Assessment of Macronutrient Level Variations on the Growth and Morphological Traits of Spinach (*Spinacia oleracea* L.)

Mehtab Rai Meghwar[1*]; Ghulam Hussain Wagan[2]; Abdul Qadir Kumbhar[3]; Ali Raza Soomro[4]; Mudassir Ali Junejo[5]; Inzamam Ali Jamali[6]; Farhan Ali Jatoi[7]; Fida Hussain Channa[8]; Kabeer Hassan Channa[9]; Li Qing[10]; Kamran Ali Mahessar[11]; Maqsood Ali Wagan[12]

[1,10]College of Life Science and Agri-forestry, Southwest University of Science and Technology, Mianyang 621010, Sichuan, China
[2]Department of Agricultural Economics, Sindh Agriculture University, Tando jam, 70060, Pakistan
[3,4,5,11]Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, 70060, Pakistan
[6]Sindh Water and Agriculture Transformation Project (SWAT), Tando Jam, 70060, Pakistan
[8,9]Department of Plant Pathology, Shaheed Zulfiqar Ali Bhutto Agricultural College, Dokri, Larkana
[7,12]Department of Horticulture, Sindh Agriculture University, Tandojam, 70060, Pakistan
*Corresponding Author

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Abstract— *This study investigates the effect of varying levels of macro nutrients on the growth and development of spinach (Spinacia oleracea L.) using a Completely Randomized Design (CRD) with two varieties: Sindhi and English spinach. Conducted during 2024, the experiment utilized a total of six nutrient combinations, including control, NPK (3g/L, 4g/L, 5g/L), and Calcium Nitrate (1g/L), applied to two per pot across replicated treatments. Parameters such as seed germination percentage, germination index, plant height, leaf metrics, root characteristics, and chlorophyll content were assessed. Results indicated a significant enhancement in growth metrics with NPK (3g/L) plus Calcium Nitrate (1g/L), particularly for Sindhi spinach, where it exhibited a germination rate of 95.16% and an increase in chlorophyll to 64.86. Conversely, higher concentrations of NPK negatively affected germination and growth in both varieties, suggesting that balanced nutrient application is crucial for optimal spinach development. This investigation underscores the importance of macro nutrients in enhancing spinach yield, contributing valuable insights for growers aiming to maximize crop productivity*.

Keywords— Spinach, Macro Nutrients, growth, Development.

I. INTRODUCTION

Spinach (Spinacia oleracea L.) is a common leafy green vegetable that belongs to the Chenopodiaceae family. Spinach is a staple leafy green vegetable known for its nutritional value as well as culinary versatility and consumed widely in many countries across the world (Cho et al., 2018). It is believed that this plant originated from southwestern Asia and has been farmed in various parts of Europe, North America, and Australia. Spinach is rich in essential vitamins and minerals, including vitamin A, vitamin C, iron, and calcium. It is low in calories and fat, while high in fiber. So, it can be said that spinach is healthy to consume in any diet (Umar et al., 2007). The ways to eat spinach include raw in salads or cooked with omelets, pastas, and soups. It can also be stuffed into pies and pastries (Max et al., 2016). Spinach has a history dating back to thousands of years ago when people used it for medicinal purposes. It is believed to possess anti-inflammatory and antioxidant properties and may prevent chronic diseases like heart disease and cancer. In short, spinach is a healthy and tasty vegetable enjoyed by many people across the globe for its nutritional benefits and taste (El-Kamony et al., 2000). Spinach is a cool-season annual crop that is often grown from seed. It can be propagated by seeds that are either broad-casted or planted in hills using dry soil (Vignesh et al., 2012). In Egypt, spinach is one of the most important leafy vegetables and has shallow roots, making it relatively easy to grow. Spinach is a very nutritious vegetable with vitamins, minerals, and antioxidants (Ahmadi et al.,

2010). It is typically consumed after boiling, either fresh or frozen, or can be eaten raw in salads. Spinach is often used in various dishes, such as omelets, pastas, and soups, and can also be used as a filling for pies and pastries. Major groupings of vegetables are green leafy vegetables, such as spinach, that have been considered to have high nutritional value and health benefits. They are also referred to as "nature's anti-aging wonders" since they can potentially reduce the risk of chronic diseases, such as heart disease and cancer. In addition, spinach has anti-inflammatory properties and may aid in digestion. Spinach is a significant and nutritious vegetable that is consumed globally (Rabie et al., 2014). Spinach is one of the common vegetables grown in most African countries, either for consumption or as a cash crop to generate money. Spinach, like other vegetables, is frequently grown in many parts of Africa, such as in rural, peri-urban, and urban areas (Mdoda et al., 2022). Smallholder farmers often cultivate spinach to increase their food security and income. Spinach is a nutrient-rich and versatile vegetable, which can be consumed in various ways; it can be eaten raw in salads or cooked in dishes like omelets, pastas, and soups. It can also be used as a stuffing for pies and pastries. Spinach has several health benefits like anti-inflammatory and antioxidant activities, thus preventing chronic diseases such as heart disease and cancer. Therefore, spinach is a valuable and delectable vegetable that everyone enjoys across the world (Khalsa, 2003).

II. MATERIALS AND METHODS

The experiment has been conducted during 2024 to Evaluate the Effect of different level of macro nutrient on the development of spinach (Spinacia oleraceae L.), Seeds of two verities were sown in pots (15) containing media soil + silt +FYM in the ratio of 1:1:1 after germination seedling were thinned out and keep two per pot three pots per treatment per verity were maintained for experiment.

2.1 Experimental design: Completely Randomize Design (CRD) – factorial:

Replications: Three (03)

Treatments = Two factors (A & B)

Factor A = micro Nutrients Combinations

N1=Control

N2 =N.P.K (3g L -1)

N3 = Calcium Nitrate (1g L-1)

N4= N.P.K (3g L-1) + Calcium nitrate (1g L-1)

N5= N.P.K (4g L-1) + Calcium nitrate (1g L-1)

N6= N.P.K (5g L-1) + Calcium nitrate (1g L-1)

Factor B = Varieties = 02

V1=Sindhi local

V2 = English

2.2 Data analysis:

We used Statistics 8.1 to perform a statistical analysis of the data (Statistics.2006). We used the LSD (Least Significant Difference) test to compare the treatments when necessary.

