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*Research Article*

# Effect of Using Two Nutrient Solutions with Different NPK Ratios on Physical Characteristics and Concentration of Bioactive Compounds in Peas (*Pisum sativum*) Cultivated Using Hydroponics Technology

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**Abstract**

*Pisum sativum*, or garden peas, were grown hydroponically in this study utilising two distinct media solutions with varying NPK (nitrogen, phosphorus, and potassium) ratios (2:1:3 and 6:1:2). The goal of the study was to identify the media solution that would provide the plants with superior physical traits, such as more leaves and branches, as well as higher levels of phytochemicals like flavonoids and phenolic acids. The study's findings demonstrated that the NPK ratio of the media solutions did significantly affect the *Pisum sativum* plants' growth and phytochemical content. These substances contain anti-inflammatory and antioxidant capabilities, among other health advantages. This study emphasises the significance of the NPK ratio and its effects on plant development and phytochemical content in hydroponic *Pisum sativum* growth. The findings imply that *Pisum sativum* may develop and produce phytochemicals more favourably under stressful conditions such as lack of nutrients etc.

**Keywords:** *Pisum sativum*, Hydroponics, Phytochemicals, NPK ratios, Nutrient media solution

## INTRODUCTION

The pea, or *Pisum sativum*, is a well-liked crop that is farmed all over the world. It is a great source of protein, vitamins, and minerals as well as dietary fibre. Peas have typically been cultivated using soil-based farming techniques; however, new developments in hydroponic gardening have shown encouraging results for this crop. With hydroponics, plants are grown without the use of soil and are fed with nutrient-rich water. In comparison to conventional soil-based agriculture, this method provides several benefits, including increased yield, less water use, and better control over nutrient uptake. (Macwan J et al., 2016)

From the media we used for seed germination, Coco peat is a natural anti-fungal, making it an excellent choice to start seedlings. Still, it is even used in rugs, ropes, brushes, and even as stuffing. Coco peat will improve water retention and even the porosity of the growing medium and coco peat makes the soil crumbly and very light, enhances productivity, and even reduces root diseases (Patil ST et al., 2020).

The composition of the nutrient solution, which is one of the most important aspects of hydroponic farming and is crucial to the growth and development of plants, is one of the most important components. Plants need high concentrations of the three macronutrients nitrogen (N), phosphorus (P), and potassium (K) to grow and develop. Each nutrient is essential for various stages of plant development and growth (Smith GS et al., 1983) **(Table 1).**

An incorrect NPK ratio may negatively impact plant growth and development. For instance, too much nitrogen might cause plants to develop too much vegetative at the price of producing fruit and flowers. A phosphorus surplus can cause nutritional imbalances, which can be harmful to the growth and development of plants. Potassium overuse can result in salt build-up, which can harm the roots of the plant and

**Table 1.** Importance of NPK.

| | |
|---|---|
| Nitrogen | The creation of chlorophyll, which gives plants their distinctive green colour and is required for photosynthesis, depends on nitrogen. It is crucial for the growth of leaves and stems and plays a significant part in protein synthesis (Olfati JA, 2015). |
| Phosphorus | The fundamental energy source for plants, ATP (adenosine triphosphate), can only be produced with the help of phosphorus. Additionally, it contributes to the formation of root systems, flowering, and fruiting (Olfati JA, 2015). |
| Potassium | Potassium plays a crucial role in the growth of robust stems and roots and in controlling the water balance in plants. It is essential for the plant's defence against disease and pests (Olfati JA, 2015). |

limit nutrient intake (Olfati JA 2015).

In this work, we looked at how two different nutrient solutions, by making the NPK ratios of the solution different i.e. 2:1:3 (Media 1, M1) and 6:1:2 (Media 2, M2) affected the development and phytochemical makeup of *Pisum sativum* grown hydroponically. The concentration of phytochemicals like flavonoids and phenolic content etc, as well as the physical traits of the plant like leaf and branch growth, were predicted to be significantly influenced by the composition of the nutrient solution. We compared the outcomes of the two treatments to identify which nutrient solution is more suitable for *Pisum sativum*'s hydroponic cultivation in terms of growth and phytochemical content.

