Effect of soil pH on plants growth, phytochemical contents and their antioxidant activity

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Abstract

Phytochemicals or secondary metabolites are non-nutritive plants derivatives required for a variety of animal bodily functions. Plant growth and available soil nutrients decide the primary and secondary metabolites. Soil pH has a significant impact on both soil nutrient availability, plant uptake, and growth. Soil pH also decide the distribution of plant species in around the world. Still, the significance of soil pH on phytochemical concentration has not been reported. The goals of this study were to find out how soil pH affects phytochemical content and their antioxidant activity. The model's accuracy in predicting phytochemical effects in various soil pH (3.8, 4.7, 5.7, 6.5, 7.6, and 8.3) was tested in a pot experiment. The soil's pH was adjusted using $Ca(OH)_2$ and HNO_3 (pH 3.8-8.3) and soil nutrients were maintained by KCl (8.3), $MgSO_4$ (2.5), $Ca(HPO_4)$ (5) (mg kg^{-1} soil. Monocot species viz Oryza sativa, and Zea mays, and dicot species viz Cicer arietinum, Macrotyloma uniflorum were selected for study. Whole plants were collected between 5^{th} to 8^{th}day and analysed for growth and phytochemicals like phenols, tannins, flavonoids, saponins, and alkaloids. The result showed acidic soil pH (5.7) and a slightly acidic pH (6.5) is suitable for O. sativa and Z. mays, C. arietinum growth respectively. Whereas slightly alkaline soil pH (7.6) is best for M. uniform growth. Phytochemical scarcity in plants was observed despite the presence of all nutrients in the soil. The quantity and quality of phytochemicals are affected by soil pH. DPPH, ABTS, and anti-lipid peroxidation activity also directly proportional to plant growth and soil pH. This suggests that soil pH has a direct impact on nutrient uptake and phytochemical constituents of plants.

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1 Introduction:

Phytochemicals are bioactive plant chemicals found in foods like fruits, seeds and vegetables that may provide basic nutrition along with additional health advantages, like lowering the risk of major chronic diseases. Nutritional therapy is an important strategy for disease prevention and/or treatment. It also contributes to individual health because of low-toxic dietary components, abundant materials, and low cost (1). The discovery of potentially beneficial effects of dietary changes sheds light on the role of naturally occurring plant compounds in promoting and maintaining health (2). It has been demonstrated to benefit from the protective effects of substances, and recent research into dietary supplements, functional foods, and natural health products has received a lot of attention (3). However, these compounds have a geographical impact and are unique to specific plant species, and they are produced in small quantities by secondary metabolic pathways (4).

The quantity of phytochemicals in plant species can be influenced by geographical location, thereby influencing the presumed activities of a medicinal plant (5-7). Soil pH is an imperative factor which has a substantial influence on plant growth and plant species distribution. The effect of soil pH is recurrently complex, making onerous to differentiate between the unswerving properties of surplus hydrogen or hydroxyl ions and the subsidiary effects of excess are connected with variations in the solubility of biologically significant mineral elements (8). Plant growth in soils is influenced by acidity-related factors rather than by acidity itself such as manganese toxicity, aluminium toxicity, and molybdenum deficiency (9). However, the root induced pH variations in rhizosphere are rather common and caused by various factors, that includes root respiration and subsequent CO_2 release; an imbalance in cation and anion uptake (10), which is especially dependent on the nitrogen source (11); organic acid secretion (10-12); or increased H+ efflux due to iron deficiency (13-14). Nevertheless, soil pH buffering capacity may be able to counteract or confine these pH changes at root surface to a narrow zone. Riley and Barber (15) reported up to 2 units of pH difference when comparing the nitrate and ammonium diets after mechanically separating rhizosphere soil (soybeans) from bulk soil. Changes in cation-anion uptake are also closely associated with a diminution of rhizosphere pH of phosphorus deficient oilseed sprouts (16-17).

The main causes of soil acidity are a variety of factors, includes decreased pH, increased manganese and/or aluminium, deficiencies of calcium and molybdenum (9). Plants get their

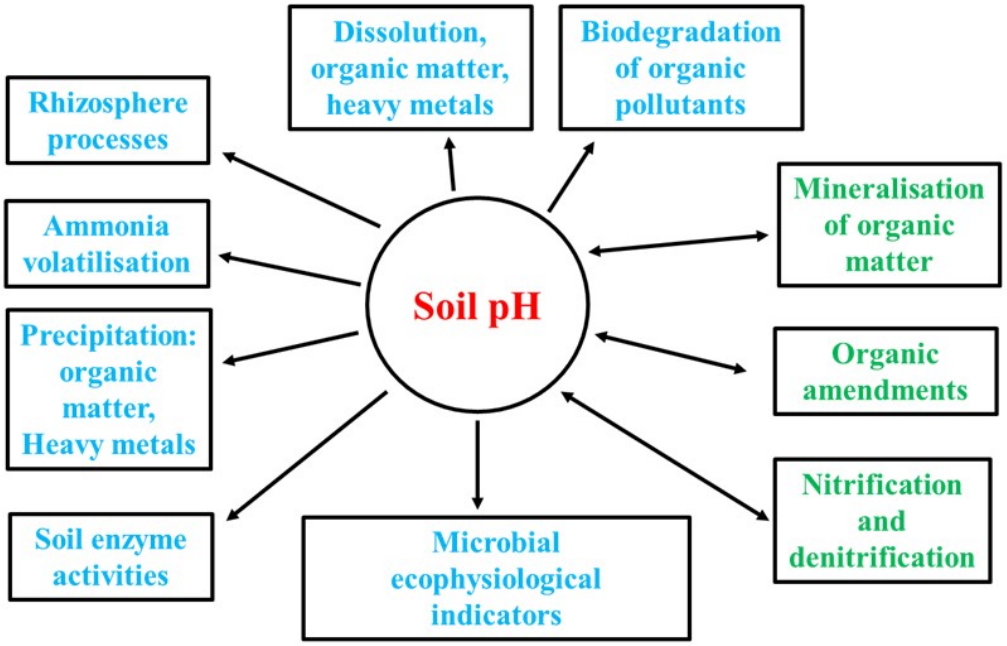

FIGURE 1

Significance of soil pH.

nutrients from soil, hence the pH of the soil is critical to understanding nutrient availability. The distribution of H^+ions between soil surfaces and the soil solution ultimately determines the pH in acidic soils. Along with soil pH, many other factors exhibit their impact on the chemical forms of various heavy metals in soil viz., electrode potential (Eh) and cation exchange capacity. Increasing the pH of the soil causes it to absorb more Cd, Zn, and Cu (18), whereas decreasing pH affects plant Cd, Zn, and Pb uptake. The heavy metal uptake on hydrous ferric oxide and Al oxides increased as the bulk solution pH increased. It was reported that the raised pH from 4.0 to 6.0 resulted in the increased concentration of calcium at the tips of the nodulated plants of various species, especially with the low-calcium treatment. Several studies have shown that pH has a significant impact on nodule formation (19). However, the soil pH effect on plants phytochemical contents has not been thoroughly investigated. The aim of current study is to resolve the complication of soil pH's effect on bioactive compounds.