III. RESULTS

Present study was carried out in 2024 to investigate the Effect of different level of micro nutrient on the development of spinach (Spinacia oleraceae L.) varieties. The experiment was set up in complete randomized design. Two varieties (Sindhi and English) were treated with different nutrient combinations to check their response on germination and vegetative growth. Observations were recorded on seed germination (%), germination index (GI), Plant height (cm), Leaf length (cm), Leaves plant^{-1}, Leaf weight (g), Leaf width (cm), Fresh biomass of root(g), Root Depth (cm) and Chlorophyll content.

3.1 Seed Germination (%):

Germination index: The germination percentage of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 1. The analysis suggested that germination percentage of spinach was significantly influenced by different levels of nutrients ($P<0.05$); and the effect of spinach varieties and interaction results were statistically significant ($P<0.05$). Seed germination (%) has a great influence on early growth and harvesting of crop. The highest germination of Sindhi Spinach seed was achieved when NPK (3g/L) + Calcium Nitrate (1g/L) was applied, with a significantly higher germination value of 95.16% compared to the control. Calcium Nitrate application alone also improved germination with a percentage of 91.23%. This indicates that the positive effect of Calcium Nitrate was noteworthy. Otherwise, an increase in the concentration of NPK in the treatments resulted in low germination in both treatments N5 and N6. The lowest percentage of germination was found at 72.78%. However, for seed of English Spinach, the control group had already achieved a very high germination percentage at 95.87%. Addition of Calcium Nitrate at 1g/L pushed up the germination to 97.47% only, and further addition of NPK at 3g/L + Calcium Nitrate at 1g/L didn't give an increase in germination and remained at 97.47%. As in the case of Sindhi Spinach, the highest concentrations of NPK in the treatments N5 and N6 decreased germination, and the lowest achieved was at 64.87%.

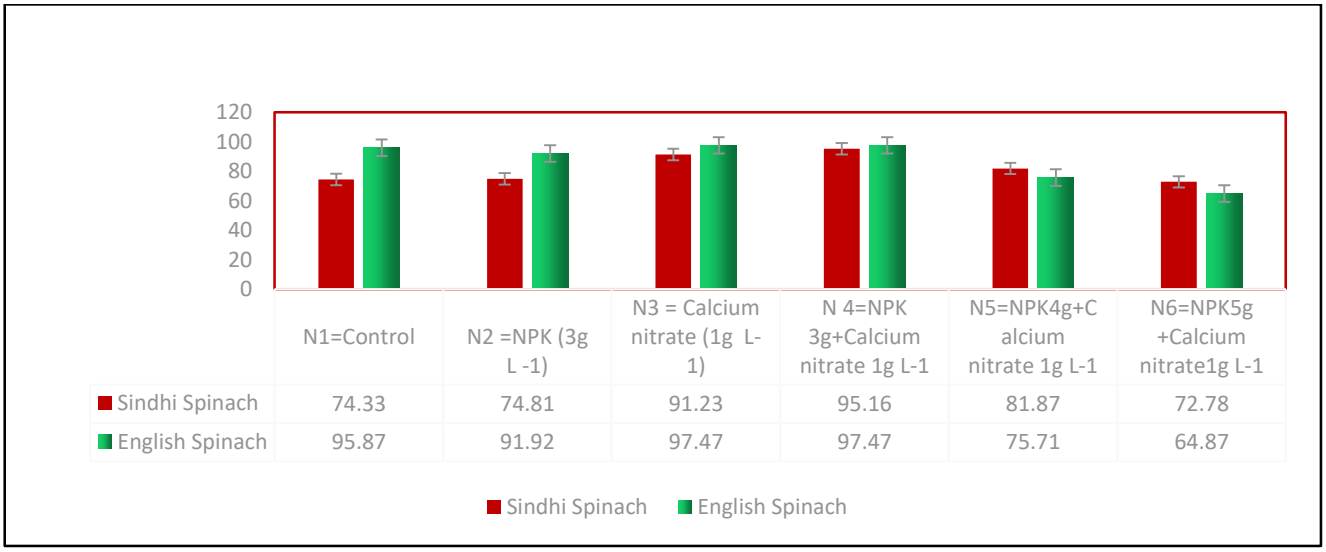

FIGURE 1: Germination % of Spinach Varieties under Nutrient combinations.

3.2 Germination index:

The germination index of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 2. The analysis suggested that germination index of spinach was significantly influenced by different levels of nutrients ($P<0.05$). The highest GI was obtained in Sindhi spinach when NPK (3g/L) + Calcium Nitrate (1g/L) was applied, and the value was significantly higher, at 17.51, compared to the control, which had a lower GI of 8.43. The application of Calcium Nitrate (1g/L) alone also showed a positive effect, and the GI value was 12.33, indicating that calcium nitrate plays a beneficial role in enhancing germination for Sindhi spinach. However, when the concentration of NPK was increased in treatments N5 and N6 (NPK 4g/L + Calcium Nitrate 1g/L and NPK 5g/L + Calcium Nitrate 1g/L) whereby the GI decreased slightly. The values reached 13.81 and 15.21 respectively. This means that extremely high concentrations of NPK are not suitable for Sindhi spinach, and an excess amount may cause damage to germination, However, for seed of English spinach, the control group had the highest GI of 22.13, meaning excellent germination under baseline conditions without added nutrients. Addition of NPK at 3g/L led to a decline in GI to 14.3, which indicated that this nutrient combination was less effective for English spinach. Similarly, the treatment with Calcium Nitrate (1g/L) alone resulted in a further reduction of GI to 7.6, which shows that English spinach may not respond well to calcium nitrate supplementation alone. The treatment N4, which was NPK (3g/L) + Calcium Nitrate (1g/L), did not enhance the GI further and resulted in a value of 9.6, which shows a limited response to this combination for English spinach variety. As with the case of Sindhi spinach, the highest values of NPK in treatments N5 and N6 diminished the GI down to 13.61 and 12.52, respectively, indicating an adverse effect on germination because of high NPK concentrations for English spinach as well.

