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Research Article

Optimization of Different Concentrations of Orthophosphate Required for Improved Growth of *Avena sativa* L.

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ABSTRACT

Orthophosphate is a naturally occurring inorganic compound found in rocks, minerals, and soil, and is also synthesized industrially for use in fertilizers, detergents, and other applications. The current study underscores two major aspects, firstly to evaluate the individual and combined effects of orthophosphate and *Parthenium* plant extract on growth and development of *Avena sativa* L. Secondly, providing scientific basis for the use of *Parthenium* extract as a sustainable fertilizer for oat plants. This study employed pre-seed sowing treatment approach to assess the effects of different orthophosphate concentrations on *Avena sativa.* Results demonstrated that moderate concentrations (2-4%) of orthophosphate significantly enhanced growth parameters, including shoot length, leaf length, root length, number of leaves and fresh weight. In contrast, lower concentration (1%) was more effective in improving chlorophyll content. The combined application of orthophosphate, sodium phosphate and *Parthenium* extract further promoted plant growth, particularly in terms of shoot development. Additionally, orthophosphate application was found to stimulate the synthesis of secondary metabolites such as alkaloids and terpenoids. Overall, the finding indicates that while higher concentrations of orthophosphate may negatively affect plant growth, its application at optimal levels (2-3%) enhances growth and physiological performance of *Avena sativa*, especially when integrated with *Parthenium* extract.

Keywords:
Orthophosphate, *Avena sativa*, Alkaloids, Terpenoids, Oat plants

1. INTRODUCTION

Phosphorus is one of the seventeen vital elements that is essential for the growth and development of plants. When it comes to restricting crop growth, it is the second most significant macronutrient after nitrogen, and it is the least accessible macronutrient. When applied as fertilizers, phosphorus is primarily adsorbed by the soil and is not available to plants that lack specific adaptations, that is why it is low in availability. Plants use inorganic orthophosphate (Pi), an insoluble form of phosphorus that diffuses slowly in soils (Balemi and Negisho, 2012). Inorganic phosphate (orthophosphate; Pi) is not readily available in the soil, which severely limits crop productivity (Wang *et al.*, 2017). Phosphorus is essential for controlling physiological reactions and improving abiotic stress tolerance in plants,

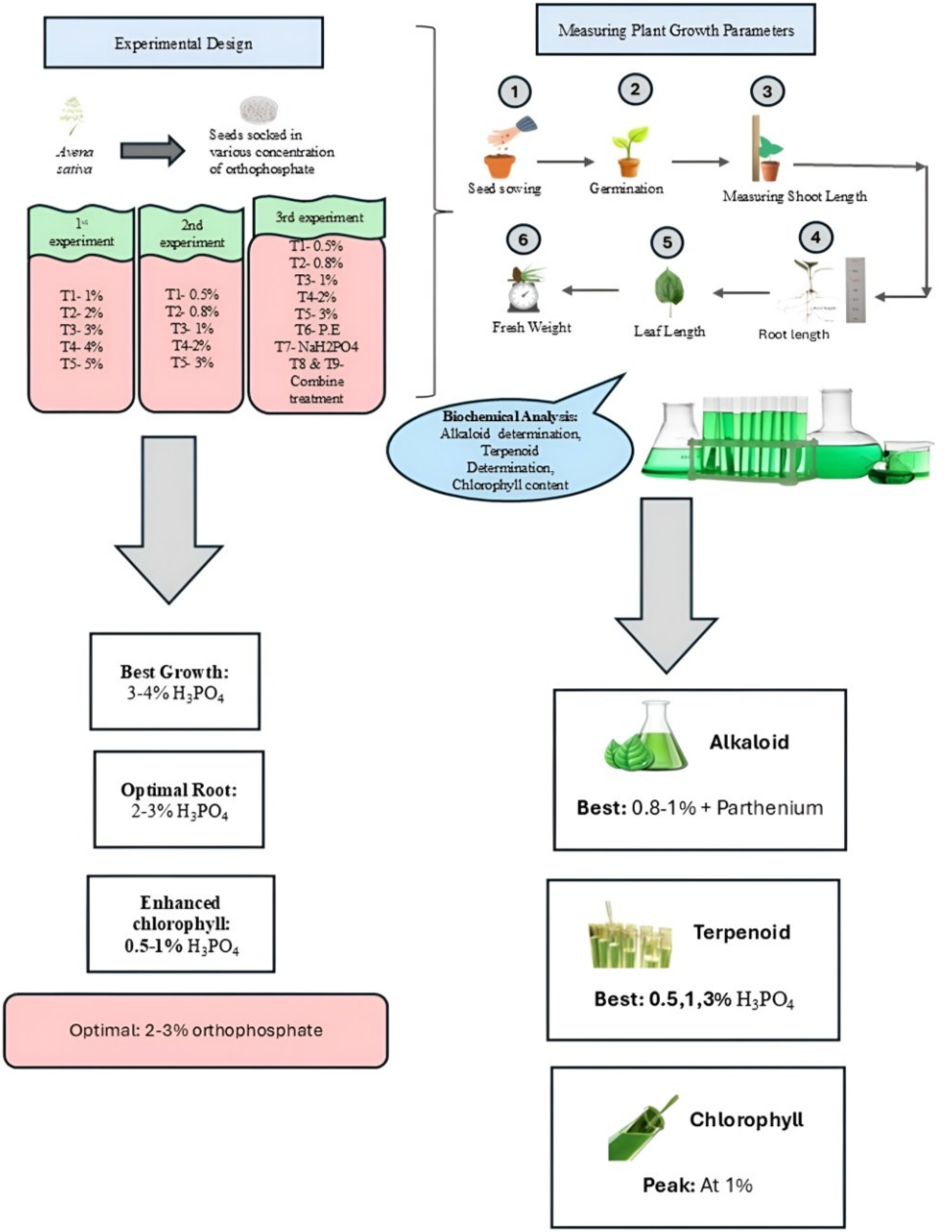

Figure 1. Overview of current study highlighting the effect of various concentrations of orthophosphate on *Avena sativa*

including high CO_2 levels, heat, salinity, drought, and waterlogging. To improve agricultural output and reduce stress tolerance, it is essential to comprehend the processes by which plants detect and react to phosphorus availability (Khan *et al.*, 2023; Guo *et al.*, 2024). Phosphorus is necessary for plant growth since it is involved in respiration, energy metabolisms, cellular transfer processes, and crop photosynthesis. Plants that do not receive enough Phosphorus, their root systems do not grow to a satisfactory length, and the plants eventually become dwarfed. The orthophosphate ions H_2PO_4- and HPO_4^{2-}, which are the only forms of phosphorus that crops can absorb, are found in soil solutions (Mardamootoo *et al.*, 2021). According to recent findings, root tips can detect variations in Pi availability in ssssthe soil locally, and they can regulate root growth by regulating cell division and expansion and gene products are most likely essential elements of root Pi sensing. According to a study, orthophosphate concentrations are typically relatively low in agricultural soil. To meet the nutritional needs of plants in P-deficient environments, P may be taken from other P pools (Ibrahim *et al.*, 2022).