### Phytochemicals

Plants contain natural bioactive substances called phytochemicals that are beneficial to human health in a number of ways. They are crucial for overall health and illness prevention even though they are not necessary nutrients. Phytochemicals give plants their vivid hues, flavours, and fragrances. They also play a crucial function in defending plants against environmental stressors including UV radiation, pests, and illnesses.

*Pisum sativum*, or garden peas, contains a wide range of phytochemicals that have various health benefits for humans. Some of the phytochemicals found in peas include flavonoids, phenolic acids, carotenoids, and saponins.

Flavonoids are a class of phytochemicals with anti-inflammatory and antioxidant effects. Quercetin, kaempferol, and catechins are just a few of the flavonoids found in peas. While kaempferol has been demonstrated to lower the risk of heart disease, quercetin has been shown to have anti-cancer and anti-inflammatory properties.

Peas' vibrant green colour is caused by carotenoids. Beta-carotene, lutein, and zeaxanthin are just a few of the carotenoids that can be found in peas. Antioxidant capabilities of these substances have been demonstrated to lower the risk of several chronic diseases, including cancer and eye conditions. (Cosson A et al., 2022).

Another group of phytochemicals present in peas is the phenolic acids (Cosson A et al., 2022). Several different phenolic acids, such as caffeic acid, ferulic acid, and p-coumaric acid, are found in peas. These substances have been demonstrated to have anti-inflammatory and antioxidant capabilities, as well as preventive benefits against cancer and heart disease.

## MATERIAL AND METHODS

### Germination of the seeds

A suitable container was taken to germinate the seeds of *Pisum sativum* which was filled with moist cocopeat seeds were spread with suitable space between them and covered again with cocopeat and were provided with water regularly. After one or two days seeds started germinating and after five days of regularly watering the seeds once daily small plants having five to six branches were seen which was the perfect time for transplanting the plants to an automated nutrient system.

### Preparation of the two different nutrient solutions

Chemicals that we selected to make the nutrient solution such that all the macro, as well as micronutrients, are provided to the plants for their perfect growth were, Ammonium Nitrate, Calcium Nitrate, Potassium dihydrogen phosphate, Potassium Sulphate, Magnesium Sulphate, EDTA, Copper Sulphate, Zinc Sulphate, Manganese Sulphate, Sodium molybdate dihydrate (Baruah N et al., 2019). Out of these chemicals, the ones that are present in Table 2 were taken in the same quantity for the nutrient solutions. These chemicals were then mixed with 1 litre of water and this solution was given to the plants in the nutrient system **(Table 2).**

After mixing these chemicals in water, Ammonium Nitrate, Calcium Nitrate, Potassium dihydrogen phosphate, Potassium Sulphate were also provided in two different quantities for different NPK ratios to make different nutrient solutions. Table 3 and Table 4 were followed to make nutrient solutions M1 and M2 respectively **(Tables 3 and 4).**

The pH of the nutrient solutions prepared was kept adjusted to be between 5.5 to 6.5 and TDS was kept between 980 to 1260 ppm.

### Hydroponic cultivation of plants

Designed and built an automated hydroponic system, NFT (Nutrient Film technique) that is appropriate for the type and size of peas having two inlets and two outlets for the two different nutrient media solutions. The system included two reservoirs for nutrient solution, pumps to circulate the solution, and containers to hold the plants. Then the containers were filled with growing medium solutions and plantlets were planted.