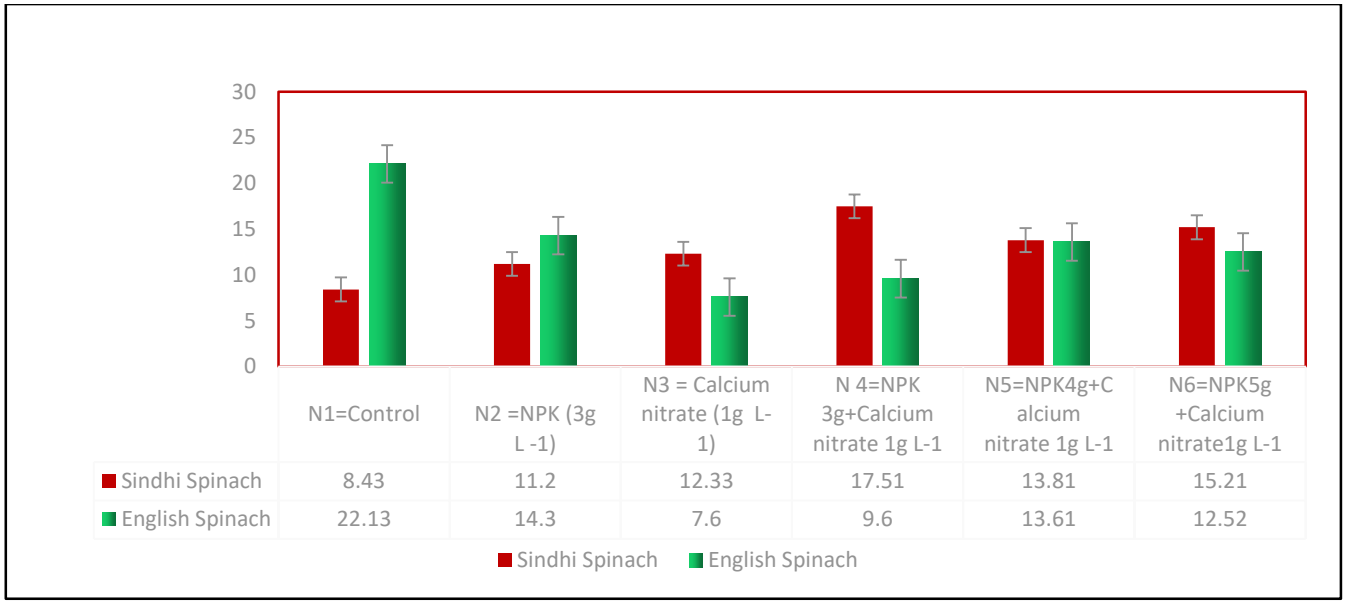

FIGURE 2: Germination index (GI) of spinach varieties under nutrient combinations

3.3 Plant height (cm):

The Plant height (cm) of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 3. The analysis suggested that Plant height (cm) of spinach was significantly influenced by different levels of nutrients ($P<0.05$); and the effect of spinach varieties and interaction results were statistically significant ($P<0.05$). The highest value for plant growth in terms of height was given by Calcium Nitrate (1g/L) (N3), which indicated a height value of 3.06cm. This was significantly higher compared to the control, which received a value of 0.96 cm; this indicates that Calcium Nitrate alone improved Sindhi Spinach growth significantly. Other treatments such as NPK (3g/L) + Calcium Nitrate (1g/L) (N4) and NPK (4g/L) + Calcium Nitrate (1g/L) (N5) resulted in lower plant heights compared to the Calcium Nitrate treatment. Thus, Calcium Nitrate seems to be the best for Sindhi Spinach in terms of height. On the other hand, English Spinach showed a different pattern. While the control with no fertilizer was only 0.39 cm, the best treatment for English Spinach was actually Calcium Nitrate at 1g/L (N3) which produced a height of 1.51 cm. That is better than the control, but significantly lower than the best for Sindhi Spinach variety. Other treatments, including NPK (3g/L) and combinations of NPK and Calcium Nitrate, failed to produce any significant benefits and, in some cases, further reduced the plant height.

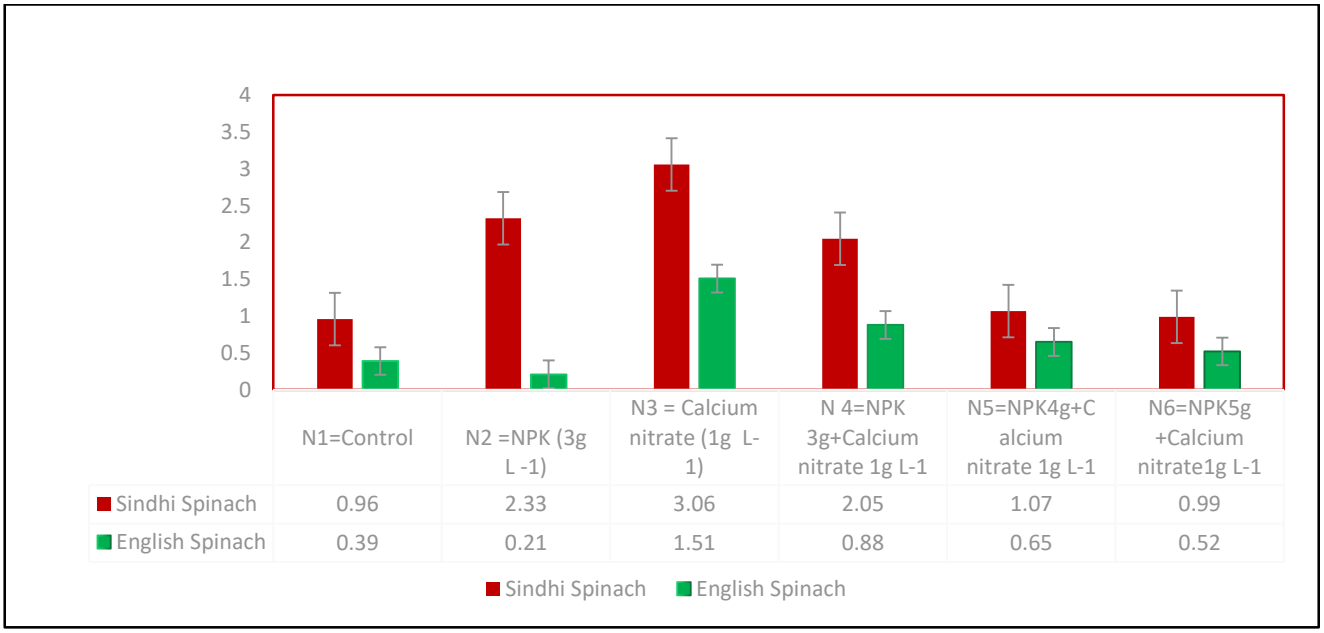

FIGURE 3: Plant Height (cm) of Spinach varieties under nutrients combinations

3.4 Leaf length (cm):

The Leaf length (cm) of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 4. The analysis suggested that Leaf length (cm) of spinach was significantly influenced by different levels of nutrients ($P<0.05$); and the effect of spinach varieties and interaction results were statistically significant ($P<0.05$). The leaf growth was observed in Sindhi Spinach to be highest with the combined treatment of NPK (3g/L) and Calcium Nitrate (1g/L), N4 which recorded a length of 8.46 cm of the leaf length. This is a much better compared to the control that had 4.5 cm of the leaf length. This means that NPK and Calcium Nitrate treatment was the best for Sindhi Spinach where there was excellent growth. However, when the concentration of NPK was increased (N5 and N6), the leaf length decreased, which indicated that higher concentrations of NPK were not as beneficial. Overall, the best treatment for Sindhi Spinach was the combination of NPK (3g/L) + Calcium Nitrate (1g/L). On the other hand, English Spinach had a much longer leaf length in the control (12.76 cm) compared to the fertilized treatments. Although the application of Calcium Nitrate (1g/L) (N3) increased the length of the leaf to 6.96 cm, it still remained shorter compared to the control treatment, and treatments with NPK (3g/L) as well as blends with higher levels of NPK had even smaller leaves. There was an apparent optimal performance in the absence of fertilization by English Spinach variety, since the leaf length was lessened in all fertilized treatments compared to the control.