2 Material and method

Tris HCl, diphenyl picryl hydrazine, aluminium chloride, trichloro acetic acid, sodium carbonate, calcium chloride, sodium nitrate, thiobarbutiric acid, copper sulphate, sodium potassium tartrate, Boric Acid, Cupric Sulphate, Zinc Sulphate, Manganese (II) Sulphate, Potassium Dihydrogen Orthophosphate, Sodium Molybdate, Folin & Ciocalteus Phenol (FC) Reagent, Gallic acid, Folin-Denis reagent, Tannic acid were procured from SRL. Remaining chemicals used in study are analytical grade.

2.1 Pot experiment

Established various stable soil pH levels viz., 3.8, 4.7, 5.7 and 6.5 by adding 8, 12, 25, and 38mM (OH^-) kg^{-1} as $Ca(OH)_2$. pH levels of 7.6 and 8.5 were established by adding 10 and 20 mM HNO_3. Micro-nutrients (mg kg^{-1} soil) were included in the following solution form: KCl (8.3), $MgSO_4$ (2.5), $Ca(HPO_4)$ (5 mg), $Na_2MoO_4.H_20$ (0.67), H_3BO_3(0.83), $CuSO_4.5H_20$ (5), $ZnSO_47H_20$ (10), $MnSO_4H_20$ (15) and KH_2PO_4 (176). To initiate the growth, 24 mg of nitrogen (NH_4NO_3) was applied. The before planting, macro and micro nutrients were incubated at field water holding capacity for 14 days (20).

2.2 Selection and planting of seeds

Seeds such as O. sativa(rice), Z. mays (maize), C. arietinum (Bengal gram) and M. uniflorum (horse gram) were purchased from an agro-centre and seed health was tested by blotting paper test. 1 gram of seeds were planted in 10 cm width pots containing 5 kg of soil. The de ionized water was used for watering. These experiments were conducted in a greenhouse from February to March. Day temperatures ranged from 27-30°C and night temperatures from 18- 22^o C. Plants were removed and the lengths of the plants were measured using a graph sheet. Aerial biomass was used to analyse the phytochemical content.

2.3 Estimation of total polyphenolic level

Level of total polyphenolic was determined colorimetrically using FC reagent method (21). To 1 ml extracts 1 ml of FC reagent (1:1) and 2ml of 10% sodium carbonate were added. After 30 mins, absorbance was read at 760 nm. The concentration of polyphenol was calculated using a standard curve and expressed as equivalents of gallic acid.

2.4 Estimation of Total flavonoid .

Level of total flavonoid was estimated using $AlCl_3$ as described by Zhishen J et.al., 1999 (22) with slight modification (23). Briefly, the reaction mixture containing 0.5 ml plant extract, 1.5 ml of ethanol (95%), 100μl of $AlCl_3$ (10%), 100μl of CH_3CO_2K (1M) were made up to 5ml with deionised water. The absorbance was taken at 415nm after incubation at room temperature for 30 minutes. was recorded for the reaction mixture. The aluminium chloride (10%) was substituted with distilled water in the blank. Flavonoids in extracts reacted with aluminium chloride were determined as described above (Labman UV-Vis spectrophotometer). Total flavonoid content calculated was expressed as quercetin equivalents (QE).

2.5 Estimation of total tannins

Total tannins level was calorimetrically assessed by measuring the intensity of blue colour formed due to the reduction of phosphotungstomolybdic acid (24). To 1 ml of extract, 5 ml of Folin Denis reagent and Na_2CO_3 solution were added and made up to 100 ml. Incubated for 30 minutes at room temperature. The OD was recorded at 760 nm. Total tannin level calculated and reported as mg tannic acid/100 g of sample (TAE).

2.6 Estimation of total alkaloids

Titrimetric methods were used to determine total alkaloids in the plant sample (25). To 1 ml of HCl (0.1N), 1 ml of plant extracts was added constant stirring for 2-3 minutes. The lower fraction is made up of alkaloids that have been neutralised with 0.1N HCl, while the upper most part was made up of n-butanol. Around 2-3 drops of methyl red were added to 1 mL of lower layer and titrated against NaOH (0.1N) till the colour turned from red to pale yellow. The neutralisation point has been identified. The total alkaloids were determined using the equivalent:

1 ml 0.1N HCl $\equiv$ 0.0162 g alkaloid

2.7 Estimation of total saponins

The total saponin level of extract was colorimetrically determined using anisaldehyde reagent (26). 0.5% anisaldehyde reagent was added to l ml plant extract and incubated for 10 minutes at room temperature. Further, 2 ml of 50% sulphuric acid was added, shaken, and kept on 65°C water bath for 10 minutes. Absorbance was recorded at 435 nm. The saponin content

was calculated using the calibration curve and diosgenin in methanol and water (10:16:4, W/V/V) was used as the standard.

2.8 DPPH scavenging activity:

The Blois method was employed to determine the DPPH radical scavenging activity of plant samples and standard ascorbic acid (27). To 500 μl of plant extract, 3ml of 0.1mM DPPH in methanol was added and made up to 4ml with methanol. Incubated for about 30 minutes at room temperature in dark. The OD was recorded at 517nm and The percentage of inhibition was calculated using formula.

Percentage scavenging activity = [(Ac-Ae)/Ac] / 100

Where,

Ac = Absorbance of the control

Ae =Absorbance in the presence of the extract

2.9 Reducing power assay

The Oyaizu method was used to determine the reducing power of plant extracts (28). To 1 ml of plant samples, 490μl of 0.2M phosphate buffer (pH 6) and 0.5ml of 1% potassium ferric cyanide were added. After 20 minutes at 50^{o}C, 0.5ml of 10% TCA was added and centrifuged at 6500rpm for 10 minutes. 1ml of the supernatant was diluted in 10ml of distilled water. At 700nm, absorbance was measured immediately after adding 0.1ml of 0.1% $FeCl_3$. An increase in absorbance when compared to the control indicates that power is being reduced (29).

2.10 Anti-lipid peroxidation activity

To measure the lipid peroxidation, a modified protocol of Halliwell and Gutteridge's (29) thiobarbituric acid-reactive species (TBARS) method was employed with slight modification (30). To summarise, 0.5ml of egg homogenate (10% v/v) and 0.1ml of extract were combined to make 1ml with distilled water. To induce lipid peroxidation, 50l of 0.07M $FeSO_4$ was added and incubated for 30 minutes. Added 1.5ml of 20% acetic acid (pH adjusted to 3.5 with NaOH), 1.5ml of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate, and 0.5ml of 20% TCA. The content was vortexed prior to getting heated at 95^{o}C for 60 minutes. After cooling, added 5.0ml of butanol to each tube and centrifuged for 10 minutes at 3000 rpm. OD was taken supernatant

at 532nm. The following formula was used to calculate the percentage anti-lipid peroxidation (%).

[1-Abs_{532}+TBA – Abs_{532}-TBA)/C] X 100

Where C is the OD of the completely oxidized control

3 Result and Discussion

Several studies reported that pH is important in the formation of plant nodules. Jensen (1944) reported that acid soil strongly inhibited N fixation by Rhizobacterium at pH 4.9-5-2 and increased approximately twice at pH 7.0-7.3 (31). Furthermore, soil pH variation reduces calcium and other cation uptake (32) and Phosphorus uptake (33). Despite this, There is no information on how soil pH affects bioactive components. The current study's goal is to determine the effect of soil pH on plant phytochemical content.