Avena sativa L. or Oats (Poaceae Family), are common cereal grains. It is grown in Europe, Russia, Sweden, Finland, and Alaska, and it is the third most important crop in the United States. It is an annual grass that reaches a maximum height of 1.5 meters. Its leaves are green, blunt, with membrane ligules and non-articulate (Kumari *et al.*, 2023; Li *et al.*, 2024). The ideal growing temperature range for oats is between 68 and 70°F. They do best in chilly, damp conditions. Canada is the biggest producer of oats in North America, with 5.2 million tons produced in 2022. Of all the countries that export oats, Canada is the biggest exporter, making up 60% of the total, most of which are sent to the United States (Ghimire *et al.*, 2024). It is rich in lipids, minerals, protein, β-glucan, flavonoids, indole alkaloids, avenanthramides, and triterpenoid saponins. The β-glucan, a significant phytocomponent, has a high concentration of non-starch polysaccharides that lower blood cholesterol and have protective reactions against the nerve necrosis virus (NNV), which is being studied for possible use in boosting vaccination (Kumari *et al.*, 2023). Oats are considered a high-value crop because they have balanced proportions of key amino acids like lysine, high levels of proteins and fats, and 2-6% of β-glucan (Kim *et al.*, 2021; Alemayehu *et al.*, 2023).

Parthenium hysterophorus belonging to family Asteraceae is indigenous to the American tropics. in India, it is commonly known as carrot weed, congress grass and it is also known as "gajar ghas" because of its resemblance to a carrot plant. To maximize crop production, Parthenium can be used at minimal expenses and to diminish the current reliance on synthetic agrochemicals that degrade the environmental quality. Allelochemicals can be used as natural herbicides, insecticides, nematicides, fungicides and plant growth regulators. Their pesticidal potential is evident through ovicidal and antifeedant activities. Additionally, they contribute to plant defense against herbivorous predators (Patel, 2011). Despite its harmful effects, this noxious weed has potential as a green manure to improve soil fertility and crop growth. It is nutrient rich, readily available, produces succulent biomass, and can thrive even in poor soil while generating large amount of organic matter (Javaid and Shah, 2010). Aim and objectives of current study is to investigate the individual and combined influence of orthophosphate, sodium phosphate and *Parthenium hysterophorus* extract on plant growth and secondary metabolite production.

2. METHODOLOGY

2.1 Impact of Orthophosphate on Growth Profile of *Avena sativa*

2.1.1 Pre-sowing Soil Analysis

Before conducting the plant growth experiment, the soil used was analyzed for its fertility. Different parameters of soil such as available organic matter, % calcium carbonate, pH of soil, electrical conductivity (EC), soil texture were analyzed.

2.1.2 Plant Growth Assay

The complete randomized block design was used for these experiments in which three replicates for each treatment were used and total 10 seeds were sown in each pot. The current experiment was conducted thrice to evaluate the optimum concentration of orthophosphate suitable for best growth of *Avena sativa* also study the impact of parthenium on growth *of Avena sativa* under treatment with orthophosphate concentration.

2.1.3 1st Experiment (Initial Screening)

In the 1st plant growth experiment the test plant, i.e. *Avena sativa,* was treated with different concentrations (1, 2, 3, 4, 5% v/v) of orthophosphate using pre-sowing seed treatment methods. The percentage-based approach allowed standard adjustments of phosphate levels to facilitate the comparison of growth responses under phosphate limited and phosphate sufficient conditions. These concentrations were selected to establish a broad experimental gradient to evaluate plant responses under varying levels of phosphate availability and to determine the threshold at which phosphate supplementation may begin to exert inhibitory effects. For priming the seeds of *Avena sativa* with different concentrations of orthophosphate Firstly, the seeds were washed with tap water using one drop of liquid detergent trailed by several wash with distilled water. Then orthophosphate solution was prepared in different concentrations (1, 2, 3, 4, 5%) and seeds were soaked in different concentrations of already prepared solutions of different concentrations for at least 1 h.

2.1.4 2nd Experiment (Secondary Screening)

Based on the results of the initial screening, it was observed that higher concentrations of orthophosphate did not provide beneficial effects on plant growth and could potentially exert inhibitory effects. In the 2nd experiment, seeds were treated with lower concentrations of orthophosphate (0.5, 0.8, 1, 2, 3% v/v) using a pre-sowing seed treatment technique to evaluate the impact of orthophosphate in lower concentrations as the higher concentrations were not proven beneficial for plant growth and development. After harvesting different biochemical tests (determination of alkaloid, determination of terpenoid and determination of chlorophyll content) were performed.

2.1.5 3rd Experiment (Final Screening)

The third plant growth experiment was conducted using only *Avena sativa* as a test plant using pre-sowing seed treatment technique. Here the impact of parthenium along with various concentrations of orthophosphate was evaluated. In Treatment T1 plants were treated with 0.5% orthophosphate. In Treatment T2 plants were treated with 0.8% orthophosphate. However, in Treatment T3 plants were treated with 1% orthophosphate. In Treatment T4 plants were treated with 2% orthophosphate. Furthermore, in Treatment T5 plants were treated with 3% orthophosphate. In Treatment T6 plants were treated with parthenium extract. While in Treatment T7 plants were treated with sodium phosphate solution. The use of sodium phosphate allowed controlled delivery of orthophosphate ions in solution form during the seed priming treatments. Its influence on plant growth largely depends on the concentrations applied in the soil or nutrients

solution. At appropriate concentrations, sodium phosphate can enhance early seedling development by improving phosphorus availability, whereas excessive levels may lead to inhibitory or phytotoxic effects. For this reason, a range of concentrations was tested in the initial experiment to evaluate plant responses and determine suitable levels for subsequent screening. In Treatment T8 plants were treated with the mixture of sodium phosphate and parthenium extract. Furthermore, in Treatment T9 plants were treated with various concentrations (0.5, 0.8, 1, 2, 3% v/v) orthophosphate and mixture of parthenium extract and sodium phosphate. However, for preparing parthenium extract and sodium phosphate mixture 20g of fresh parthenium leaves were boiled in 200ml of distilled water for 50mins. After that 30ml of sodium phosphate was added in 40ml of parthenium extract and kept in dark for two days (Fig. 2).

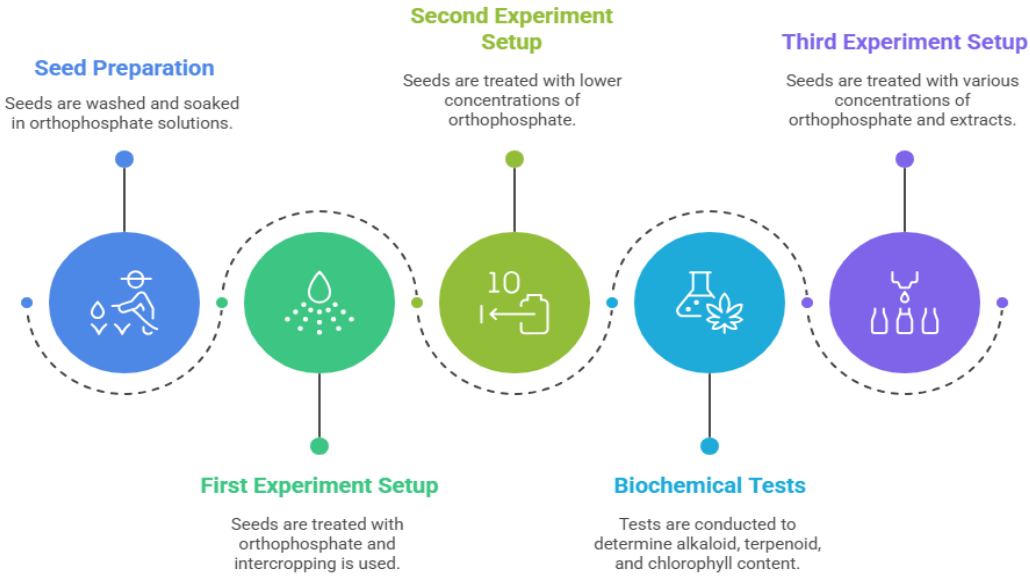

Figure 2. Plant growth Experiment Sequence

2.2 Growth Profile

In 1st, 2nd and 3rd experiments, germination percentage of control and treated plants were recorded after one week of sowing seeds and after 21 days of experiment, various growth parameters of control and treated plants were recorded.

