.52
Drought with biochar (T3)	3±0.40	4.16±0.11	1±0	1.23±0.10	41.16±2.81	75.33±2.7	3.16±0.40	6.48±0.53

Data represent mean values ± standard error (SE) of triplicate measurements, as determined by analysis of variance (ANOVA)

YIELD CHARACTERS

For SKP, the control treatment yielded 4 pods and 9 seeds per plant. In drought, the number of pods did not change, but the number of seeds per plant was reduced by 22.2%. With biochar in drought, the pod number was reduced by 25% and the number of seeds per plant by 33.3% relative to control, and by 14.3% relative to drought. As opposed to it, Malakand-96 under control conditions also possessed 4 pods and 8 seeds per plant. Both pod and seeds per plant under drought treatment were kept at levels of the control with no percentage difference. Treatment with biochar under drought significantly raised the number of pods by 25% and seeds per plant by 25% as compared to the control and drought conditions. These findings show that drought and biochar had adverse effects on SKP, decreasing seed yield, whereas Malakand-96 Variety 2 was resistant to drought and showed great improvement with the application of biochar. [Fig.16,17, Table 3]

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Print ISSN 3007-3189
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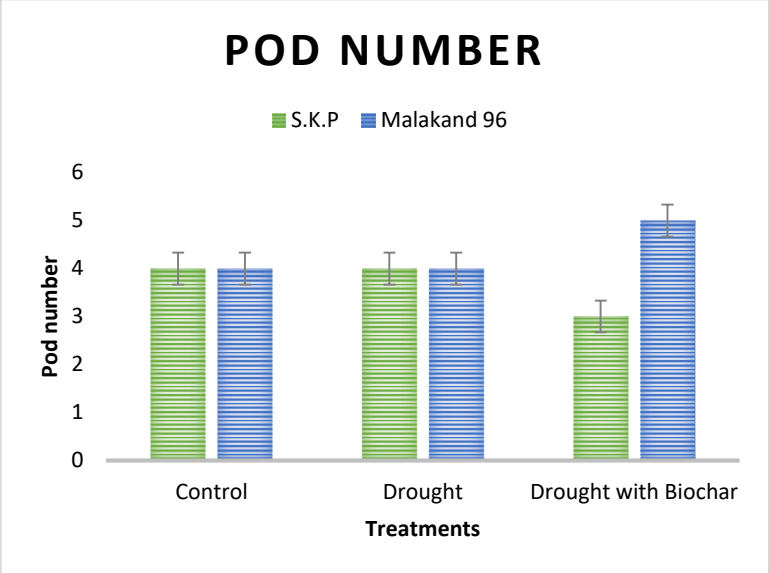


Figure 16: Effect of drought on the pod number after applying 2% biochar. Three replicas are averaged to create the data.

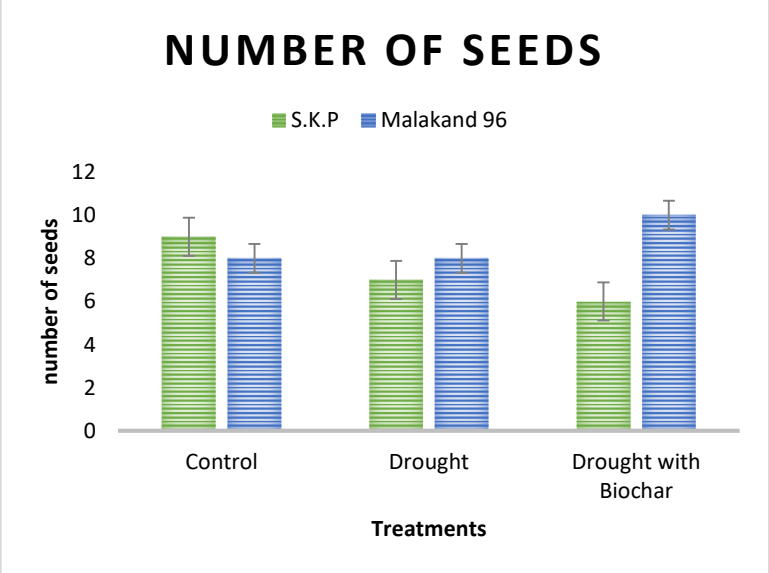


Figure 17: Effect of drought on number of seeds with application of 2% biochar. Data are the average of 3 replicas.