Initially, the soil pH was carefully adjusted by adding $Ca(OH)_2$ or HNO_3, and this pH was maintained throughout the experiment by supplying water with the same pH. The micro and macro nutrients were carefully maintained by adding $Na_2MoO_4.H_20$, H_3BO_3, $CuSO_4.5H_20$, $ZnSO_4.7H_2O$, $MnSO_4.H_2O$, and KH_2PO_4. Nitrogen was only applied as NH_4NO_3 (24mg $kg\text{-}^{l}$) at the beginning of the experiment.

The seeds were chosen in such a way that the plants would have to be short-day plants and germinate within the timeframe specified. Two monocots, O. sativa (34), Zea mays (35), and two dicots, C. arietinum (36) and M. uniflorum (37), were chosen. The quality of the seeds was determined using a blotting paper test, and the impact of water pH on germination was also investigated separately. Seeds were viable and free of microbial contamination in both cases (Figures 1 and 2), and water pH had no effect on seed germination (Figure 2).

Following that, exactly 1g of seeds were planted in a pot labelled with the soil pH and subjected to 12 hours of light and 12 hours of darkness while carefully monitoring the soil pH by adding appropriate pH water. The whole plant of O. sativa, Z. mays, and C. arietinum was collected between the 5th and 8th day, and the whole plant of M. uniflorum was collected between the 5th and 7th day, without damaging root and shoot. The length of the root and shoot was measured on graph paper. The last-day root and shoot lengths of all the plants are shown in Table 1. The O. sativa growth good at an acidic pH (pH 5.7), Z. mays and C. arietinum growth good at a slightly acidic pH (pH 6.5), and M. uniflorum growth good at a slightly basic pH (pH 7.6). Supporting to this, it has been reported that pH 5.0-6.5 is optimal for monocot growth and pH 6.0-7.5 is optimal for dicot growth (38).

Plants were tested for tannins, alkaloids, glycosides, saponin, phenolics, terpenoids, and flavonoids using qualitative phytochemical analysis. In general, phenolics and flavonoids are most diverse secondary metabolite content in the plants. Because of their red-ox function, hydrogen donors, and singlet oxygen quenchers, phenolics and flavonoids have considered as potential natural antioxidants due to their radical scavenging and metal-chelating activities (39-40). Using a puzzle and mortar, 1g of each plant (root and shoot combined) was crushed in phosphate buffer, and the filtrate was subjected to phytochemical analysis. The O. sativa plant extract exhibited phenolics, glycosides, tannins, and saponins contents at pH 5.7. The phytochemical content was randomly reduced in acidic and alkaline pH but constant up to neutral pH (Table 2). Z. mays and C. arietinum Phenolics, flavonoid, glycosides, tannins and saponins are more at slightly acidic pH (pH 6.5) and randomly reduced in basic pH. M. uniflorum Phenolics followed by flavonoids, glycosides, tannins, terpenoids, saponins, and alkaloids at pH 7.6 and constant over pH 7.6-8.3 (Table 2).

This finding implies that the phytochemical content and plant growth are directly correlated. O. sativa, Z. mays, and C. arietinum showed the phytochemicals in acidic or slightly acidic soil, while M. uniflorum showed in alkaline soil. The availability of nutrients in the rhizosphere and absorption of nutrients by plant is directly influenced by pH. When the pH is between 6.0 and 6.5, macronutrients like nitrogen, potassium, calcium, magnesium, and sulphur are readily available, whereas micronutrients are less readily available at higher, alkaline pHs (pH $>$ 7.0) (41). Supporting this, levels of cadmium, zinc, and lead in the exchangeable form elevated and levels of iron manganese oxide forms slightly decreased when soil pH were reduced (pH 7.0 to 4.55). Additionally, a decrease in soil pH was accompanied by increase amount of metals in plants (42). However, in alkaline soils, the ratios of total nitrogen to organic phosphorus were higher (43). This could be as a result of an increase in phosphate-solubilizing bacteria, which is necessary to increase and maintain the supply of available phosphorus in neutral, alkaline, and saline soils. Rhizobia associated with M. uniflorum were found to be highly salt tolerant, and their inoculation with nitrogen-fixing rhizobia significantly increased the growth and yield of legumes like M. uniflorum (44). Therefore, M. uniflorum benefits from slightly alkaline soil pH. The preliminary phytochemical tests revealed that the plant contained phytoconstituents such as phenolic compounds, tannins, flavonoids, saponins, alkaloids, glycosides, and terpenoids. among all the samples phenols, tannins, flavonoids, and saponins are common, hence these phytochemicals are considered for qualitative estimation in all pH shoot biomass.

According to the quantitative analysis, the phenol content O. sativa (1.6 mg/ml) and Z. mays (2.1 mg/mL) is higher than that of tannins, saponins and flavonoids, (Table 3). C. arietinum

showed phenolic content (2.6 mg/mL) was higher than that of tannins, saponins, and flavonoids. In comparison to other plants, M. uniflorum demonstrated the highest phytochemical contents such as phenolics (3.1 mg/mL), followed by flavonoids, tannins and saponins. This suggests that soil pH has a direct impact on phytochemical content and change in soil pH affected the quality and quantity of phytochemicals.

The quantitative phytochemical analysis revealed that the above-taken plant showed a good concentration of phenols, tannins, flavonoids, and saponins. Plants' antioxidant effects are also attributed to phenolic compounds such as flavonoids, tannic acids, and phenolic compounds (45). Antioxidants are compound which prevents the synthesis of free radical, ROS, lipid oxidation in a living system. Many phytochemicals from dietary or medicinal plants are proven as antioxidants. Furthermore, the anti-oxidant activity of O. sativa, Z. mays, C. arietinum, and M. uniflorum extracts were subjected to DPPH, FRAP and ALP Assay.

DPPH free radical is a nitrogen centred stable free radical usually used for antioxidant activity of compound or plant extract radical scavenging activity. Purplish DPPH free radical solution reduced to yellow-coloured diphenyl picrylhydrazine radical solution after accepting an electron from the antioxidant compound which is measured spectrometrically. Antioxidants and thus radical scavengers are substances that are capable of performing this reaction (46). DPPH radical scavenging activities of last day plant were estimated at 1mL concentration using ascorbic acid as standard. Among all the plant extracts horse gram exhibit good DPPH scavenging activity (Figure 6). The percentage scavenging activity is in the order of M. uniflorum (pH 7.6), C. arietinum (pH 6.5), Z. mays (pH 6.5) and O. sativa (pH 5.7) respectively.

The electron donating activity of various compounds mediates Fe (III) reduction, an important antioxidant action mechanism (47). Reductones exert a phytochemical's reducing ability, which includes antioxidant activity via free radical chain breakage via hydrogen atom donation (48). Among all the plant extracts M. uniflorum (pH 7.6) exhibit reduction of Fe^{3+} ferricyanide complex to the Fe^{2+} followed by C. arietinum (pH 6.5), Z. mays (pH 6.5) and O. sativa (pH 5.7) (Figure 7). Thus demonstrating the ability to reduce power.