2.2.1 Germination Percentage

Germination of control and treated plants was checked using germination percentage formula.

Germination percentage = Total No. of seeds sown/ Total No. of seeds grown × 100

2.2.2 Growth Parameters Measurement

The shoot length of control and treated plants was recorded. The root length of control and treated plants was observed from the basal region of shoot where exactly root starts to the tip of root. In case of leaves, the leaf length of control and treated plants was recorded from the base of the leaf to the tip of leaf. Number of leaves were also observed and compared in control and treated plants. Moreover, fresh

weight of control and treated plants was also recorded.

2.3 Biochemical Profiling of *Avena sativa* under Various Concentrations of Orthophosphate

2.3.1 Determination of Alkaloids (Mayer's test)

Mayer's reagent is freshly prepared by dissolving mercuric chloride and potassium iodide in 100ml water. Few drops of Mayer's reagent were added in 1ml of plant extract. Yellowish white precipitate formed, indicating the presence of alkaloids.

2.3.2 Determination of Terpenoids

Two ml of trichloroacetic acid was added in 1 ml of plant extract, and the presence of terpenoids was confirmed by red precipitate formation.

2.3.3 Determination of Chlorophyll

One gram of plant material was homogenized in 10 ml of 80% acetone and kept for 24 h in the dark. The extract was then centrifuge at 5000 rpm for 10 min to obtain a clear supernatant. The absorbance of the supernatant was recorded at 648nm and 663nm using spectrophotometer. Chlorophyll content was calculated using standard equations.

3. RESULTS

3.1 Impact of Various Concentrations of Orthophosphate on Growth Profile of *Avena sativa*

Plant growth experiments were conducted to evaluate the impact of various concentrations of orthophosphate on the growth profile of *Avena sativa.*

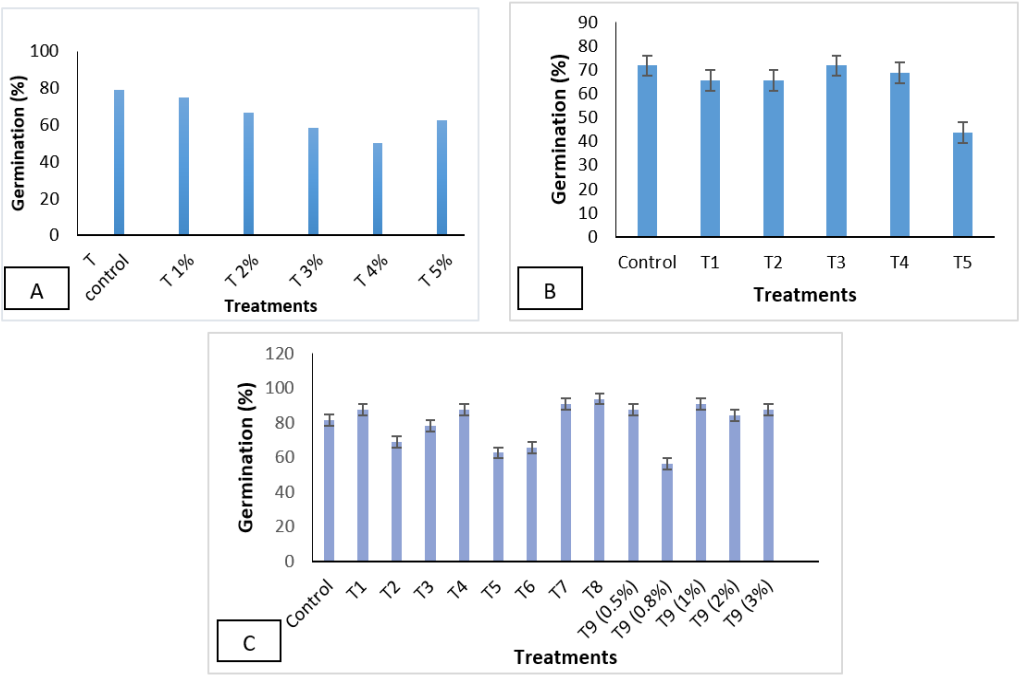

Figure 3. Germination of control and treated plants of *Avena sativa.* A: Germination % of first plant growth experiment, B: Germination % of second plant growth experiment, C: Germination % of third plant growth experiment

3.2 Soil Analysis

Soil analysis was conducted, and was found that he loamy soil contained 0.22% organic matter, 0.011% nitrogen and 5% calcium carbonate. Moreover, the pH of the soil was 7.99 and electrical conductivity of soil was 1.10 ds/m.

3.3 Growth Profile

Different growth parameters of harvested plants were checked and compared with control plants such as shoot length, root length, Number of leaves, leaf length and fresh weight to understand the impact of various concentrations of orthophosphate on growth of *Avena sativa*. Three different experiments were conducted with the Avena sativa for clear understanding of overall growth and suitable concentration of orthophosphate for optimum growth.

3.4 Experiment (Initial Screening)

3.4.1 Germination Percentage

In first plant growth setup no significant increase in germination percentage of *Avena sativa* was observed in plants that were treated with orthophosphate in Treatment T1(1%), T2(2%), T3 (3%), T4(4%), T5(5%) which was recorded to be up to 25.02%, 33.3%, 41.7%, 50.0%, 37.5% respectively, when compared with control plants (Fig. 3).