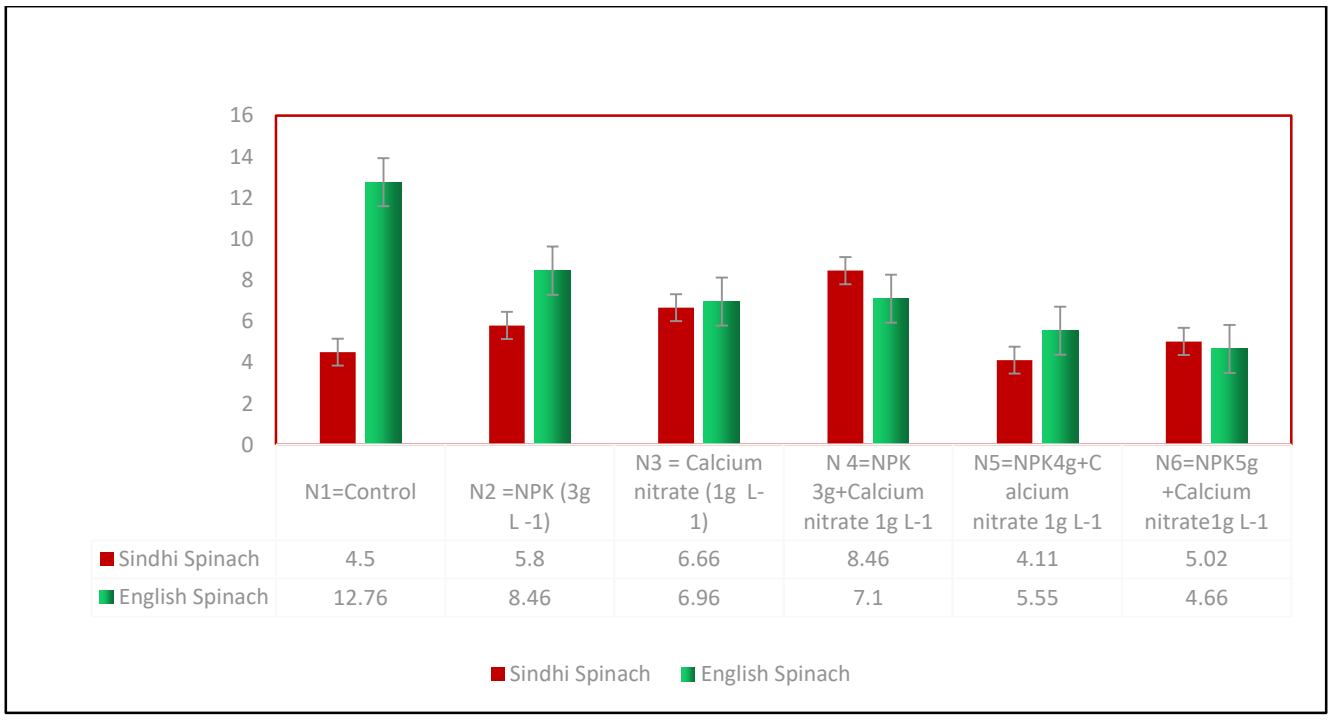

FIGURE 4: Leaf length (cm) of spinach varieties under nutrients combinations

3.5 Leaves plant^{-1}:

The Leaves plant^{-1} of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 5. The analysis suggested that Leaves plant^{-1} of spinach was significantly influenced by different levels of nutrients ($P<0.05$); and the effect of spinach varieties and interaction results were statistically significant ($P<0.05$). The control treatment (no fertilizer) yielded 4.33 leaves per plant. The highest increase in leaf number was with the combination of NPK (3g/L) and Calcium Nitrate (1g/L) (N4), where the number of leaves increased significantly to 10. This combination outperformed all other treatments, showing that Sindhi Spinach benefits most from a balanced nutrient mixture. Interestingly, the number of leaves decreased with increasing concentration of NPK, especially in NPK (5g/L) + Calcium Nitrate (1g/L) (N6). This suggests that higher concentrations of NPK may not be as beneficial for Sindhi Spinach. On the other hand, English Spinach had a higher leaf count in the control, which was significantly better than the other fertilized treatments at 8.33 leaves per plant. While the addition of NPK (3g/L) (N2) and NPK (3g/L) + Calcium Nitrate (1g/L) (N4) resulted in 7 leaves, they still did not surpass the control. As with Sindhi Spinach, increasing the concentration of NPK (N5 and N6) caused a decrease in the number of leaves, with the lowest being 4 leaves per plant for both treatments.