Lipid peroxidation is generally recognised as major toxicological event. That caused by the production of free radicals from a various sources, such as organic hydroperoxides, redox cycling compounds, and iron containing compounds (49). Among all, extracts of M. uniflorum (pH 7.6) capable to prevent the formation of MDA followed by C. arietinum (pH 6.5), Z. mays (pH 6.5) and O. sativa (pH 5.7) (Figure 8). The biological activity mainly depends on the phytochemical contents and the quality and quantity of phytochemicals is decided by soil pH.

4 CONCLUSION:

Antioxidant properties have been demonstrated in a large number of natural compounds found in food. Among phytochemical phenolics, tannins, flavonoids, saponins and alkaloids are major components. The quantity of these phytochemicals is mainly depending on available soil nutrients, minerals, environment water availability etc. although presence of all nutrients in different pH soil leads plants to phytochemical scarcity. The present study reveals that the soil pH plays an imperative role in accumulation on phytochemicals and plant growth. Initially, effect of water pH alone was examined, but there is no change in the plant growth and phytochemicals. The addition of lime to these soils in the cultivation of O. sativa, Z. mays, and C. arietinum raises the pH, reducing the solubility of aluminium and manganese and providing calcium (50). In general, the availability of most micronutrients decreases at higher soil pH levels. This data suggests that soil pH affect plant growth and phytochemicals content directly. Although, field experiments are needed to clarify the effects of various physico-chemical factors like soil type and pH on nutrient availability and the threshold.

Figure and table

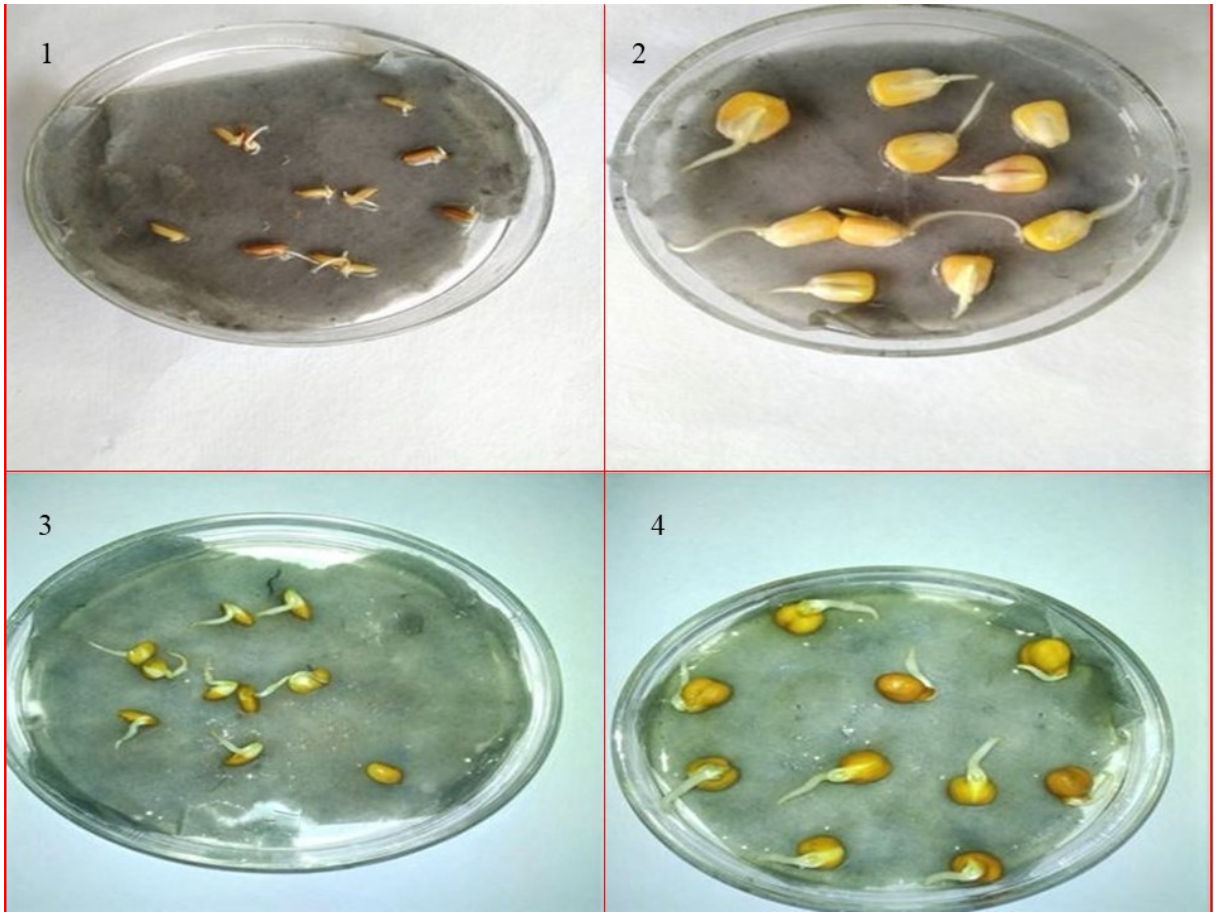

FIGURE 2

Blotter test for seed health analysis. The selected seeds were placed on petri plates containing wet blotting paper and incubated for 3 days at 25°C.

The whole plants of O. sativa, Z. mays, C. arietinum and M. uniflorum were collected on 7^{th} day at various pH (3.8 to 8.3) and measured using graph sheet.

The whole plants were collected on 7^{th} day at various pH (3.8 to 8.3) and subjected for phytochemical analysis.

The 7^{th} day whole plants were selected with respective to their optimal growth pH and subjected for phytochemical analysis.