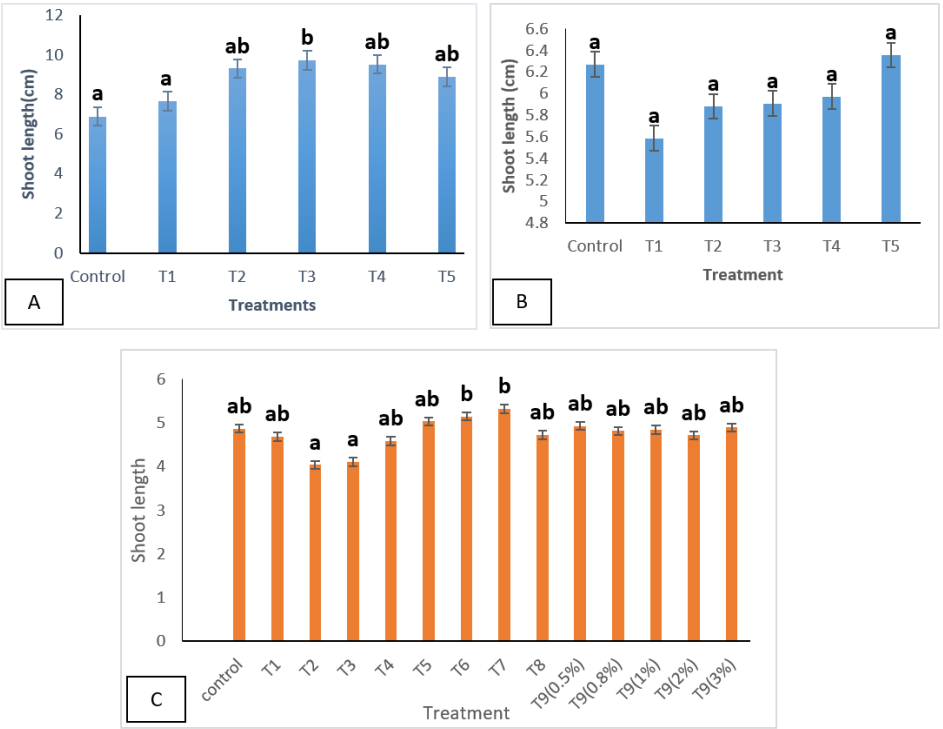

Figure 4. A: Effect of various concentrations (1, 2, 3, 4, 5%) of orthophosphate on shoot length, B: Effect of various concentrations (0.5, 0.8, 1, 2, 3% orthophosphate on shoot length, C: Effect of various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate, parthenium extract, sodium phosphate solution, mixture of sodium phosphate solution and parthenium extract and various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate with the mixture of sodium phosphate solution and parthenium extract on shoot length of *Avena sativa*. Different letters indicate significant difference between treatments using DMR test (p=0.05)

3.4.2 Shoot Length

In first plant growth experiment significant increment in shoot length was recorded in Treatment T1, T2, T3, T4, T5 (1, 2, 3,4, 5%) up to 92.3%, 90.6%, 90.2%, 90.4% and 90.1% respectively when compared with control plants (Fig. 4).

3.4.3 Root Length

In first plant growth experiment significant decrease in root length of germinated plants was observed in Treatment T1, T2, T3, T4, T5 (1, 2, 3, 4, 5%) which is found to be up to 95.5%, 93.8%, 94.4%, 95.3% and 94.8% respectively when compared with control plants (Fig. 5).

3.4.4 Leaf Length and Number of Leaves

In first plant growth experiment significant enhancement in the number of leaves was recorded in Treatment T1, T2, T3, T4, T5 (1, 2, 3, 4, 5%) which is found to be up to 98.6%, 98.3%, 98.1%, 98.3% and 98.5% respectively when compared with control plants (Fig. 6). Moreover, significant increment was recorded in leaf length with Treatment T1, T2, T3, T4, T5 (1, 2, 3, 4, 5%) which is found to be up to 79.6%, 73.9%, 75% and 77% as compared to the control plants (Fig. 7).

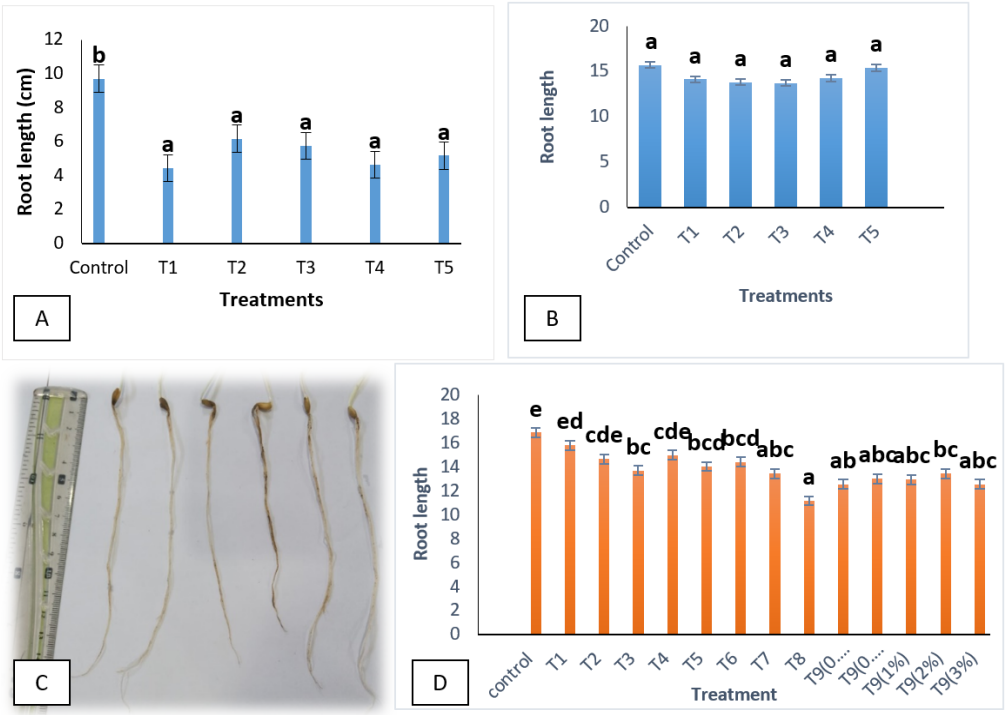

Figure 5. A: Effect of various concentrations (1, 2, 3, 4, 5%) of orthophosphate on root length, B: Effect of various concentrations (0.5, 0.8, 1, 2, 3%) orthophosphate on root length, C: comparison of root length of treated plants with control plant under less concentrations of orthophosphate (0.5, 0.8, 1, 2, 3%), D: Effect of various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate, parthenium extract, sodium phosphate solution, mixture of sodium phosphate solution and parthenium extract and various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate with the mixture of sodium phosphate solution and parthenium extract on shoot length of *Avena sativa.* Different letters indicate significant difference between treatments using DMR test (p=005)

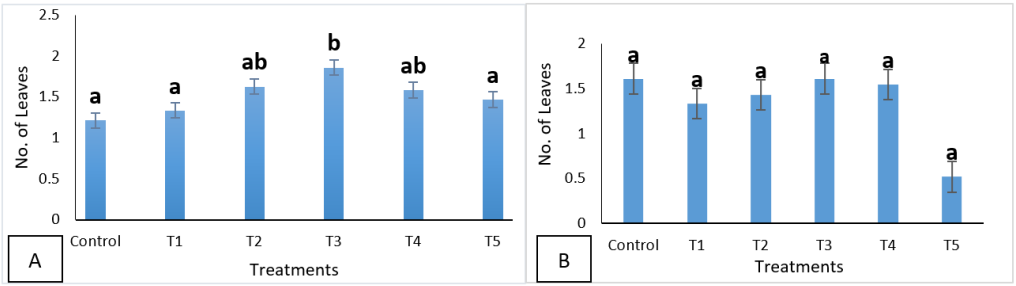

Figure 6. A: Effect of various concentrations (1, 2, 3, 4, 5%) of orthophosphate on number of leaves, B: Effect of various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate on number of leaves. Different letters indicate significant difference between treatments using DMR test (p=0.05)