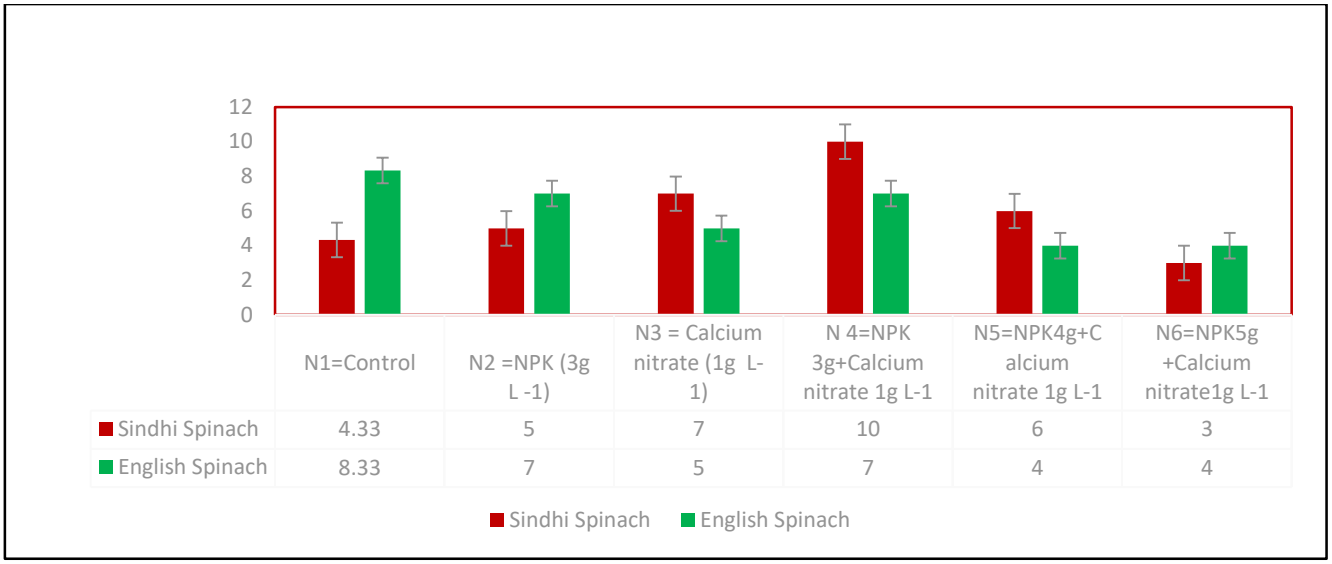

FIGURE 5: Leaves per plant of spinach varieties under nutrient combinations

3.6 Leaf weight (g):

The Leaf weight (g) of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 6. The analysis suggested that Leaf weight (g) of spinach was significantly influenced by different levels of nutrients ($P<0.05$); and the effect of spinach varieties and interaction results were statistically significant ($P<0.05$). The control treatment had a low leaf weight, with 6.31 g as compared with some of the fertilized treatments. The increase in leaf weight was most profound when NPK at 3g/L in combination with Calcium Nitrate at 1g/L was given as N4 with a value of 8.46g. This leaf weight was the highest compared to that of the control. This implies that Sindhi Spinach reacted to this nutrient mix best by achieving maximum increase in the leaf weight. However, increasing the NPK concentrations further at N5 and N6 results will decline the leaf weight, implying that at these higher NPK levels, there was no gain in the leaf growth. In contrast, with regard to English Spinach, a different trend will be presented. The control treatment produced a leaf weight of 13.95 g that was much greater than that produced by Sindhi Spinach. It means that the leaf weight in English Spinach is greater by nature compared to Sindhi Spinach without applying any nutrient supplement. Applying NPK at the rate of 3g/L or Calcium Nitrate at the rate of 1g/L (N2 and N3) reduced the leaf weight with the reduction up to 10.73 g and 9.28 g, respectively. The additive effect of NPK (3g/L) + Calcium Nitrate (1g/L) (N4) showed a slight increase to 8.79 g but was still not as high as the control. The increased concentration of NPK led the leaf weight to continue in decline, and the minimum value was recorded in the treatment NPK (5g/L) + Calcium Nitrate (1g/L) (N6), at a value of 6.66 g.

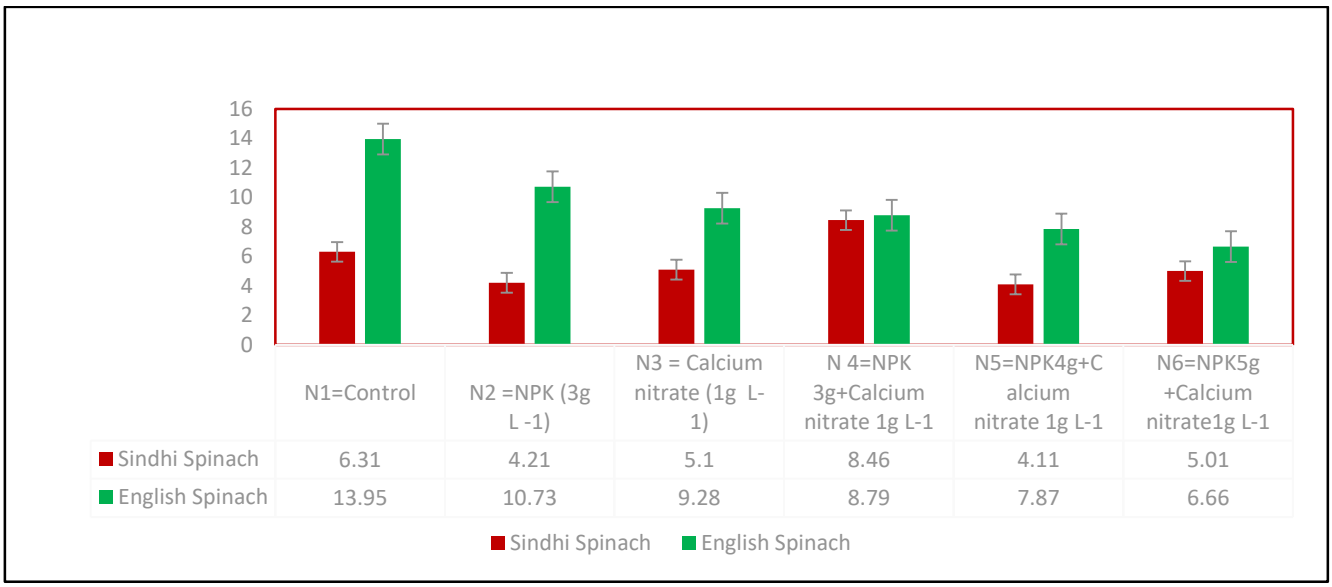

FIGURE 6: Leaf weight (g) of spinach varieties under nutrients combinations

3.7 Leaf width (cm):

The Leaf width (cm) of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 7. The effect of spinach varieties and interaction results were statistically significant ($P<0.05$). The control treatment without fertilizer resulted in a leaf width of 3.21 cm. The addition of Calcium Nitrate at 1g/L increased the leaf width to 3.8 cm with a positive response. The highest improvement was found with the addition of NPK at 3g/L along with Calcium Nitrate at 1g/L (N4), where the leaf width increased to 6.46 cm, which was a huge improvement over the control. This combination of nutrients proved to be the most effective in promoting leaf width in Sindhi Spinash. Interestingly, with an increase in the concentration of NPK (N5 and N6), the leaf width decreased, with the lowest being 2.3 cm for NPK (4g/L) + Calcium Nitrate (1g/L) (N5), suggesting that excessive NPK was harmful to the leaf width in Sindhi variety. In contrast, English Spinach had a larger leaf width in the control (5.6 cm) than Sindhi Spinach. However, the addition of NPK (3g/L) (N2) reduced the leaf width slightly to 5.06 cm, and Calcium Nitrate (1g/L) (N3) further decreased it to 4.36 cm. The smallest leaf width was 3.16 cm, significantly lower than the control, with the combination of NPK (3g/L) + Calcium Nitrate (1g/L) (N4). This nutrient combination was not favorable for English Spinach. With the increase in the concentration of NPK (N5 and N6), the leaf width decreased further, with the lowest being 2.16 cm for NPK (4g/L) + Calcium Nitrate (1g/L) (N5).