**Table 2.** Type of chemicals used in nutrient system of plants.

| Chemical | Quantity |
|---|---|
| $MgSO_4$ | 1.89 g |
| EDTA | 0.08 g |
| $MnSO_4$ | 0.032 g |
| $CuSO_4$ | 0.0064 g |
| $ZnSO_4$ | 0.08 g |
| $Na_2MoO_4$ | 0.0064 g |

**Table 3.** Media 1 (M1), NPK = 2:1:3.

| Chemical | Quantity |
|---|---|
| $NH_4NO_3$ | 1.2 g |
| $Ca(NO_3)_2$ | 3.7 g |
| $KH_2PO_4$ | 1.89 g |
| $K_2SO_4$ | 1.32 g |

**Table 4.** Media 2 (M2), NPK = 6:1:2.

| Chemical | Quantity |
|---|---|
| $NH_4NO_3$ | 1.65g |
| $Ca(NO_3)_2$ | 16.87 g |
| $KH_2PO_4$ | 1.89 g |
| $K_2SO_4$ | 0.75 g |

Data was collected on the growth and development of the plants for 30 days.

## Analysis of physical characteristics

For analysis of the physical characteristics of the two types of plants, two parameters were taken into account, the average number of leaves and the average number of branches.

The branches and leaves were counted routinely for 30 days and graphs were plotted for the average number of leaves vs days and the average number of branches vs days for the nutrient media solutions and the results were compared.

## Extract preparation

After three to four weeks, all the aerial parts of the two types of plants were collected and dried in an oven for around twelve hours at 60 °C (Kawatra N et al., 2023). A mechanical grinder was used to grind the dried plant, and 100 mg of the powdered sample of each plant type was separated out and mixed with 0.1% acetic acid and 70% methanol solutions. An ultrasonic water bath was then used for the sonication of the two sample solutions for 10 minutes (Zhen J et al., 2016) and was later placed in an incubator shaker overnight at 37 °C. The samples were then centrifuged at 5000 rpm for 10 minutes and the supernatant was separated out which was used later used in the experimental analysis as plant extracts.

## Analysis for phytochemical compounds

**Total flavonoid concentration:** The aluminium chloride colourimetric method (Derakhsan Z et al., 2018) was used to identify the content of flavonoids present in the peas extract. For the estimation, the standard curve of rutin was plotted and the results were reported in mg Rutin Equivalent (RE). To prepare the standard curve, dilutions of rutin of 0.5, 1.5, 2.5, and 4 mg/ml in 80% methanol were prepared in test tubes (Jain KL et al., 2017). The curve was plotted by recording the absorbance at 415 nm.

0.5 ml of each plant extract was taken in 3 test tubes. In each test tube, 2% 0.5 ml aluminium chloride was mixed and then the solutions were kept at room temperature for about 40 minutes. The absorbance of mixtures in each test tube was recorded at 415 nm and was compared.

**Total flavonol concentration:** The above method of aluminium chloride colourimetric was used again for the preparation of the samples for the determination of flavanols content (Derakhsan Z et al., 2018), the only difference is that the absorbance was recorded at 440 nm and that too after keeping the sample mixtures for 150 min at room temperature.

**Total sugar concentration:** According to the method described by Singh (2016), for estimating the total concentration of sugar present in the plant extract 1 ml of both the extracts was taken in three different test tubes and 1 ml of 5% phenol was mixed into it. 5 ml of conc. sulphuric acid was mixed while constantly stirring and allowed to stand for 30 minutes at room temperature. Absorbance has noted at 490 nm as the colour of the reaction mixture changed to slight orange.

For the standard curve of glucose, first, we prepared the dilutions of glucose of 0.5, 1.5, 2.5, and 4 mg/ml in test tubes and mixed them with 1 ml of DNS (3, 5-Dinitrosalicylic Acid). Then the tubes were heated in a water bath for at least 5 minutes and after some time absorbance was noted at 490 nm and the curve was plotted that was used to estimate the amount of sugar that is present in the plant extract samples **(**Zhang P et al., 2019).

**Total phenolic concentration:** Follin-Ciocalteu reagent was used to determine the total phenolic content present in the plant extracts (Derakhsan Z et al., 2018). Gallic acid was used to prepare the standard curve to determine the concentration. First, we prepared the dilutions of gallic acid of 0.5, 1.5, 2.5, and 4 mg/ml in methanol in test tubes. Then, 500 µl of water and 100 µl of Follin-Ciocalteu reagent were added and after a few minutes 7% of 1 ml sodium carbonate were added and absorbance was recorded at 765 nm.