Table 3: Impact of poultry litter biochar amendments on the number of seeds and pods of the Malakand-96 and SKP varieties during drought events.

Environmental/ condition	Pod number/plant (SKP)	Pod number/plant (Malakand-96)	No. of seeds/plant (SKP)	No. of seeds/plant (Malakand-96)
control (T1)	4±0	4±0	9±0	8±0.40

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Drought (T2)	4.33±0.23	4±0.40	7±0.70	8.33±0.62
Drought with biochar (T3)	3±0.40	4.66±0.23	6±0.81	10.33±0.84

Data represent mean values ± standard error (SE) of triplicate measurements, as determined by one way analysis of variance (ANOVA)

DETERMINATION OF PHYSIOLOGICAL PARAMETERS

Table 2 indicated that two soybean varieties (SKP Variety 1 and Malakand 96 Variety 2) exhibited considerable differences in carotenoid and chlorophyll content. For Variety 1, the control treatment had the highest content with chlorophyll a at 2.669 mg/g, chlorophyll b at 4.859 mg/g, total chlorophyll at 7.528 mg/g, and carotenoids at 28.994 mg/g. During drought, Variety 1 had decreases of 34.7% in chlorophyll a, 34.8% in chlorophyll b, 34.7% in total chlorophyll, and 36.0% in carotenoids than in the control, these results are associated with reports of (Guo, L, et al., 2021). Using biochar during drought improved these declines, raising chlorophyll a by 8.6%, chlorophyll b by 11.4%, total chlorophyll by 10.4%, and carotenoids by 16.5% over drought but below that of the control. For Malakand 96 Variety 2, drought treatment surprisingly enhanced chlorophyll a by 63.7%, chlorophyll b by 78.8%, total chlorophyll by 73.3%, and carotenoids by 29.1% over its control (chlorophyll a: 1.919 mg/g, chlorophyll b: 3.335 mg/g, total chlorophyll: 5.254 mg/g, carotenoids: 25.052 mg/g). But the use of biochar during drought for Variety 2 lowered chlorophyll a by 32.2%, chlorophyll b by 35.2%, total chlorophyll by 34.2%, and carotenoids by 37.3% over drought alone, though rates remained marginally higher than control for chlorophylls (10.9% increase for chlorophyll a, 15.9% increase for chlorophyll b, 14.0% increase for total chlorophyll) but lower for carotenoids (19.0% drop). These findings pinpoint differing drought and biochar responses in the two varieties, where Variety 1 had an advantage due to biochar during drought, but Variety 2 revealed higher pigment content during drought but decreased levels when biochar was added. [Fig.18-21, Table 4]