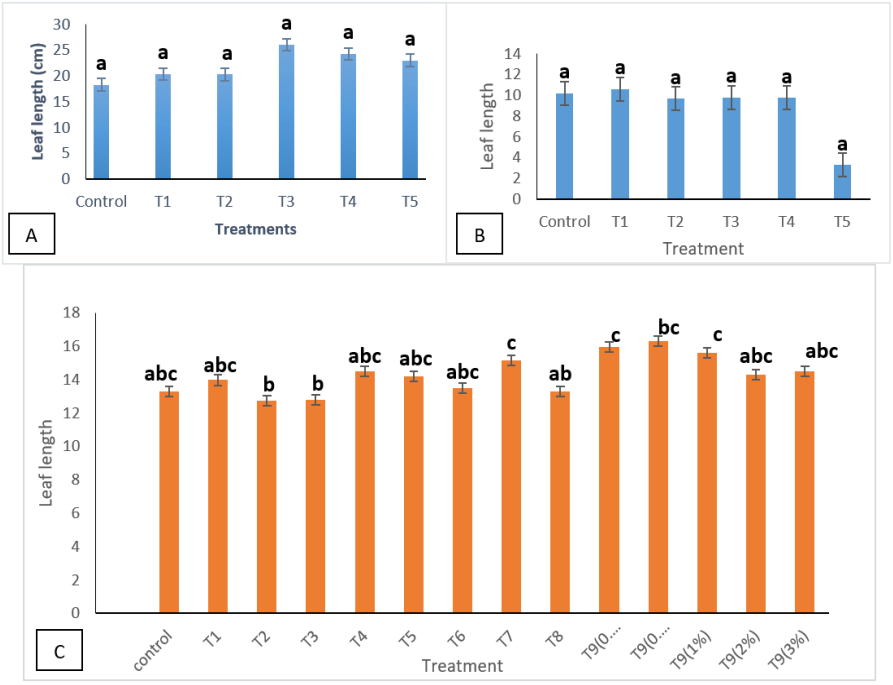

Figure 7. A: Effect of various concentrations (1, 2, 3, 4, and 5%) of orthophosphate on leaf length, B: Effect of various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate on leaf length, C: Effect of various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate, parthenium extract, sodium phosphate solution, mixture of sodium phosphate solution and parthenium extract and various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate with the mixture of sodium phosphate solution and parthenium extract on leaf length of *Avena sativa*. Different letters indicate significant difference between treatments using DMR test (p=005)

3.4.5 Fresh Weight

In first plant growth experiment significant increment in fresh weight was recorded in Treatment T3 (3%), i.e., up to 99.6% while significant decrease was observed in Treatment T1, T2, T4, T5 (1, 2, 4, 5%) which was found to be up to 99.8% when compared with control plants (Fig. 8).

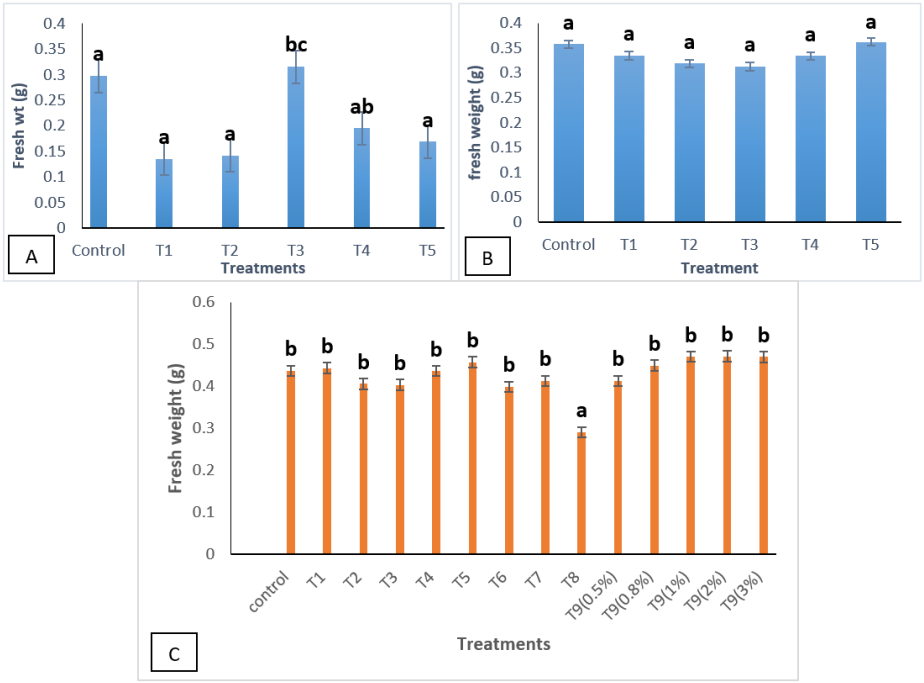

Figure 8. A: Effect of various concentrations (1, 2, 3, 4, 5%) of orthophosphate on fresh weight, B: Effect of various concentrations (0.5, 0.8, 1, 2, 3% orthophosphate on fresh weight, C: Effect of various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate, parthenium extract, sodium phosphate solution, mixture of sodium phosphate solution and parthenium extract and various concentrations (0.5, 0.8, 1, 2, 3%) of orthophosphate with the mixture of sodium phosphate solution and parthenium extract on fresh weight of *Avena sativa*

3.5 2nd Experiment (Secondary Screening)

After careful evaluation of the first experimental setup results another set of experiments was planned in which lower concentrations of orthophosphate were also assessed for detailed analysis.

3.5.1 Germination Percentage

However, in second plant growth experiment there was a significant decrease in germination percentage recorded. In Treatment T3 when 1% orthophosphate was used to inoculate the seeds up to 28.1% and significant decrease was observed in Treatment T1, T2, T4, T5 (0.5, 0.8, 2, 3%) up to 34.3%, 34.3%, 31.2%, 43.7% respectively, when compared with control plants (Fig. 3).

3.5.2 Shoot Length

In second plant growth experiment significant enhancement in shoot length was recorded in Treatment T5 (3% orthophosphate) up to 93.6% while when in shoot length was observed in Treatment T1, T2, T3, T4 (0.5, 0.8, 1, 2%) which is found to be up to 94.4% and 94% respectively when compared with control plants. The results indicated that higher concentrations i.e. 2% and 3% and 4% of orthophosphate are found to be promising for promoting the growth of shoots in oat plants (Fig. 4).

3.5.3 Root Length

In second plant growth experiment slight decrease in root length of germinated plants was recorded in Treatment T1, T2, T3, T4, T5

(0.5, 0.8, 1, 2, 3%) up to i.e. 85.85%, 86.1%, 86.2%, 86.1% and 85.8% respectively when compared with control plants (Fig. 5).

3.5.4 Leaf Length and Number of Leaves

In second plant growth experiment there was a significant increment in leaf length with Treatment T1 (0.5%) which was found to be up to 89.3% while it was also observed that in Treatment T2, T3, T4, T5 (0.8, 1, 2, 3%) produce to 90.3% and 90.2% and 96.7% increase respectively when compared with control plant (Fig 6,7). Moreover, significant decrease in the number of leaves was recorded in Treatment T1, T2, T4, T5 (0.5, 0.8, 2, 3%) that is up to 98.6%, 98.5%, 98.4% and 99.4% while in Treatment T3 (1%) in which plants were treated with 1% orthophosphate no change (increase/decrease) was recorded in the number of leaves when compared with control plants (Fig. 6).