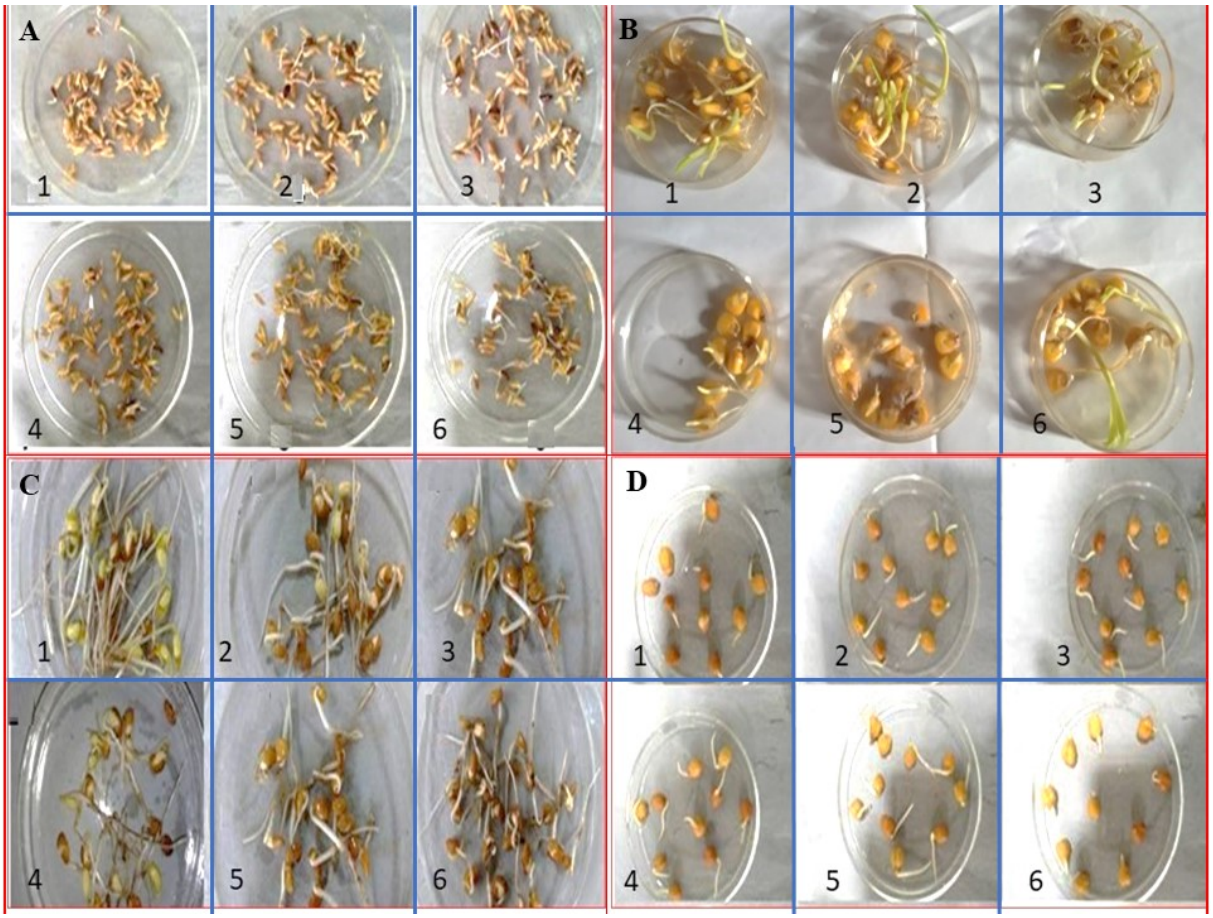

FIGURE 3

Effect of Water pH on seed germination. A blotter test was done to check the effect of different water pH of 1) pH 3.8, 2) pH 4.7, 3) pH 5.7, 4) pH 6.5, 5)7.6, and 6)8.3 on A) O. sativa, B) Z. mays, C) C. arietinum and D) M. uniflorum seed germination.

TABLE 1

Plant growth in different soil pH.

pH	Plants name and their root and shoot length in cm							
of	O. sativa		Z. mays		C. arietinum		M. uniflorum	
soil	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
3.8	1.3	1.6	1.9	2.1	2.30	2.2	1.9	3.1
4.7	2.4	2.3	2.1	2.2	3.0	2.8	2.0	3.3
5.7	2.8	2.9	2.3	2.9	3.20	3.3	2.4	2.9
6.5	2.0	2.4	3.1	3.2	3.3	3.8	2.4	3.9
7.6	2.0	2.2	2.4	2.9	3.0	2.6	2.8	4.8
8.3	1.8	2.1	2.1	2.8	2.9	3.0	2.5	3.4

TABLE 2

Qualitative phytochemical analysis of O. sativa, Z. mays, C. arietinum and M. uniflorum grown at different soil pH.

pH	Plants name	Name of the phytochemicals						
		Tannins	Alkaloids	Glycosides	Saponin	Phenolics	Terpenoids	Flavonoids
3.8	O. sativa	-	-	-	+	-	-	-
	Z. mays	-	-	-	-	-	-	-
	C. arietinum	+	-	-	-	-	-	-
	M.uniflorum	-	-	-	-	-	-	-
4.7	O. sativa	+	-	+	+	+	-	-
	Z. mays	-	-	-	-	-	-	-
	C. arietinum	+	-	+	-	+	-	-
	M.uniflorum	-	-	+	-	-	-	-
5.7	O. sativa	+	-	+	+	+	+	-
	Z. mays	-	-	+	+	+	-	-
	C.arietinum	+	-	+	+	+	-	-
	M.uniflorum	+	-	+	-	+	-	-
6.5	O. sativa	+	-	+	+	+	+	+
	Z. mays	+	-	+	+	+	+	+
	C.arietinum	+	-	+	+	+	-	+
	M.uniflorum	+	-	+	-	+	-	+
7.6	O. sativa	+	-	+	+	+	-	+
	Z. mays	+	-	+	+	+	+	+
	C.arietinum	+	-	+	+	+	-	-
	M.uniflorum	+	-	+	+	+	+	+
8.3	O. sativa	-	-	+	+	-	+	-
	Z. mays	+	-	+	-	-	-	-
	C.arietinum	+	-	+	-	-	-	-
	M.uniflorum	+	-	+	+	+	-	-