Around 100 µl of the plant of both the extract samples were taken in three test tubes each and then approximately 500 µl of Follin-Ciocalteu reagent was mixed in the test tubes. 400 µl of 7.5% sodium carbonate solution was added, and the tubes were allowed to stand for 30 min at room temperature. The Absorbance of the sample was measured using a UV-spectrophotometer at 765 nm and the results were reported in mg Gallic Acid Equivalent (GAE) based on the gallic acid standard calibration curve.

**Total protein concentration:** The method described by Bradford, 1976, was used to determine the concentration of protein in the plant extract samples. 1 mg Coomassie Brilliant Blue G-250 was dissolved in 0.5 ml 95% ethanol. After that 1 ml 85% (w/v) phosphoric acid was also mixed. Dilute to 10 ml when the dye has completely dissolved, and filter before use. For the preparation of the standard curve, the dilutions of BSA (Bovine Serum Albumin) of concentrations 0.5, 1.5, 2.5, and 4 mg/ml were prepared by using distilled water. Also, 5-10 µl of the samples were also taken in separate test tubes. After that 1 ml of the reagent that was prepared earlier was also mixed in the test tubes. And then they were incubated for 10 to 15 minutes. The absorbance of all the tubes was recorded at 595 nm. A standard curve was built for BSA which was further utilised to determine the concentration of the protein in the plant extract samples.

# RESULT AND DISCUSSION

## Physical characteristics

The results of the study indicated that M1 resulted in better physical characteristics compared to M2. The *Pisum sativum* plants grown in M1 had a significantly higher number of leaves and branches than those grown in M2 **(Figures 1 and 2**).

These results suggest that the NPK ratio of M1 is better suited for the hydroponic growth of *Pisum sativum* compared to M2.

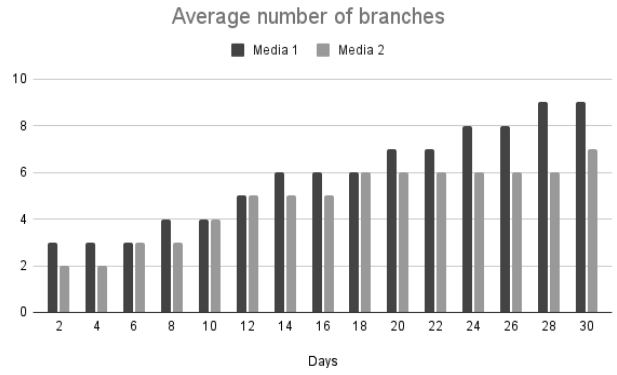


**Figure 1.** Average number of branches vs days.

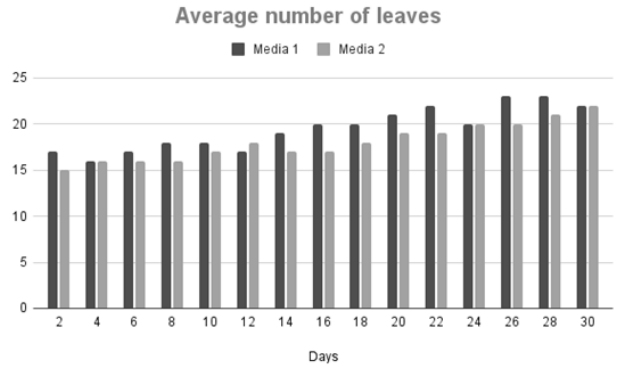


**Figure 2.** Average number of leaves vs days.

## Bioactive Compound Concentrations

**Total flavonoid concentration:** Rutin was used as the standard, and it was dissolved in methanol before being serially diluted to create a standard curve **(Table 5).** After plotting the standard curve, a straight line with a slope of 0.176 and a y-intercept of 0.284 was produced. The line's equation is y = 0.176x+0.284 **(Figure 3).** The quantity of flavonoids in these plants was calculated using three separate runs.