3.5.5 Fresh Weight

In second plant growth experiment significant enhancement in fresh weight was observed in Treatment T5 (3%) up to 99.6% while slight decrease in fresh weight was recorded in Treatments T1, T2, T3, T4 (0.5, 0.8, 1, 2%) i.e., up to 99.6% when compared with control plants (Fig. 8).

3.6 Biochemical Profiling of *Avena sativa* under Various Concentrations of Orthophosphate

3.6.1 Alkaloid Determination

In second plant growth experiment under less concentrations of orthophosphate (0.5, 0.8, 1, 2, 3%) results indicate the presence of alkaloid in Treatment T2, T3 (0.8, 1%) by showing yellowish white colour (indication of alkaloid) while alkaloid was found to be absent in control plants and in those plants that were treated with 0.5, 4 and 5% orthophosphate (Fig. 9).

3.6.2 Terpenoid Determination

Under low concentrations of orthophosphate in Treatment T1, T3 and T5 (0.5, 0.8, 1, 2, 3%) results indicate the presence of terpenoid by showing red color while terpenoid was absent in control and in plants that were treated with 0.8 and 2% orthophosphate (Fig. 10).

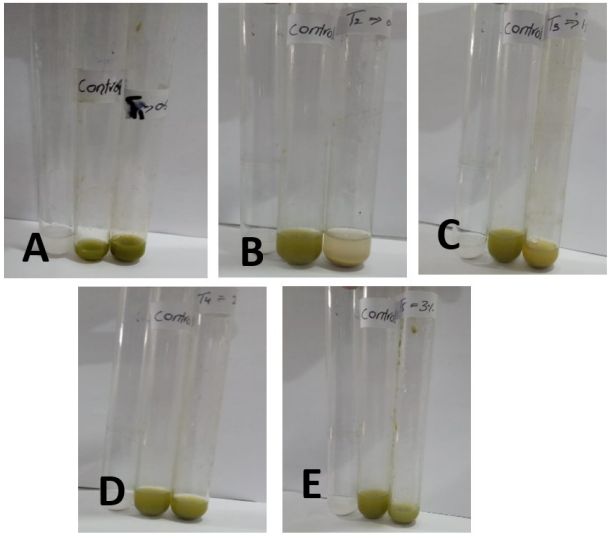

Figure 9. Alkaloid determination after 2nd Experiment (Mayer's reagent solution) A: control, B: Treatment T1 (0.5%), C: T2 (0.8%), D: T3 (1%), E: T4 (2%), T5 (3%)

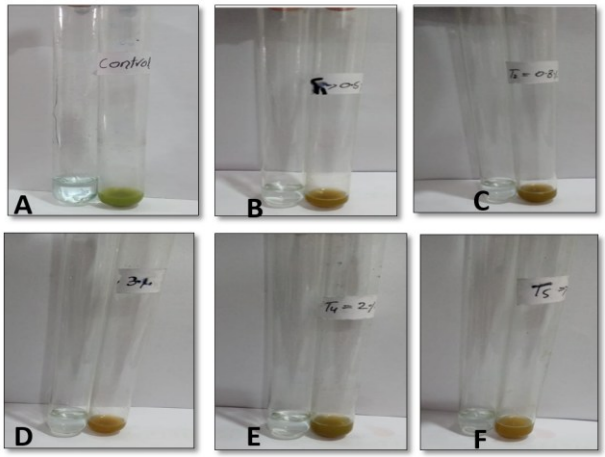

Figure 10. Terpenoid determination after 2nd Experiment [trichloroacetic acid, A: control, B: Treatment T1 (0.5%), C: Treatment T2 (0.8%), D: Treatment T3 (1%), E: Treatment T4 (2%), F: Treatment T5 (3%)

Table 1. Chlorophyll content in *Avena sativa* after 2nd experiment [chlorophyll a, b and total chlorophyll content]

S. No.	Treatments	Chlorophyll (a) in (mg/g)	Chlorophyll (b) in (mg/g)	Chlorophyll (a+b) in (mg/g)
01	Control Tº	1.931	43.432	45.432
02	Treatment T1 (0.5%)	0.455	9.331	9.786
03	Treatment T2 (0.8%)	1.641	0.355	1.996
04	Treatment T3 (1%)	5.941	53.411	59.352
05	Treatment T4 (2%)	0.235	2.141	2.376
06	Treatment T5 (3%)	0.911	4.331	5.242

3.6.3 Chlorophyll Content

Chlorophyll content showed notable variations across treatments. The control (Tº) exhibited chlorophyll a, b and total chlorophyll values of 1.931, 43.432 and 45.432 mg/g respectively. Among treatments, T3 (1%) recorded the highest chlorophyll levels (5.941 mg/g for a, 53.411 mg/g for b and 59.352 mg/g total). In contrast, T1 (0.5%), T2 (0.8%), T4 (2%) and T5 (3%) showed comparatively lower chlorophyll contents, with total chlorophyll ranging from 1.996 to 9.786 mg/g (Table 1).

3.7 3rd Experiment (Final Screening)

3.7.1 Germination Percentage

In third plant growth experiment in Treatment T1, T4 when 0.5 and 2% orthophosphate treatment was used, T7 (sodium phosphate), T8 (mixture of sodium phosphate and parthenium extract), T9 (0.5, 1, 2, 3% orthophosphate along with the mixture of sodium phosphate and parthenium extract) significant increment was observed up to 12.5%, 12.5%, 9.5%, 6.3%, 12.5%, 9.4%, 15.7%, 12.5% respectively, while in Treatment T2, T3, T5 (0.8, 1, 3% orthophosphate), T6 (parthenium extract), T9 (0.8% orthophosphate along with the mixture of sodium phosphate and parthenium extract) significant decrease was observed up to 31.3%, 21.9%, 37.5%, 34.4%, 12.5% respectively, when compared with control plants (Fig 3).

3.7.2 Shoot Length

In third plant growth experiment in Treatment T5 (3% orthophosphate), T6 (parthenium

extract), T7 (sodium phosphate solution) significant enhancement was recorded in shoot length which is found to be up to 94.9%, 94.8% and 94.6% respectively while significant decrease was observed in Treatment T1 (0.5% orthophosphate), T4 (2% orthophosphate), T8 (mixture of sodium phosphate solution and parthenium extract), T9 (2% orthophosphate and mixture of sodium phosphate and parthenium extract), Treatment T2 (0.8%), T3 (1%) orthophosphate up to 95.3%, 95.4%, 95.2% and 95.2%, 95.9% and 95.8% respectively when compared with control plants. However, in Treatment T9 (0.5, 0.8, 1, 3% orthophosphate and mixture of sodium phosphate and parthenium extract) there was no change (increase/decrease) recorded in shoot length (Fig. 4).