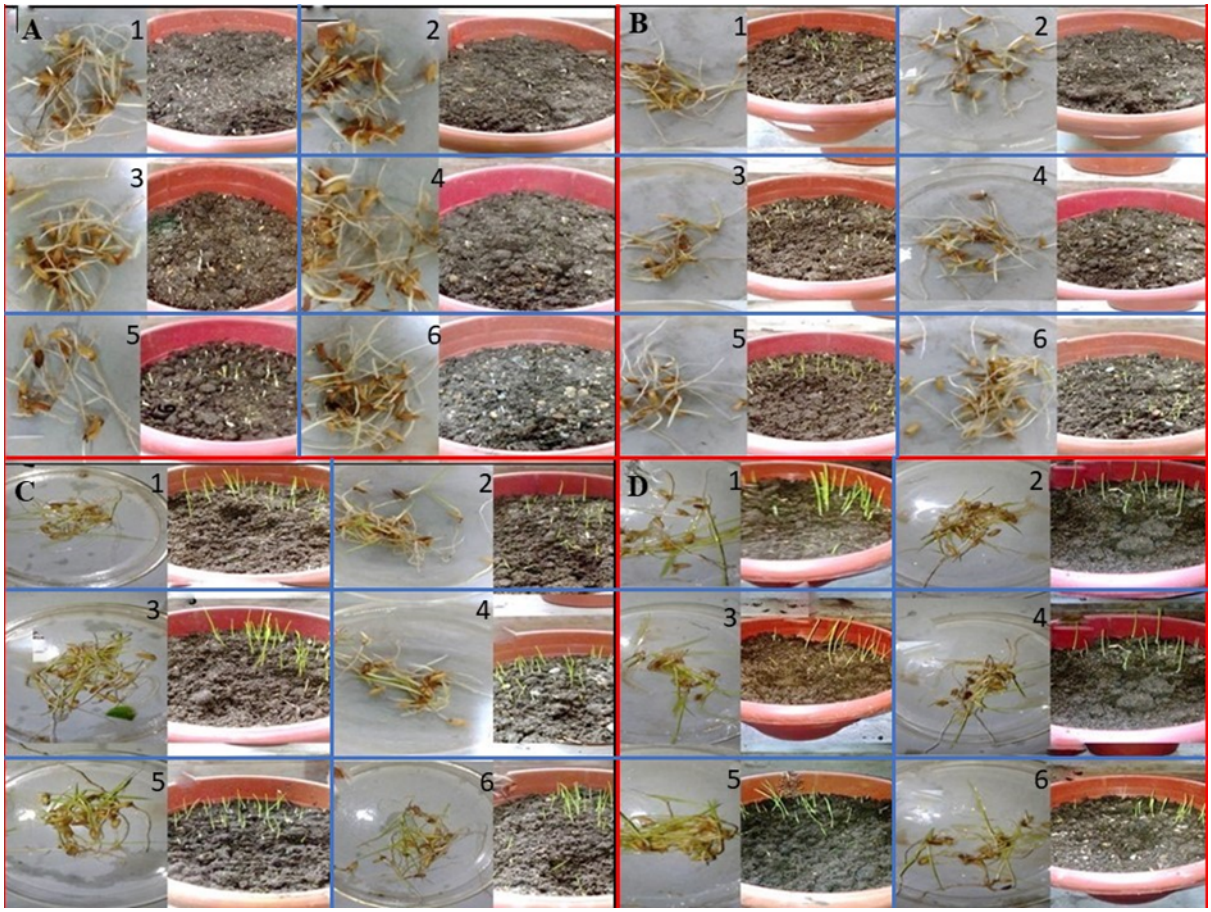

FIGURE 4

Effect of soil pH on O. sativa growth. One gram seed were planted on pot containing different soil pH 1) pH 3.8, 2) pH 4.7, 3) pH 5.7, 4) pH 6.5, 5)7.6, and 6)8.3 whole plants were collected at A) 5^{th} B) 6^{th} C) 7^{th} and D) 8^{th} day and growth were measured by using graph sheet.

TABLE 3

Quantitative phytochemical analysis of O. sativa, Z. mays, C. arietinum and M. uniflorum .

Phytochemicals	Macrotyloma uniflorum / DAYS							
	O. sativa		Z. mays		C. arietinum		M. uniflorum	
pH	**7.0**	**5.7**	**7.0**	**6.5**	**7.0**	**6.5**	**7.0**	**7.6**
Phenolics (GAE)	1.10	1.6	1.9	2.1	2.30	2.6	2.9	3.1
Tannins (TAE)	0.7	0.9	1.1	1.2	1.40	1.8	1.6	1.7
Flavanoids (QE)	0.3	0.6	0.6	0.7	1.0	1.3	2.0	2.3
Saponins (DE)	0.5	0.8	0.6	0.8	0.5	0.6	0.5	0.6

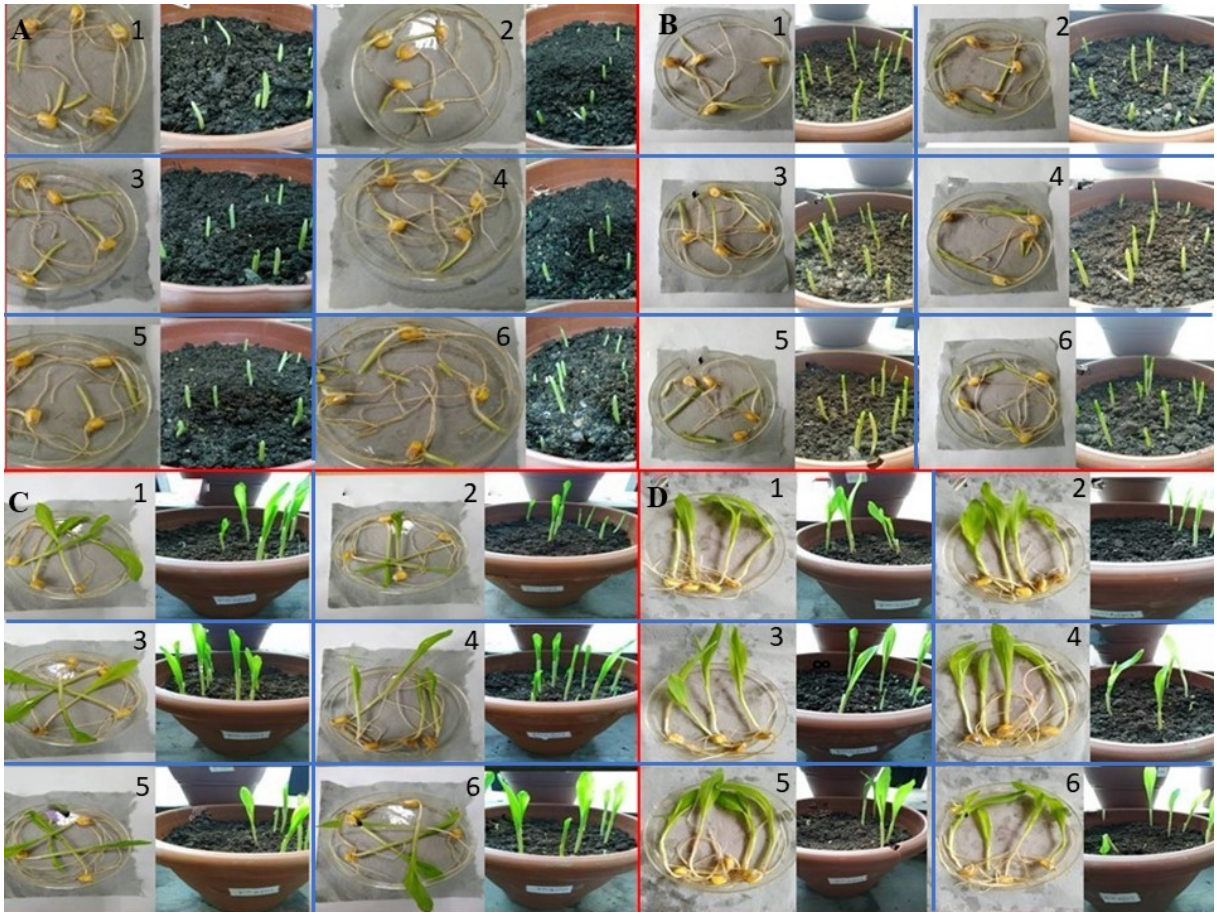

FIGURE 5

Effect of soil pH on Z. mays growth. One gram seed were planted on pot containing different soil pH 1) pH 3.8, 2) pH 4.7, 3) pH 5.7, 4) pH 6.5, 5)7.6, and 6)8.3 whole plants were collected at A) 5^{th} B) 6^{th} C) 7^{th} and D) 8^{th} day and growth were measured by using graph sheet.

FIGURE 6

Effect of soil pH on C. arietinum growth. One gram seed were planted on pot containing different soil pH 1) pH 3.8, 2) pH 4.7, 3) pH 5.7, 4) pH 6.5, 5)7.6, and 6)8.3 whole plants were collected at A) 5^{th} B) 6^{th} C) 7^{th} and D) 8^{th} day and growth were measured by using graph sheet.

