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From Dormancy to Growth: Decoding the pH Window for *Momordica charantia* Seed Germination

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ABSTRACT

Momordica charantia is commonly known as bitter melon or bitter gourd, which belongs to family of *Cucurbitaceae*. It has been used as folk medicine to treat various diseases, including diabetes, dysmenorrhea, eczema, jaundice, leprosy, leucorrhea, and piles. Soil pH influences a myriad of soil biological, chemical, and physical properties and processes that affect plant growth and biomass yield. This study was conducted to evaluate the acidic and basic solution effects on *M. charantia* seed germination in 2024. The trial was conducted at the Department of Botany, Government Postgraduate College Pahar Pur, Dera Ismail Khan, Pakistan. Two types of solutions, including acidic and basic, were used in this research. Before sowing seeds, the seeds were soaked in water for 24 hours to facilitate the germination process and to break dormancy due to hard seed coat of *M. charantia*. Morphological and physiological parameters are studied in this research. The highest shoot length, number of leaves, and root length were observed in *M. charantia* which was grown at slightly acidic to neutral pH (6 to 7), resulting in shoot length of 9.9 and 8.6 inches, number of leaves 2, and root length was 2 .9 and 2.4 inches, respectively. Similarly, leaf weight was maximum at slightly acidic to neutral pH (6 to 7), fresh weight was 4.2 g and 3.5 g, and dry weight was 2.8 g and 2.5 g, respectively. The highest chlorophyll a, chlorophyll b, and carotenoids are present at pH 6 and pH 7. We have identified the optimal conditions under which *M. charantia* seeds give a high germination percentage, ensuring maximum production.

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KEYWORDS

Momordica charantia, pH, Acidic, Alkaline, Soil, Seed germination, Chlorophyll

INTRODUCTION

Momordica species are vegetable crops, belonging to the family of Cucurbitaceae (also commonly referred as cucumber, gourd, melon or pumpkin family), which comprises of medium-sized plants that grow abundantly in warmer regions of the world [1]. Among the crop genera, Momordica is much known for its bitter gourd species consumed for dietary and medicinal benefits across the world. There are 60 species of Momordica [2], the majority of which remain under-utilized, less known and restricted to the wild in spite of huge medicinal importance [3]. Among them, *Momordica charantia* commonly known as bitter melon or bitter gourd is tropical or subtropical climber of the family of cucurbitaceae [4,5]. It is mostly available in China, Malaysia, India, and tropical Africa. The herbaceous, tendril bearing plant grows to six meters or longer. It bears simple, alternate leaves 4-12 cm across, with 3-7 deeply separated lobes. The lobes are mostly blunt but have small marginal points. Stipules are absent. Flowers are actinomorphic and always unisexual. Perianth has a short to prolonged epigynous zone; yellow on short (female) or long (male) peduncles that are short-lived. Fruit has ovoid, ellipsoid or spindle-shaped usually distinct, warty-looking exterior and an oblong shape. It is hollow in cross-section with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seed and pith [6]. Seeds in size 8-13mm, long compressed, corrugated on the margin, sculptured on both faces [7]. All parts of the plant, including the fruit taste very bitter [8], as it contains a bitter compound called momordicin that is believed to have a stomachic effect.

The main constituents of bitter melon (Karela) are triterpene, protein, steroid, alkaloid, inorganic, lipid, and phenolic compounds [9]. Different parts of *M. charantia* (Karela) are recommended for many diseases like; cholera, bronchitis, anemia, blood diseases, ulcers, diarrhea, dysentery, sexual tonic, and as a cure for gonorrhea [10]. The last few decades, several hundred studies have been carried with *M. charantia*. *M charantia* possesses antidiabetic, antiviral, antitumor, antileukemic, antibacterial, anthelmintic, antimutagenic, anti-mycobacterial, antioxidant, antiulcer, anti-inflammatory, hypocholesterolemic, hypotriglyceridemic, hypotensive, immunostimulant, and insecticidal properties [11].