3.7.3 Root Length

In third plant growth experiment slight decrease was recorded in all the Treatments [T1 T2, T3, T4, T5 (1, 2, 3, 4, 5% orthophosphate), T6 (parthenium extract), T7 (sodium phosphate solution), T8 (mixture of sodium phosphate solution and parthenium extract) T9 (0.5, 0.8, 1, 2, 3% of orthophosphate solution along with the mixture of sodium phosphate solution and parthenium extract)] which is found to be up to 84.1%, 85.3%, 86.2%, 84.9%, 85.9%, 85.5%, 86.5%, 88.8%, 87.4%, 86.9%, 87.0%, 86.5% and 87.4% respectively when compared with control plants (Fig. 5).

3.7.4 Leaf Length and Number of Leaves

In third plant growth experiment significant increment was recorded in Treatment T1 (0.5% orthophosphate), T4 (2% orthophosphate), T5 (3% orthophosphate), T6 (parthenium extract), T7 (sodium phosphate solution), T9 (0.5, 0.8, 1, 2, 3% orthophosphate along with mixture of sodium phosphate and plant extract) which is found to be up to 86.0%, 85.4%, 85.8%, 86.4%, 84.8%, 84.0%, 83.7%, 84.4%, 85.7% and 85.5% respectively while moderate decrease was observed in Treatment T2 (0.8% orthophosphate), T3 (1% orthophosphate), T8 (mixture of sodium phosphate and parthenium extract) up to 87.2%, 86.6% respectively when compared with control plants (Fig. 6).

3.7.5 Fresh Weight

In third plant growth experiment significant enhancement in fresh weight was recorded in Treatment T5 (5% orthophosphate), T9 (1, 2, 3% orthophosphate along with mixture of sodium phosphate and parthenium extract) up to 99.7% respectively while significant decrease in fresh weight was observed in Treatment T8 (mixture of sodium phosphate and parthenium extract), T2 (0.8% orthophosphate), T3 (1% orthophosphate), T6 (parthenium extract), T9 (0.5% orthophosphate and mixture of sodium phosphate and parthenium extract) which is found to be up to 99.7%, 99.5% and 99.6% respectively when compared with control plants. Furthermore, In Treatment T1 (0.5% orthophosphate), T4 (2% orthophosphate), T7 (solution of sodium phosphate), T9 (0.8% orthophosphate and mixture of sodium phosphate and parthenium extract) there was no significant change (increase/decrease) in fresh weight (Fig. 8).

3.8 Biochemical Profiling of *Avena sativa* under Various Concentrations of Orthophosphate

3.8.1 Alkaloid Determination

In third plant growth experiment results indicate that alkaloid was absent in all the treated plants except those plants that were treated with Treatment T6 (parthenium extract), T9 (2%, 3% orthophosphate along with the mixture of parthenium extract and sodium phosphate) respectively (Table 2).

Table 2. Presence of alkaloid in *Avena sativa* After 3rd Experiment indicated by color

Treatment	Color of plant extract	Presence of alkaloid
Control	Light green	Absent
T1	Light green	Absent
T2	Light green	Absent
T3	Light green	Absent
T4	Light green	Absent
T5	Light green	Absent
T6	Yellowish white	Present
T7	Light green	Absent
T8	Light brown	Absent
T9 (0.5%)	Light green	Absent
T9 (0.8%)	Light green	Absent
T9 (1%)	Light green	Absent
T9 (2%)	Yellowish white	Present
T9 (3%)	Yellowish white	Present

Table 3. Presence of Terpenoid in *Avena sativa* After 3rd Experiment indicated by color

Treatment	Color of plant extract	Presence of terpenoid
Control	Brown	Absent
T1	Red	Present
T2	Red	Present
T3	Light green	Absent
T4	Red	Present
T5	Red	Present
T6	Red	Present
T7	Brown	Absent
T8	Brown	Absent
T9 (0.5%)	Brown	Absent
T9 (0.8%)	Red	Present
T9 (1%)	Brown	Absent
T9 (2%)	Light green	Absent
T9 (3%)	Red	Present

3.8.2 Terpenoid Determination

In third plant growth experiment results indicate the presence of terpenoid in plants that were treated with Treatment T1 (0.5%), T2 (0.8%), T4 (2%), T5 (3%) orthophosphate and T9 (0.8% and 3% orthophosphate along with the mixture of sodium phosphate and parthenium extract) indicated by red color of plant extract while terpenoid was absent in control plants and in Treatment T3 (1% orthophosphate), Treatment T7 (sodium phosphate solution) and Treatment T8 (mixture of parthenium extract and sodium phosphate) Treatment T9 (0.5, 1, 2% orthophosphate along with mixture of parthenium extract and sodium phosphate) respectively (Table 3).

3.9 Spectral Analysis of Different Samples used in 3rd Plant Growth Experiment

In 3rd plant growth experiment Spectral analysis were performed to record the absorbance of different samples using

spectrophotometer. The absorbance attributes of parthenium extract, sodium phosphate, and the combination of parthenium extract and sodium phosphate in the ultraviolet range were determined via UV spectroscopy analysis. A spectrophotometer was utilized to record spectrum in the 600–800 nm range. The parthenium extract's UV absorbance spectrum demonstrated a maximum absorbance peak with an absorbance value of 0.7974 at a wavelength of 450 nm. On the other hand, sodium phosphate's UV absorbance showed the highest absorption peak at a wavelength of 300 nm, with an absorbance value of 0.5951. Moreover, a combination of parthenium extract and sodium phosphate showed a UV absorbance spectrum with a significant absorbance peak at 400 nm wavelength and an absorbance value of 0.7561 (Fig. 11).

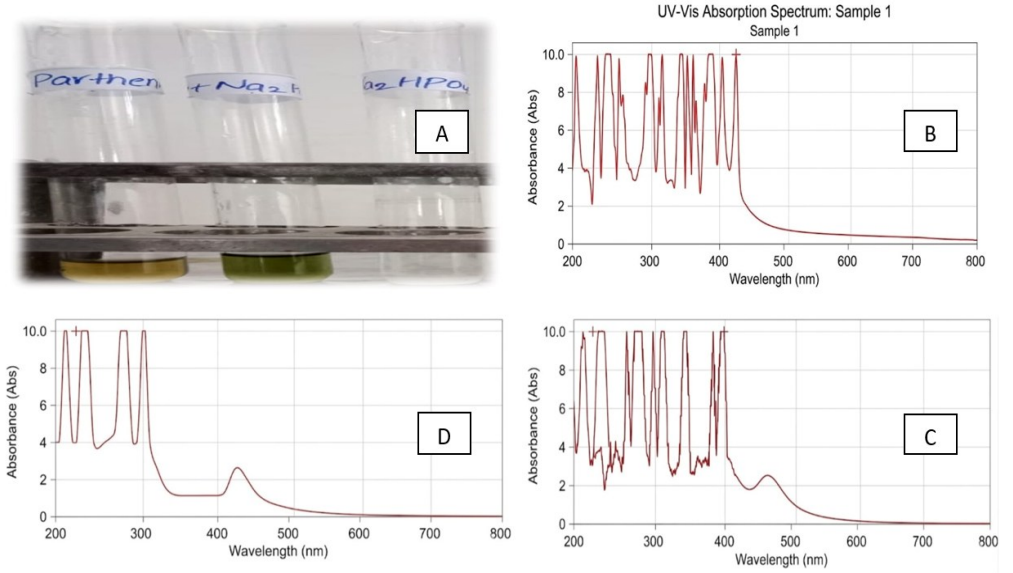

Figure 11. A: Synthesis of extracts (1-parthenium, 2-parthenium extract + sodium phosphate, sodium phosphate), B: Spectral analysis of parthenium extract, C: Spectral analysis of sodium phosphate, D: Spectral analysis of mixture (parthenium extract + sodium phosphate)