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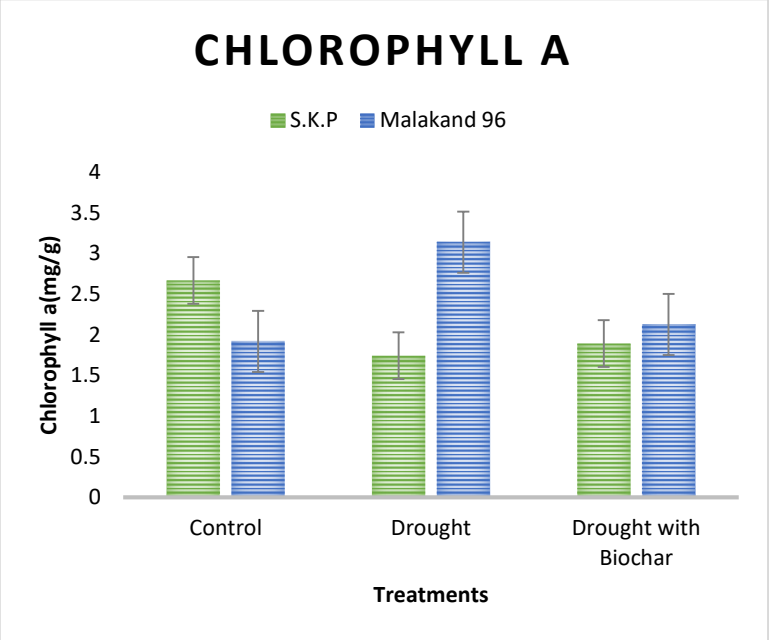


Figure 18: Effect of drought on chlorophyll a (mg/g) with application of 2% biochar. Data are the average of 3 replicas.

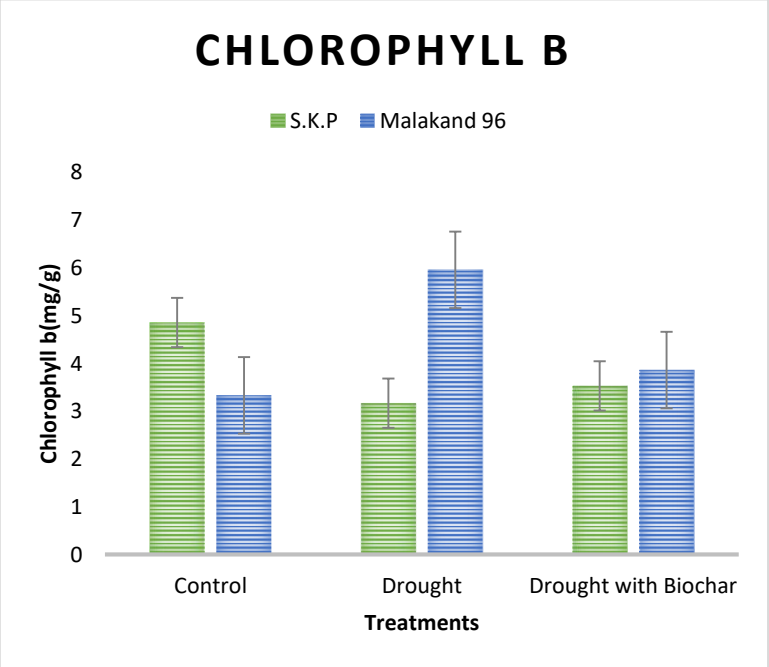


Figure 19: Effect of drought on chlorophyll b (mg/g) with application of 2% biochar. Data are the average of 3 replicas.

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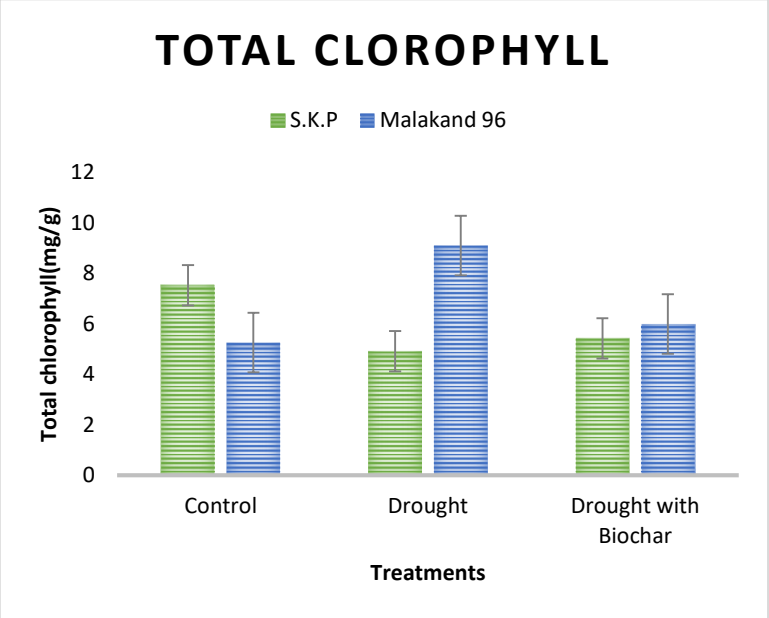