In the natural environment, the pH of the soil has an enormous influence on soil biogeochemical processes. Soil pH is, therefore, described as the "master soil variable" that influences myriads of soil biological, chemical, and physical properties and processes that affect plant growth and biomass yield [12,13]. Soil pH controls the solubility, mobility, and bioavailability of trace elements, which determine their translocation in plants [14]. At low pH, trace elements are usually soluble due to high desorption and low adsorption. At intermediate pH, the trend of trace element adsorption increases from almost no adsorption to almost complete adsorption within a narrow pH range called the pH-adsorptionedge. From this point onwards, the elements are completely adsorbed [15]. Soil pH increases the solubility of soil organic matter by increasing the dissociation of acid functional groups [16] and reducing the bonds between the organic constituents and clays. Soil pH is essential for the proper functioning of enzyme activity in the soil [17,18], and may indirectly regulate enzymes through its effect on the microbes that produce them [19]. Soil pH influences biodegradation through its effect on microbial activity, microbial community and diversity, enzymes that aid in the degradation processes as well as the properties of the substances to be degraded. Soil pH was the most important soil property in the degradation of atrazine [20]. Soil pH controls mineralization in soils because of its direct effect on the microbial population and their activities. Additionally, at a higher soil pH, the mineralizable fractions of C and N increase because the bond between organic constituents and clay is broken [21]. The experiment was conducted to evaluate the acidic and basic effects on Momordica in 2024.

MATERIALS AND METHODS

Study Location

This trial was carried out on 4 April 2024 at the Department of Botany, Government Postgraduate College Pahar Pur, Dera Ismail Khan, Pakistan.

Experimental Materials

The seeds of *M. charantia* were obtained from a local shop in Pahar Pur. The seeds of *M. charantia* were soaked in water for 24 hours at room temperature to break seed dormancy. The above-mentioned seeds were sown in 13 earthen pots, in which 2 kg of soil was placed in each pot. Then named these pots as 1,2,3,4,5,6,7,8,910,11,12, and13.Each pot contained different pH like pot 1 ,2 contain pH 5.5, pot 3,4 contain pH 5, pot 5,6 are pH 6, pot 7,8 are pH 9, pot 9,10 are pH 10, pot 11,12 are pH 11, pot 13 is the control group. Fertile soil was collected from field where sugar cane was grown earlier. About two kilograms of soil were put into each pot. The pH of soil was neutral. Distilled water was used for irrigation of plants, which was obtained from the laboratory of Botany Department of Government Degree College Pahar Pur.

The chemicals required for research were sodium hydroxide and carbonic acid (acidic bottles). The instruments required for research were a thistle funnel, beaker, conical flask, bottle, test tubes, weight balance, aluminium sheet, pH meter, centrifuge machine, and spectrophotometer.

Preparation of Acidic and Basic Solutions

In research, two types of solutions were used, in which one was acidic and other was basic. The acidic bottles were obtained from the local factory by dissolving carbon dioxide into these bottles. Such acidic bottles have pH 4. By dilution of this acid, various ranges of pH are obtained. In preparation of basic solution small amount of sodium hydroxide (1g) is dissolved in distilled water to make 250 ml solution whose pH is 12. By dilution of this basic solution various ranges of basic solutions are obtained, such as pH11, pH10, pH9, respectively. To check the acidity or basicity of solution, pH meter is used for this purpose.

Seed Hydropriming and Seed Sowing

Before seed sowing, the seeds were soaked in water for 24 hours to facilitate the germination process and to break dormancy due to the hard seed coat of *Momordica charantia*, and also to check the viability of seeds. Seeds were sowed in each pot by scattering them on upper layer of soil and adding a small amount of soil over them to hide them into soil about 250 ml of each solution was given to this pot according to their pH concentrations, and simple distilled water to the normal pot at that time.