Absorbance recorded for plant extract was put in the equation and the concentration was calculated. For M1 average flavonoid concentration was 0.587 mg RE/g and for M2, 1.771 mg RE/g.

**Total flavanol concentration:** Rutin served as the reference material for flavonols, and a standard curve was produced by dissolving it in methanol and serially dilution **(Table 6**). The standard curve was plotted, resulting in a straight line with a 0.191 slope and a 0.199 y-intercept. y = 0.191x+0.199 **(Figure 4)** is the equation for the line. Three different runs were used to determine how many flavonols each plant extract contained.

Absorbance recorded for plant extract was put in the equation and the concentration was calculated. For M1

**Table 5.** Rutin standard curve readings for flavonoids.

| Concentration (mg/ml) | Absorbance |
|---|---|
| 0.1 | 0.258 |
| 0.5 | 0.355 |
| 1.5 | 0.588 |
| 2.5 | 0.813 |
| 4 | 0.922 |

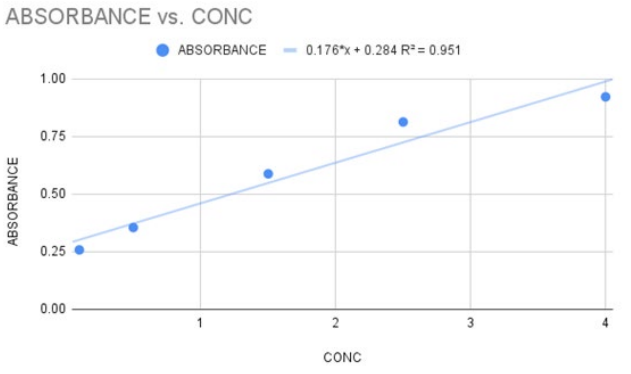


**Figure 3.** Rutin standard curve for flavonoids.

**Table 6.** Rutin standard curve readings for flavanols.

| Concentration (mg/ml) | Absorbance |
|---|---|
| 0.1 | 0.201 |
| 0.5 | 0.277 |
| 1.5 | 0.571 |
| 2.5 | 0.623 |
| 4 | 0.968 |

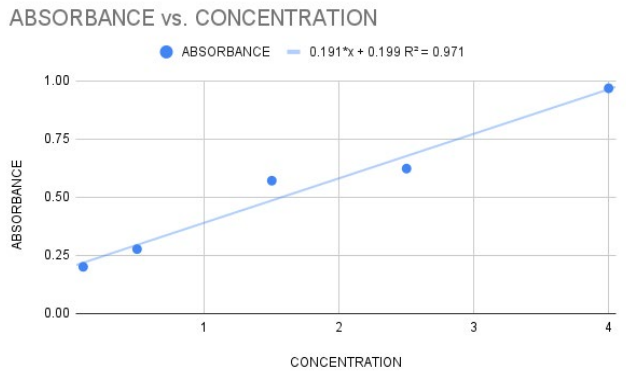


**Figure 4.** Rutin standard curve for flavonols.

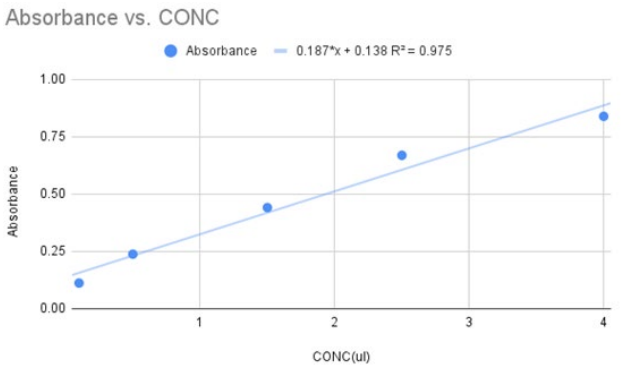


**Figure 5.** Glucose standard curve.

**Table 7.** Glucose standard curve readings.

| Concentration (mg/ml) | Absorbance |
|---|---|
| 0.1 | 0.112 |
| 0.5 | 0.238 |
| 1.5 | 0.441 |
| 2.5 | 0.669 |
| 4 | 0.839 |