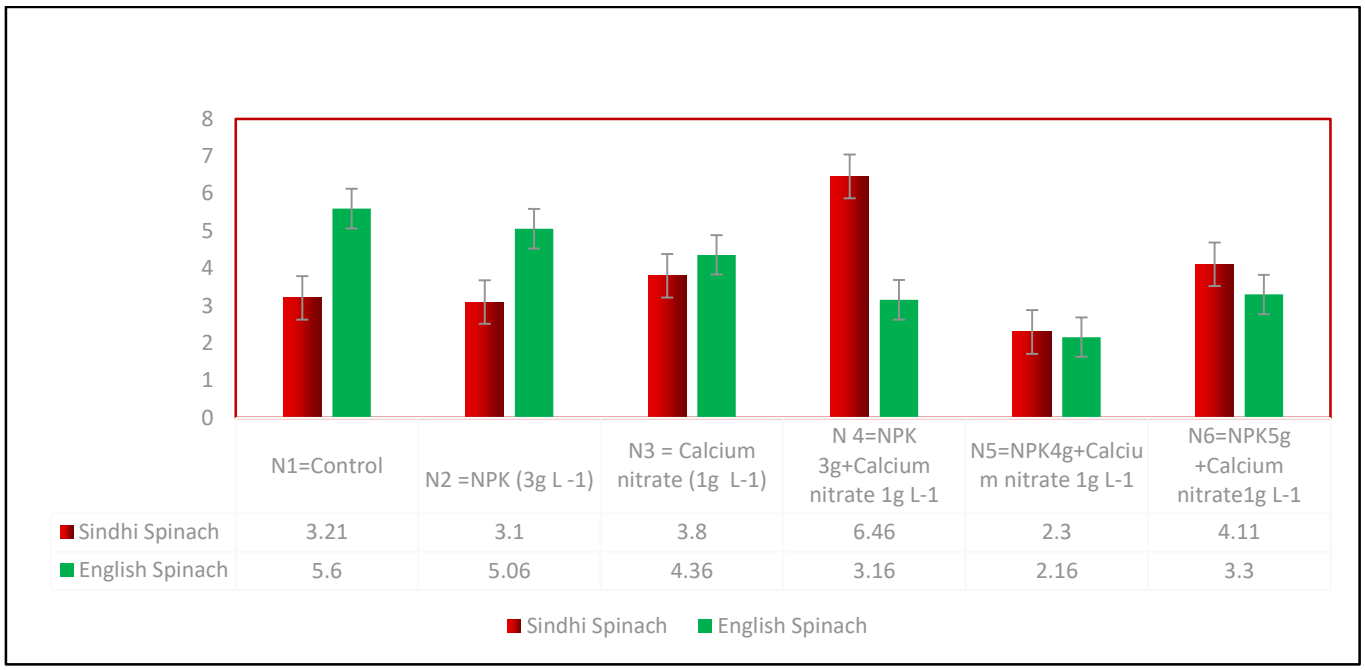

FIGURE 7: Leaf width (cm) of Spinach varieties under nutrients combinations

3.8 Fresh biomass of root (g):

The Fresh biomass of root (g) of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 8. The analysis of variance (ANOVA) suggested that Fresh biomass of root (g) of spinach was significantly influenced by different levels of nutrients ($P<0.05$); and the effect of spinach varieties and interaction results were statistically significant ($P<0.05$). The control (N1) treatment resulted in a root biomass of 0.38 g from Sindhi Spinach, which was relatively low. The addition of NPK (3g/L) (N2) caused a significant increase to 0.66 g, making it the best treatment for promoting root biomass in Sindhi Spinach. Even the combination of NPK (3g/L) + Calcium Nitrate (1g/L) (N4), which produced a root biomass of 0.64 g, performed well compared to the control. However, an increase in the concentration of NPK (N5 and N6) resulted in a reduction in root biomass at 0.43 g and 0.45 g, respectively, which indicated that higher doses of NPK were not beneficial for root growth in Sindhi Spinach. For English Spinach, the control produced the highest root biomass at 0.77 g, which was significantly higher than that of Sindhi Spinach in the control. Fertilization generally did not improve root biomass in English Spinach. For instance, NPK (3g/L) (N2) resulted in 0.73 g, a slight decrease, and Calcium Nitrate (1g/L) (N3) led to 0.54 g, further lowering the root biomass. The NPK (3g/L) + Calcium Nitrate (1g/L) (N4) produced 0.69 g, which is still lower than the control. Even at higher concentrations of NPK (N5 and N6), the root biomass was not higher than the control at 0.45 g and 0.63 g, respectively.