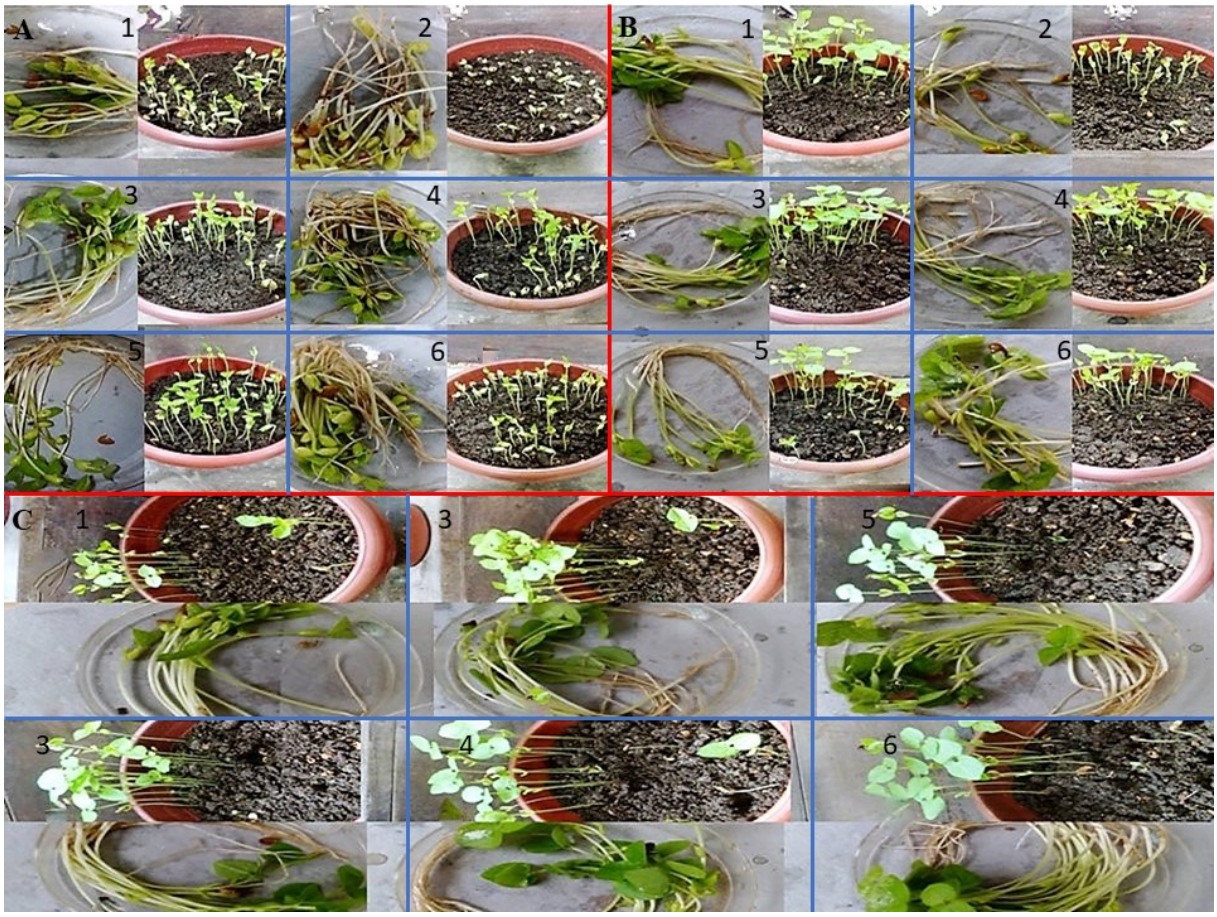

FIGURE 7

Effect of soil pH on and M. uniflorum growth. One gram seed were planted on pot containing different soil pH 1) pH 3.8, 2) pH 4.7, 3) pH 5.7, 4) pH 6.5, 5)7.6, and 6)8.3 whole plants were collected at A) 5^{th} B) 6^{th} and C) 7^{th} day and growth were measured by using graph sheet.

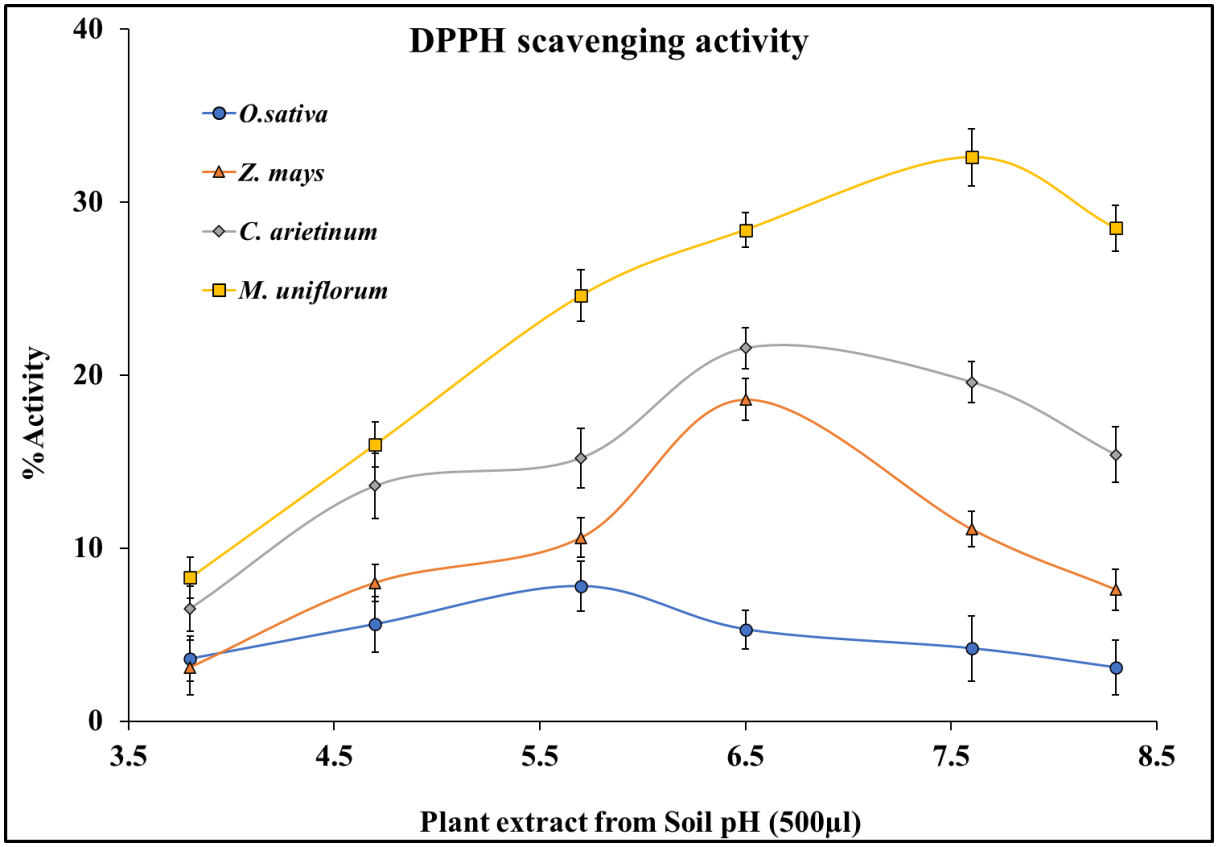

FIGURE 8

DPPH activity ofO. sativa, Z. mays, C. arietinum and M. uniflorum plant. 500μl plant extracts from different soil pH were subjected for DPPH scavenging activity. The OD was recorded at 517nm. n=3, mean $\pm$ standard deviation.