4. DISCUSSION

This study deals with the effect of various concentrations of orthophosphate on the growth and development of *Avena sativa.* The results indicated that the orthophosphate positively affects *Avena sativa*. Our study indicated that higher concentrations i.e. 2% and 3% and 4% of orthophosphate are found to be best for promoting the growth of shoots in oat plants. In the presence of highest dose of organic- mineral concentrate, shoot count was highest in the variation receiving 3 L ha^{-1}. A statistically significant increase in the number of shoots was seen in the second version, with 2 L ha^{-1}, in comparison to the control plot. 9.0% variance was seen in the number of shoots Radkowski *et al.* (2024) also reported significant increment in the number of shoots of *Lolium perenne* plants when treated with organic minerals including phosphate. Moreover, in chickpea plants When it came to phosphorus levels, the tallest plants (29.7 cm) were discovered to be treated with 90 kg P ha^{-1}, followed by the plants treated with 60 kg P ha^{-1} Ahmed *et al.* (2023) also reported that

higher concentration of orthophosphate is found to be suitable for promoting height of plants (shoot length). Shoot length of plants that were treated with Treatment parthenium extract and sodium phosphate solution showed maximum growth. In contrast, increment in shoot length was observed when chickpea was treated with 2.5% parthenium extract. Shafiq *et al.* (2020) also reported that parthenium extract was found to be suitable for promoting shoot length. It might be possible that the increased nutrient availability encouraged cell division, which led to the growth of taller plants (Radkowski *et al.*, 2024). There was a significant enhancement in root length of those plants which were treated with 2-5% orthophosphate highlighting that higher concentrations were suitable for promoting root length. Previous study showed that, in wheat plant Ortho-P considerably enhanced root dry weight, length, surface area, and volume by 55, 101, 147 and 210%, respectively, in comparison to the unfertilized group, according to an analysis of root morphological characteristics. Bourak *et al.* (2023) also reported that orthophosphate play a great role in promoting root length of plants. The increase in root length, surface area, volume might be because in many crops, roots play a major role in enhancing the efficiency of P accumulation (Pu *et al.*, 2023). The increase in root length might be due to the efficient uptake of phosphorus (Wen *et al.*, 2019). The results investigated that 0.5, 0.8, 3, 4% orthophosphate is suitable for leaf length of oat plants. And 1, 2, 3% orthophosphate is found to be suitable for number of leaves. The increased number of leaves at 1-3% orthophosphate may be attributed to the role of phosphorus that supports ATP production, cell division and photosynthetic activity, thereby promoting leaf initiation and vegetative development. However, excessive phosphate level may disrupt nutrient balance and reduce growth performance (Shukla *et al.*, 2017). In soybean plants the main stem's leaf areas increased by 109% at P60 and 105% at P120 at 65 DAS, and by 43% at P60 and 48% at P120 at maturity. Feng *et al.* (2021) also reported that application of phosphorus was found to be good for promoting the growth of leaf area, leaf length and number of leaves. Previous studies showed that increase in leaf length might be due to the key role of phosphorus in preserving electron transfer efficiency during photosynthetic processes and in stimulating ribulose-1,5-bisphosphate carboxylase (Rubisco) activity to lengthen leaves (Nguyen *et al.*, 2022). With 40% parthenium root extract, or 31.88% at 25 DAS, 34.69% at 45 DAS, and 33.79% at 65 DAS, the greatest increase in leaf count and leaf length was seen. Srivastava *et al.* (2015) also reported that parthenium extract is found to be helpful in promoting leaf length. The increase in leaf length might be due to the increase in number of nodes on main stem (Singh *et al.*, 2018). Higher concentrations, i.e. 2%, 3% and 4% of orthophosphate are required to enhance fresh weight of oat plants. In all parthenium extract treatments, especially in 2.5% as compared to control, there was an increase in shoot fresh weight (biomass) (Shafiq *et al.*, 2020). Moreover, tuber numbers increase when phosphorus was applied as 100% orthophosphate or 100% polyphosphate Ndou and Pieterse (2023) also reported that higher concentration of orthophosphate is found to be essential for increasing biomass of plants. The increase in biomass might be due to the most readily assimilated forms of phosphorus are first taken up by plant roots, increasing plant biomass (Shen *et al.*, 2016). Low to moderate concentrations of orthophosphate, particularly with *Parthenium* extract, enhanced alkaloid production. In *Catharanthus roseus* the maximum alkaloid output was seen in pH 5.82 medium with elevated phosphate levels and a total nitrogen concentration of 3710.10 mg/L (Mishra *et al.*, 2019). Mishra *et al.* (2019) also reported that orthophosphate help in promoting the production of alkaloid. The increase in

alkaloid production might be due to orthophosphate modifying the availability of nitrogen, which impacts the synthesis of alkaloids. Our findings showed that orthophosphates at low concentrations (1%) was effective in raising the amount of chlorophyll in oat plants. At the blooming stage, the highest relative chlorophyll rate was measured in wheat, which corresponds to Z55, 59.3 ± 5, and 57.6 ± 6 mol/m2 leaves that received Poly-P and ortho-P treatments, respectively when compared with unfertilized plants. Bourak *et al.* (2023) also reported that phosphorus (Poly-P and ortho-P) is found to be effective in raising the chlorophyll content in plants. Increment in chlorophyll content might be due to the involvement of Phosphorus in the synthesis of chlorophyll a and b (as synthesis of chlorophyll a, b increased, the chlorophyll content also increased. Terpenoids were detected in plants treated with 0.5-3% orthophosphate and in those receiving *Parthenium* extract alone or combined with orthophosphate and sodium phosphate across both experiments. In contrast, a study in *Rosmarinus officinalis* reported a favorable relationship between the amounts of total terpenoid and leaf phosphorus (Bustamante *et al.*, 2020) also reported that phosphorus application positively effects terpenoid production in plants. The exhibition of terpenoid is due to participation of phosphorus in the activation of terpene synthases and other enzymes necessary for terpenoid production (Bergman *et al.*, 2019).

5. CONCLUSION

These findings suggest that *Avena sativa* is a highly responsive plant to orthophosphate and prefers to be supplied with parthenium extract, which appeared to enhance germination, growth, and biomass accumulation. The combining influence of orthophosphate and parthenium extract compound also promoted chlorophyll formation, secondary metabolites and increasing alkaloid and terpenoid production could be reliable indicators of the advantageous effects of orthophosphate on the whole plant growth performance.

Conflict of Interest

The Authors declare that there is no conflict of interest

Author contributions

AG: Investigation, Formal analysis, Writing – original draft. **AB:** Investigation, Formal analysis, Writing – original draft. SW: Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing.

Ethics approval

No ethical approval was required for this study as it did not involve human or animal subjects.

Generative AI Disclosure

The authors confirm that no generative artificial intelligence tools were used in this manuscript.

Availability of data

Data will be furnished upon request.

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