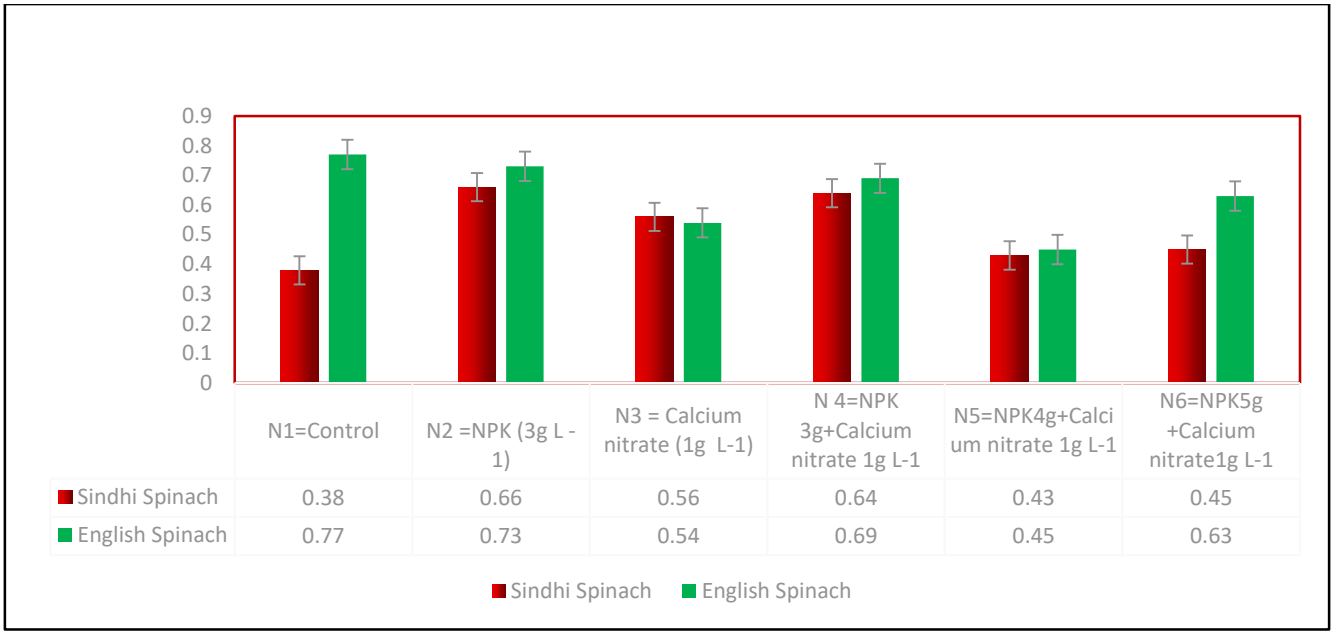

FIGURE 8: Fresh biomass of root(g) of spinach varieties under nutrients combinations

3.9 Root Depth (cm):

The Root Depth (cm) of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 9. The analysis suggested that Root Depth (cm) of spinach was significantly influenced by different levels of nutrients ($P<0.05$); and the effect of spinach varieties and interaction results were statistically significant ($P<0.05$). The control (N1) resulted in a root depth of 11 cm of Sindhi Spinach. The root depth increased slightly with the addition of NPK (3g/L) (N2) to 11.74 cm and further with Calcium Nitrate (1g/L) (N3) to 13.06 cm. However, the highest increase in root depth was with the combination of NPK (3g/L) + Calcium Nitrate (1g/L) (N4), which showed a very significant increase in root depth to 20.46 cm, much deeper than in the control. Higher concentrations of NPK at N5 and N6 recorded slight decreases of root depth: 11.02 cm, and 14.46 cm, respectively, which may indicate a limitation of using excessive NPK for root deepening in Sindhi Spinach. On the other hand, English Spinach had its deeper root compared to Sindhi Spinach that was recorded from the control with a root depth of 17.43 cm. However, with the application of NPK (3g/L) (N2), the root depth was reduced to 13 cm. The addition of Calcium Nitrate (1g/L) (N3) resulted in a slight increase to 13.36 cm. The combination of NPK (3g/L) + Calcium Nitrate (1g/L) (N4) led to a modest reduction in root depth to 16.1 cm, which was still greater than the control. With increasing NPK concentration, the depth of the root was further decreased with 15.1 cm and 14.46 cm, respectively at N5 and N6.

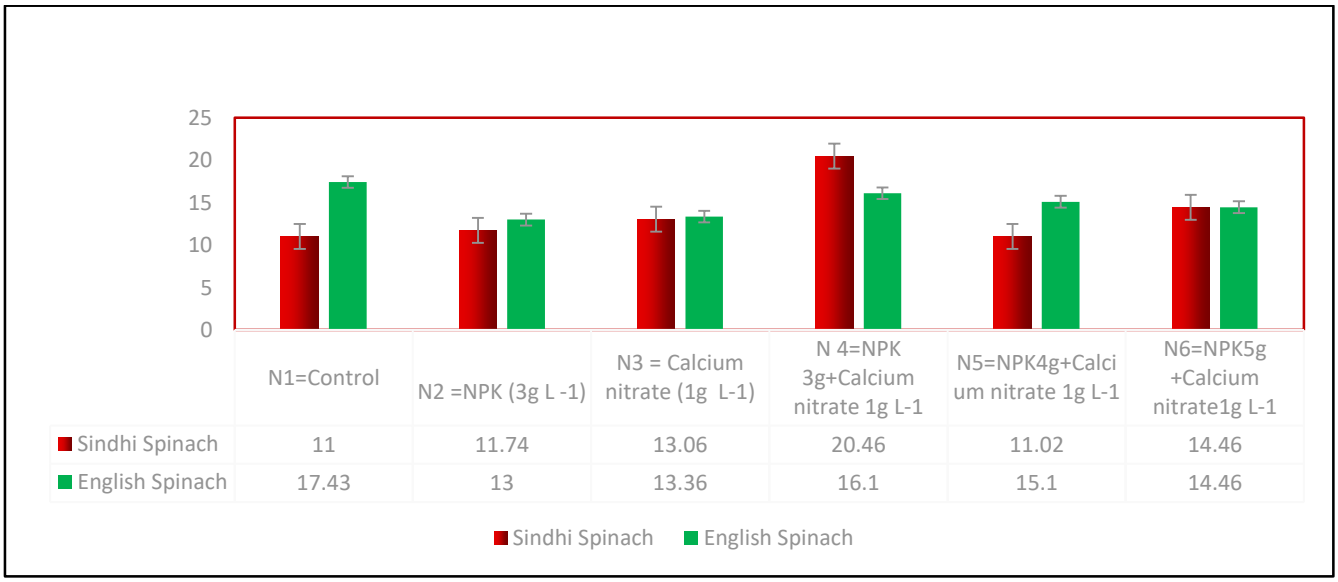

FIGURE 9: Root Depth (cm) of spinach varieties under nutrient combinations

3.10 Chlorophyll content (SPAD):

The Chlorophyll content (SPAD) of spinach varieties was calculated under the impact of different levels of nutrients and the findings are given in Figure 10. The effect of spinach varieties and interaction results were statistically significant ($P<0.05$).