Figure 20: drought's impact on total chlorophyll (mg/g) when 2% biochar is applied. The data is the mean of three copies.

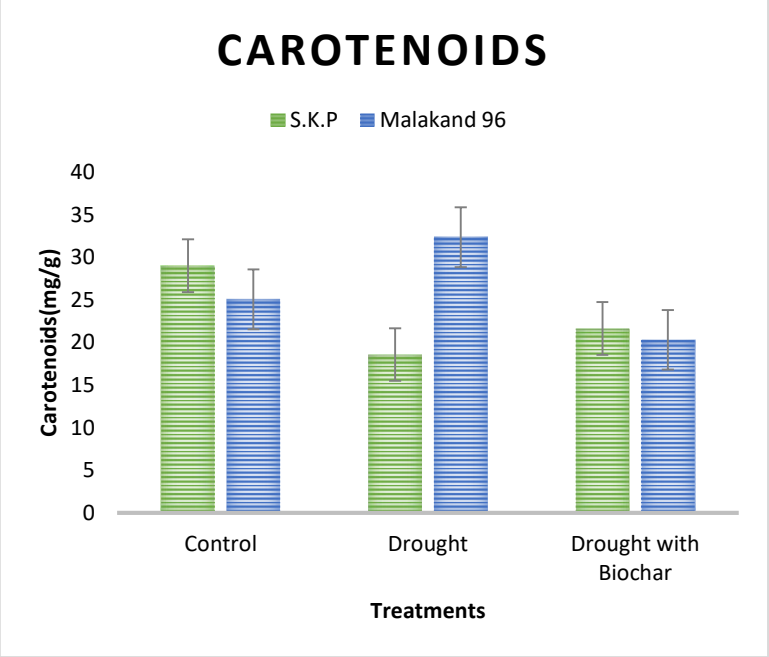


Figure 21: Effect of drought on total carotenoids (mg/g) with application of 2% biochar. Data are the average of 3 replicas.

Table 4: Effect of poultry litter biochar amendments on chlorophyll a, b and total chlorophyll content of SKP variety and Malakand-96 variety under drought conditions.

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Environment /condition	Chlorophyll a mg/g (SKP)	Chlorophyll a mg/g (Malakand-96)	Chlorophyll b mg/g (SKP)	Chlorophyll b mg/g (Malakand-96)	Carotenoids mg/g (SKP)	Carotenoids mg/g (Malakand-96)	Total Chlorophyll mg/g (SKP)	Total Chlorophyll mg/g (Malakand-96)
control (T1)	0.1±0.011	0.01±0.002	0.1±0.011	0.01±0.002	0.16±0.019	0.05±0.006	2.16±0.13	3.03±0.2
Drought (T2)	0.04±0.001	0.14±0.038	0.04±0.002	0.14±0.039	0.08±0.002	0.19±0.038	3.7±0.39	2.09±0.01
Drought with biochar (T3)	0.06±0.02	0.046±0.009	0.05±0.02	0.04±0.01	0.12±0.06	0.05±0.006	2.6±0.05	2.1±0.32

Data represent mean values ± standard error (SE) of triplicate measurements, as determined by one way analysis of variance (ANOVA)