**Table 8.** Gallic acid standard curve readings.

| Concentration (mg/ml) | Absorbance |
|---|---|
| 0.1 | 0.11 |
| 0.5 | 0.283 |
| 1.5 | 0.421 |
| 2.5 | 0.59 |
| 4 | 0.881 |

average flavonol concentration was 0.905 mg RE/g and for M2, 2.192 mg RE/g.

**Total sugar concentration:** A standard curve was created by serial dilution using glucose as the reference material for sugar **(Table 7).** When the standard curve was plotted, the line had a slope of 0.187 and a y-intercept of 0.138. The equation for the line is y = 0.187x+0.138 **(Figure 5).** The sugar concentration in each plant extract was counted using three separate runs.

Absorbance recorded for plant extract was put in the equation and sugar concentration was calculated. For M1 average sugar concentration was 3.787 mg/g and for M2, 2.384 mg/g.

**Total phenolic concentration:** By serial dilution and utilizing gallic acid as the reference material for phenolic content, a standard curve was produced **(Table 8).** The line's slope and y-intercept were 0.186 and 0.137, respectively, when the standard curve was shown. Y = 0.186x+0.137 **(Figure 6)** is the equation for the line. Three different runs were used to tally the total phenolic content of each plant extract.

Absorbance recorded for plant extract was put in the equation and total phenolic content was calculated. For M1 average total phenolic content was 1.632 mg GAE/g and for M2, 2.543 mg GAE/g.

**Total protein concentration:** By serial dilution and utilizing bovine serum albumin (BSA) as the reference material for protein concentration, a standard curve was produced **(Table 9).** The line's slope and y-intercept were 0.117 and 0.424, respectively, when the standard curve was shown. Y = 0.117x+0.424 is the equation for the line. Three different

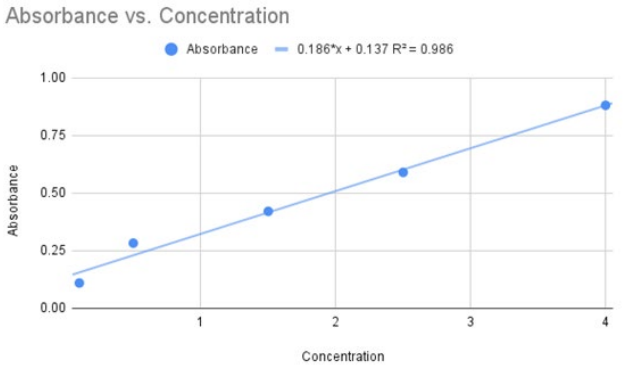


**Figure 6.** Gallic acid standard curve.

**Table 9.** BSA standard curve readings.

| Concentration (mg/ml) | Absorbance |
|---|---|
| 0.1 | 0.331 |
| 0.5 | 0.587 |
| 1.5 | 0.606 |
| 2.5 | 0.734 |
| 4 | 0.866 |

runs were used to tally the protein concentration of each plant extract.

Absorbance recorded for plant extract was put in the equation and total phenolic content was calculated. For M1 average total phenolic content was 2.157 mg/ml and for M2, 3.487 mg/ml.

# CONCLUSION

The increased number of leaves and branches in plants

grown in M1 could be attributed to the higher concentration of essential nutrients in this media solution. Plants feel stress if proper conditions are not present and in that condition, it tends to produce some secondary metabolites or it starts flowering at an early stage which may be the condition that the plant with M2 has higher concentrations of phytochemicals (phenols, flavonoids, flavanols) and the plant with M1, have a higher concentration of sugar and proteins. Further analysis could be conducted to determine the specific NPK ratio that is optimal for the growth of *Pisum sativum* in a hydroponic system.

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