The control treatment was 28.1 from the Sindhi Spinach, very low. NPK at 3g/L had a little better result, and that was about 34.06. The treatment with Calcium Nitrate at 1g/L had the most dramatic improvement, as the chlorophyll level reached 64.86, far beyond the control and other treatments. This indicates that in using Calcium Nitrate, the plants have been more amplified for chlorophyll enhancement in Sindhi Spinach. While the combination of NPK (3g/L) + Calcium Nitrate (1g/L) increased to 35.8, it was lower than the boost seen with Calcium Nitrate alone. Other treatments like NPK (4g/L) + Calcium Nitrate (1g/L) and NPK (5g/L) + Calcium Nitrate (1g/L) also increased the chlorophyll content to 57.43 and 42.63, respectively, but these were still lower than the Calcium Nitrate treatment alone. The control treatment for English Spinach showed a chlorophyll content of 27.43, slightly lower than the control of Sindhi Spinach. The NPK (3g/L) treatment caused an abrupt increase to 54.41, and thus NPK alone was found to be very effective for raising the chlorophyll content of English Spinach. However, when Calcium Nitrate (1g/L) was applied, the chlorophyll content decreased to 33.36, which indicates that Calcium Nitrate was not as beneficial for English Spinach as it was for Sindhi Spinach. The combination of NPK (3g/L) + Calcium Nitrate (1g/L) led to a chlorophyll content of 46.1, which was an improvement over the control but still not as high as the response to NPK (3g/L) alone. The other treatments of higher NPK concentrations produced the following; namely, N5 and N6, gave results of 35.1 and 44.46 respectively lower than NPK (3g/L) treatment.

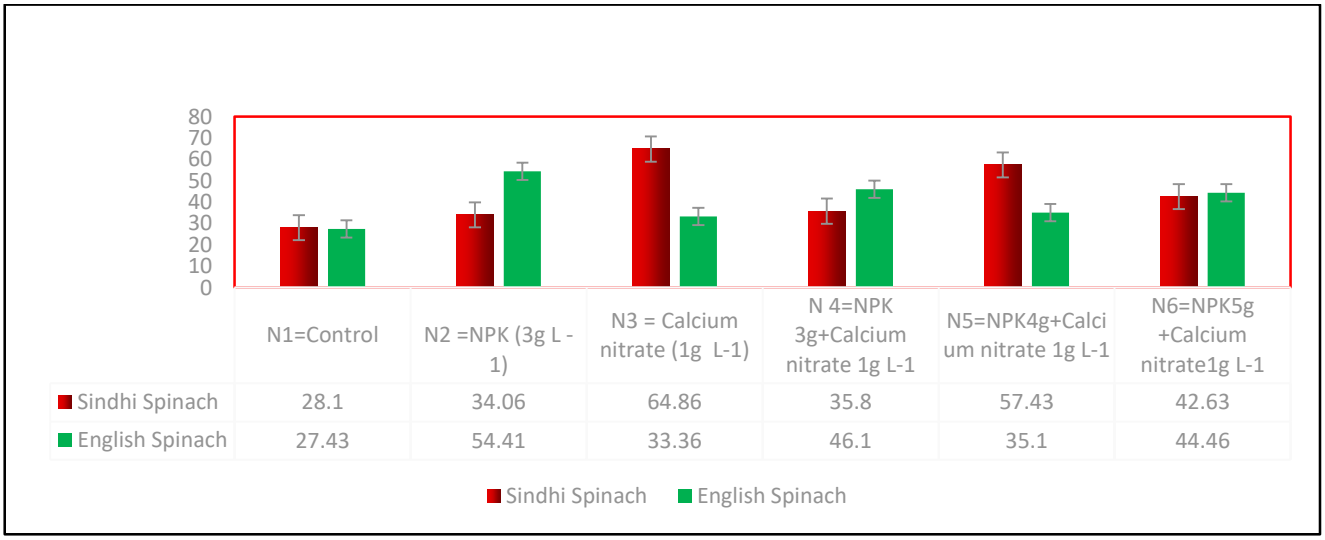

FIGURE 10: Chlorophyll content (SPAD) of spinach varieties under nutrient combinations

IV. DISCUSSION

The macro nutrients have been used to enhance of vegetables and fruiting behaving of horticultural crops. These macro and micro nutrients have triggered various physiological characteristics and plants. The leaves of green factories where photosynthesis produces compounds needed for growth. These are absorbed right at the site they are used acting fast. The study established that crop yield in spinach is enhanced when macro nutrients were applied combined rather than used separately. NPK and calcium nitrate will provide maximum net return to growers (Ali at al., 2024). In this present study the effect of nutrient on production of spinach was tried by using the commercial product named as NPK which was a powder containing diverse essentially needed macro nutrients. NPK was applied at the concentration of NPK 3g L^{-1} and calcium nitrate 1g L^{1} and control was maintained to check the plants performance. Maximum result in spinach were obtained under NPK 3g L^{1} + calcium nitrate 1g L^{1}. It produces most of the observed parameters in a maximum value as compared to other treatment. A significant amount of research work has been reported from different parts of the world on the aspect under the study. The result of the present investigation are in concurrence with the result of (Abgad at al., 2015) used a compound powder form fertilizer containing most macro nutrients along with NPK and calcium nitrate these fertilizers provide nutrients to the plant these fertilizers are completely soluble in water. Further, our results are in accordance with the finding of (Robinson, D. (1994) which found maximum plant height (14.78 cm), leaves plant^{1} (7.50), leaf length (7.63) determined the effect of fertilizer of NPK (20-20-20+TE) and calcium nutrient and concluded that plant yield and total uptake of nutrient by plant varied significantly with respect to plant population and nutrient levels.

A comparative analysis of the findings of the present study and findings of past workers indicated that there is great scope for improving the yield of spinach through NPK and calcium nitrate of macro nutrients.

V. CONCLUSION

The Present study showed the significant influence of NPK and calcium nitrate on spinach growth. NPK (3g/L) and calcium nitrate (1g/L) positively impacted seed germination, plant height, leaf metrics, root characteristics, and chlorophyll content, especially for Sindhi spinach. Higher NPK concentrations negatively affected germination and growth in both varieties, highlighting the importance of balanced nutrient application for optimal spinach development. These findings offer valuable insights for maximizing crop productivity and emphasize the significance of macro nutrients in enhancing spinach yield.

ACKNOWLEDGEMENTS

An acknowledgement section may be presented after the conclusion, if desired.

AUTHORS' CONTRIBUTIONS

MRM and MAW: Conceived and designed the study, and prepared the initial draft of the manuscript. MRM, MAW, MAJ: Carried out the experimental work and collected the data. GHW: Provided technical guidance, assisted in data analysis, and contributed significantly to manuscript refinement. LQ: Offered continuous technical support throughout the research process. IAJ and FHC: Assisted in data organization, tabulation, and preliminary compilation. FAJ and FHC: Contributed to statistical analysis and graphical data representation. KHC and KAM: Provided input on experimental layout and contributed to manuscript writing. AQK and ARS: Critically reviewed and revised the manuscript for intellectual content.

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