DETERMINATION OF SEED CONTENTS

The research examined the impact of drought and drought with biochar on seed quality attributes in two soybean genotypes. In the case of S.K.P, drought lowered moisture (5.01% compared with 5.27% control) and oil content (17.01% compared with 17.65% control), whereas protein was quite constant (38.05% compared with 38.43% control). In contrast, drought with biochar not only improved moisture (5.12%) but also enhanced oil (18.04%) and protein (39.71%) content above control. Equally, in Malakand 96, drought reduced slightly the moisture (5.04% vs. 5.39%) and the oil (17.36% vs. 17.65%), although with little effect on protein (38.67% vs. 38.78%). Interestingly, drought with biochar restored the moisture to control levels (5.38%) and strongly promoted both oil (18.17%) and protein (39.95%) content. same results are reported by (Bashir, M. K. et al., 2019); (Maqsood, M. A., et al., 2020). these results show that application of biochar under drought stress can enhance seed quality, especially oil and protein content, in both soybean lines, with slightly higher improvement in Malakand 96. [Fig.22-24, Table 5]

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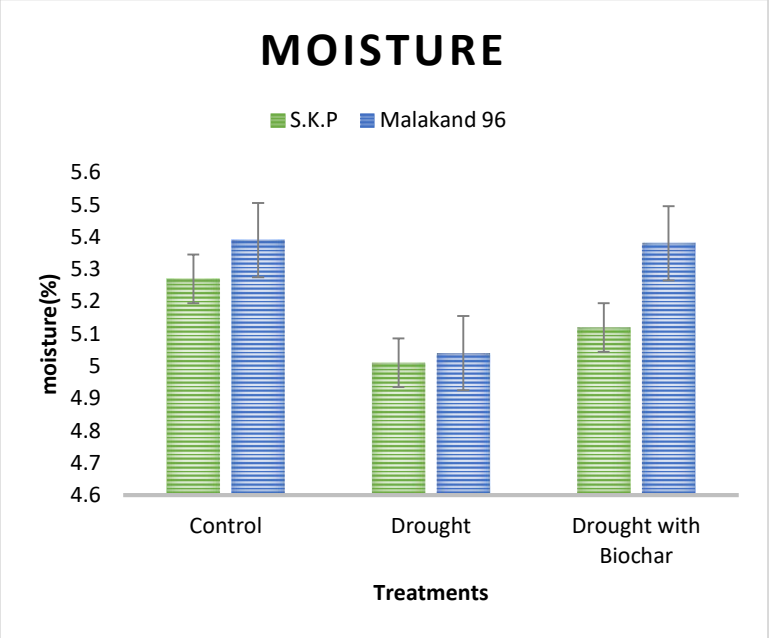


Figure 22: Effect of drought on seed moisture with application of 2% biochar. Data are the average of 3 replicas.