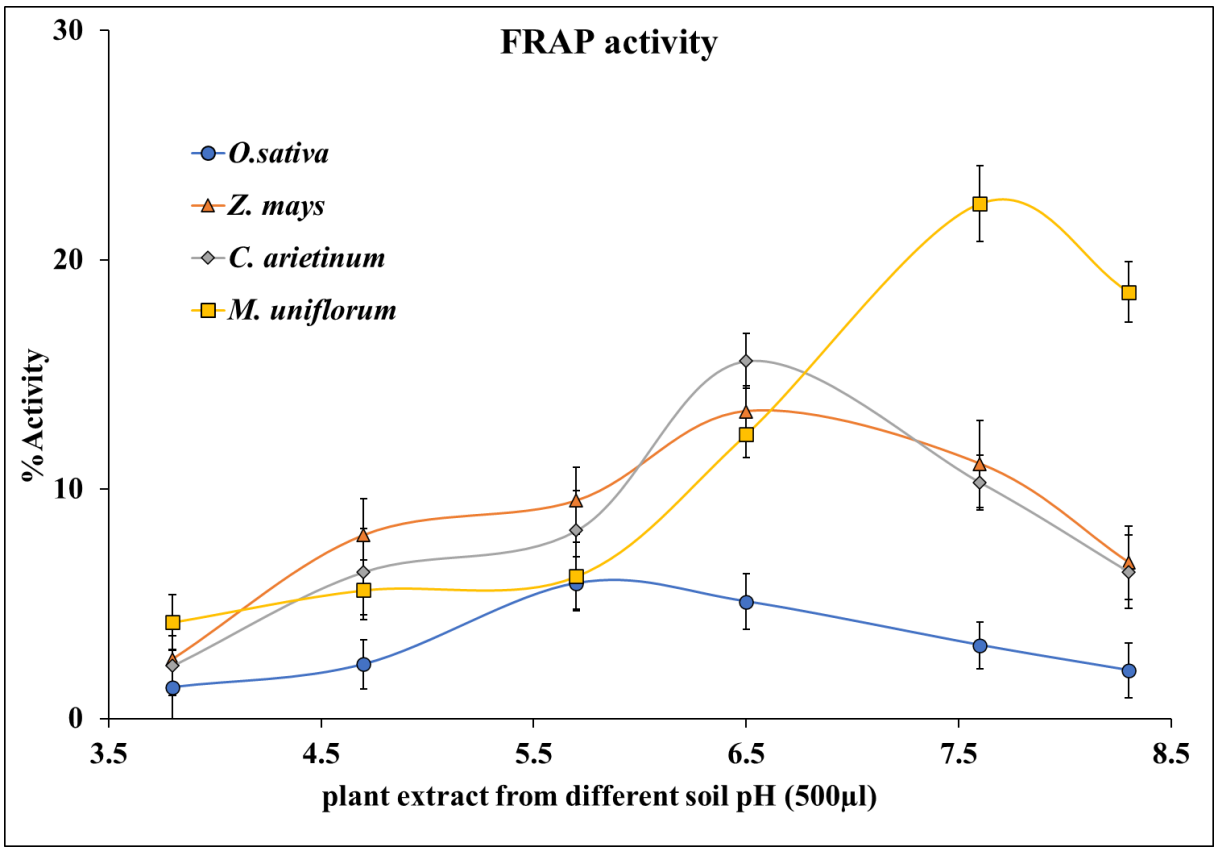

FIGURE 9

Reducing power activity ofO. sativa, Z. mays, C. arietinum and M. uniflorum plant. 500μl plant extracts from different soil pH were subjected for FRAP activity. OD was recorded at 700nm. n=3, mean ± standard deviation.

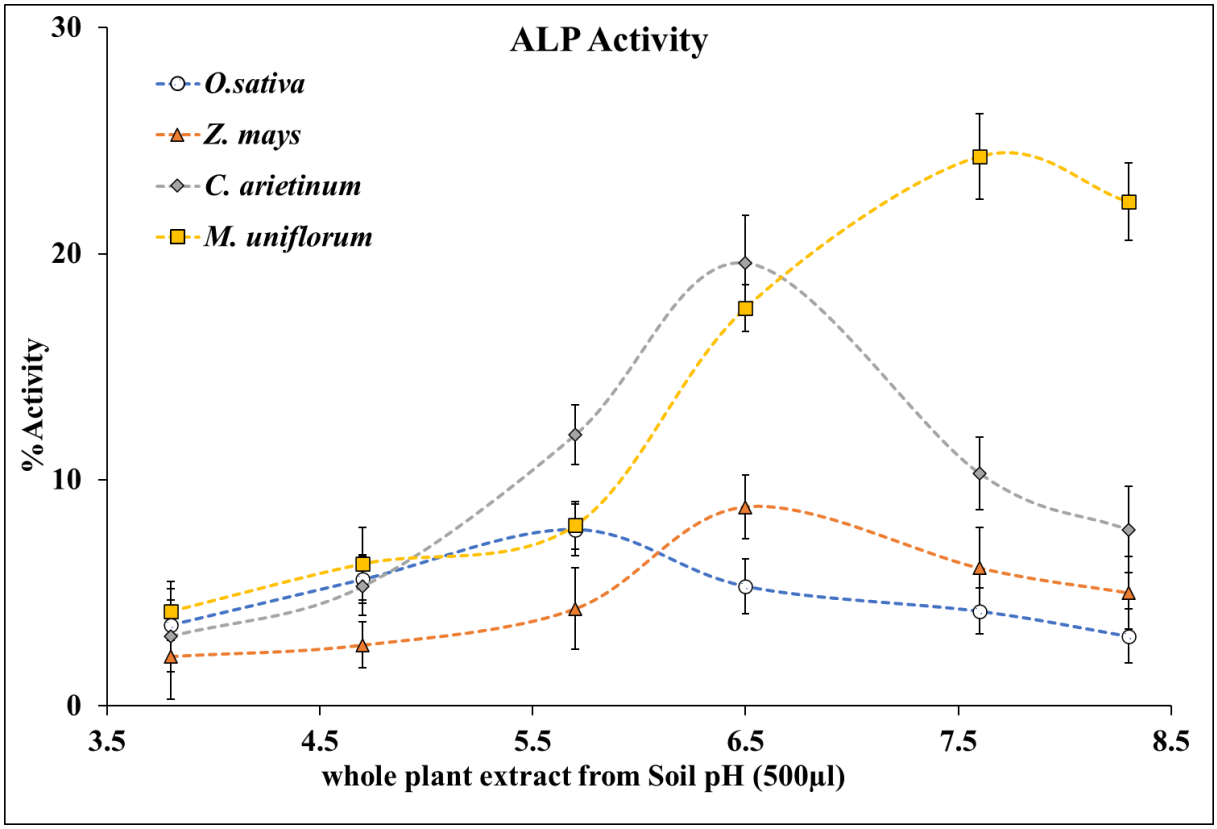

FIGURE 10

Anti-lipid peroxidation activity ofO. sativa, Z. mays, C. arietinum and M. uniflorum plant. 500μl plant extracts from different soil pH were subjected for ALP activity by TBARS method. OD was recorded at 532nm. n=3, mean $\pm$ standard deviation.

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