Method of Irrigation

The plants were watered daily after two days by adding 250 ml solution to each pot with respect to their pH levels and added distilled water in the normal pots. But after few weeks, water was added to them whenever required.

Parameter to be Studied

Morphological and physiological parameters are studied in this research.

Measurements of Morphological Parameters

Length of roots and shoots, length and broadness of leaves were measured by using a measuring scale. The fresh weight of the root and shoot was measured through a weight balance. Root and shoots are dried for 10 days for dry weight.

Chlorophyll Analysis

For analysis of chlorophyll a and b, 2 grams of leaf were taken from each replica, crushed, and soaked in 10 ml 100% methanol for 24 hours. After 24 hours, the extract was centrifuged to remove the hanged particles, and the supernatant was collected for UV analysis in the range of 400-750 nm. For each specimen, the absorbance was measured at 665, 652, and 470 for chlorophyll a, b, and carotenoids, respectively.

Statistical Analysis

All descriptive statistics were measured in mean and standard error. Additionally, all data were analyzed using SPSS software (version 16.0).

RESULTS

Seed Germination

In seed germination, *M. charantia* grows at pH 5, 5.5,6,7,9,10 while null germination occurs at pH 11. Fast germination was shown at acidic and neutral pH.

Shoot Length

Statistical analysis of different concentrations of pH on *M. charantia* in the current study the highest shoot length was observed in *M. charantia* which was at slightly acidic to neutral pH (6 to 7). The shoot length was 9.9 and 8.6 inches, respectively. So, it is concluded that the slightly acidic to neutral pH was best for shoot length (Table 1).

Stem length	pH 5	pH 5.5	pH 6	pH 7	pH 9	pH 10	pH 11
After10 days	0.47±0.1	0.63±0.1	0.8± 0.1	0.7± 0.1	0.57± 0.15	0.37± 0.06	0
After 20 days	1.3±0.058	1.5±0.058	1.84± 0.06	1.74±0.05	1.34± 0.21	1.07± 0.12	0
After 30 days	2.24±0.05	0.67±0.05	2.83±0.05	2.5±0.1	2.34±0.21	2.1±0.12	0
After 50 days	3.84±0.05	4.54±0.15	4.97± 0.05	4.57±0.15	4.37±0.32	3.9±0.1	0
After 63 days	6.1±0.7	6.13±0.32	6.83±0.1	6.5±0.1	6.17±0.25	5.8±0.1	0
After 80 days	6.83±0.20	7.6±0.2	8.2±0.1	7.87±0.15	6.74±0.31	6.7±0.1	0
After 98 days	7.2±0.2	8.4±0.1	9.9±0.1	8.67±0.15	7.47±0.45	6.87±0.05	0

Table 1: Impact of pH level on shoot length of *Momordica charantia*.

Number of Leaves

Statistical analysis of different concentrations of pH on *M. charantia* shows maximum number of leaves at pH 6 to 7 while at other different pH it shows different number of leaves. The number of leaves was reduced at pH extremes acidic and basic (Table 2).

Number of leaves	pH 5	pH 5.5	pH 6	pH 7	pH 9	pH 10	pH 11
After 10 days	1.67±0.58	2±0	2±0	2±0	1.67±0.58	1.34±0.58	0
After 20 days	3.34±0.58	3.34±0.58	3.67±0.58	3.34±0.58	2.67±0.58	2.34±0.58	0
After 30 days	4±1	5.34±0.58	6.67±0.58	6.34±0.58	4.67±0.58	4.34±0.58	0
After 50 days	5±1	6.34±0.58	8±0	7.34±0.58	5.67±0.58	5.34±0.58	0
After 63 days	6±1	7±0	9±0	8.34±0.58	6.67±0.58	6.38±0.58	0
After 80 days	7±1	7.67±0.58	11±1	10.34±0.58	7.67±0.58	6.67±0.58	0
After 98 days	8±1	8.32±0	12±1	11.22±0.58	8.67±0.58	7.56±0	0

Table 2: Impact of pH on the number of leaves of *Momordica charantia*.