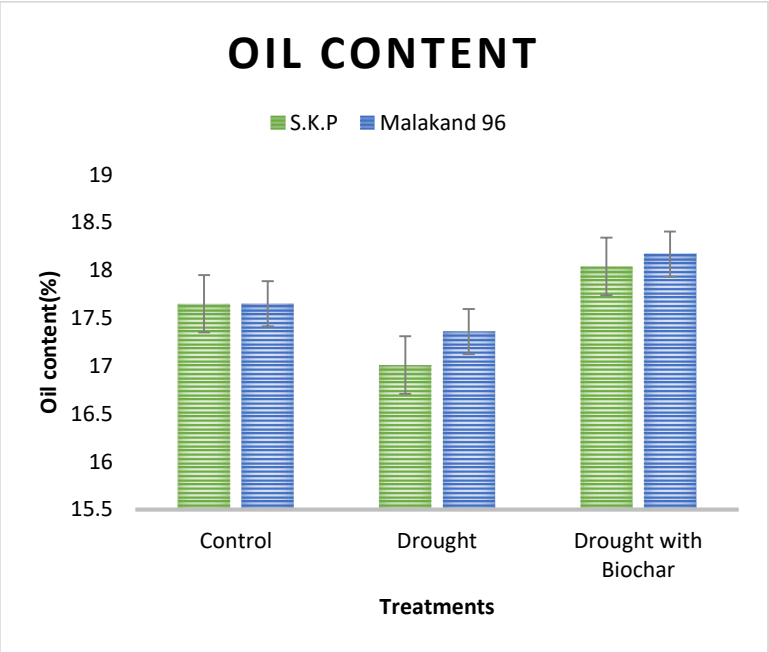


Figure 23: Effect of drought on total seed oil content with application of 2% biochar. Data are the average of 3 replicas.

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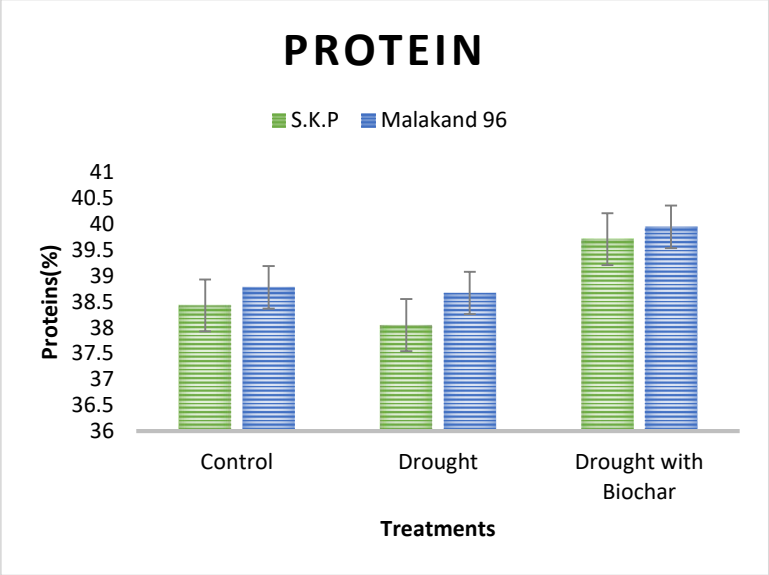


Figure 24: Effect of drought on total seed protein %age with application of 2% biochar. Data are the average of 3 replicas.

Table 5: Effect of poultry litter biochar amendments on Seed Oil and protein %age of SKP variety and Malakand-96 variety under drought conditions

Environment/ Condition	Moisture % (SKP)	Moisture % (Malakand-96)	Oil content% (SKP)	Oil content% (Malakand-96)	Protein% (SKP)	Protein% (Malakand-96)
control (T1)	5.27±0.43	5.39±0.41	17.65±0.42	17.65±0.39	38.10±0.39	38.78±0.40
Drought (T2)	5.01±0.41	5.04±0.41	17.01±0.41	17.36±0.42	38.5±0.02	38.66±0.42
Drought with biochar (T3)	5.11±0.45	5.38±0.41	18.04±0.42	18.17±0.4	39.71±0.40	39.95±0.41

Data represent mean values ± standard error (SE) of triplicate measurements, as determined by analysis of variance (ANOVA)

CONCLUSION AND RECOMMENDATION

The research findings have been shown that there are significant differences in both SKP. and Malakand-96 soybean varieties with regard to the response to drought stress as well as biochar amendment. With drought stress, a greater decrease was observed in most parameters for both varieties but SKP was particularly affected in all parameters. Regardless, it can be observed that biochar treatment increased some of the negative drought effects and this was more effective on Malakand-96, indicating more growth with greater chlorophyll and carotenoid levels than SKP. With plants that matured in biochar, the oil and protein contents were significantly elevated due to a higher seed

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quality. In this regard, biochar had a more favorable outcome than malakand-96 suggesting that this genotype had a better adaptive mechanism on drought conditions. Further investigations are required particularly in root architecture, water uptake, and stress signaling in future.

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