Root Length

M. charantia shows maximum root length at pH 6 to 7 which was 2 .9 (inch) and 2.4 (inch), respectively. While reduced root length was observed at pH extremes i.e. at acidic pH 5,5.5 observed root length was 1.1 (inch) and 1.9 (inch) respectively, and at basic pH 9,10 observed root length was 1.4 (inch) and 1.1 (inch) respectively (Table 3).

Root length	pH 5	pH 5.5	pH 6	pH 7	pH 9	pH 10	pH 11
	1.2±0.1	1.8±0.1	2.7±0.25	2.1±0.11	1.3±0.1	1.1±0.1	0

Table 3: Impact of pH on root length of *Momordica charantia*.

Fresh Weight and Dry Weight

Statistical analysis of different concentrations of pH on *M. charantia*. In current study, the fresh weight was measured in *M. charantia* which was maximum at slightly acidic to neutral pH (6 to 7). The fresh weight was 4.2 g and 3.5 g, respectively. So, it is concluded that the slightly acidic to neutral pH was best for fresh weight (Table 4).

Similar results were obtained for dry weight in *M. charantia* under different concentrations of pH. Dry weight was maximum at pH 6 to 7 but it reduced at pH extremes acidic and basic. Dry weight was 2.8 g at pH 6 and 2.5 g at pH 7 which indicates that plant growth was maximum at this pH range.

pH levels	Fresh weight	Dry weight
pH 5	2.3±0.23	1.34±0.15
pH 5.5	2.5±0.5	2±0.26
pH 6	4.23±0.25	2.97±0.15
pH 7	3.63±0.32	2.54±0.32
pH 9	2.27±0.23	1.3±0.1
pH 10	1.67±0.15	1.03±0.23
pH 11	0	0

Table 4: Impact of pH on fresh weight and dry weight of *Momordica Charantia*.

Physiological Characteristics

In physiological characteristics, we just examine their chlorophyll content due to time limitations.

Chlorophyll Content

Statistical analysis of different concentrations of pH on *M. charantia*. In current study, the highest chlorophyll a, chlorophyll b, and carotenoids are present at pH 6 and pH 7 (Table 5).

pH levels	Chlorophyll a	Chlorophyll b	Carotenoids
pH 5	1.162±0.07	1.49±0.07	0.45±0.07
pH5.5	1.36±0.04	1.81±0.07	1.55±0.07
pH 6	2.92±0.07	2.86±0.04	2.25±0.07
pH 7	2.4±0.06	2.42±0.02	1.85±0.07
pH 9	1.11±0.014	1.37±0.03	0.55±0.07
pH 10	0.96±0.02	1.09±0.007	0.35±0.07
pH 11	0	0	0

Table 5: Impact of pH on chlorophyll content of *Momordica charantia*.

DISCUSSION

The results obtained from our research about the reaction of seeds of *M. charantia* to pH variations showed that seeds of *M. charantia* able to germinate at acidic pH range from 5 to 6 and neutral medium, and basic or alkaline pH 9 to 10, while *M. charantia* showed null germination and growth.

There were no differences in germination at pH values from 5 to 11, when data were averaged over species. Germination was negligible at pH 3 for most weed species. Tall morning glory, Florida beggar weed, Brazil pusley, ivy leaf morning glory, Johnson grass and strangler vine had no germination at pH 3, but similar germination was observed at pH 5, 7, 9 and 11. Variations in germination were observed among the weed species, but pH from 5 to 11 had similar germination of the test species.

Germination of redroot pigweed, hairy beggar ticks and sickle pod was similar at pH of 5, 7, 9 and 11; none of them germinated at pH 3. Common ragweed, though had lowest germination among the tested weed species, germination was higher at pH 5 and decreased with increase in pH; there was no germination at pH 3. In case of prickly sida, highest germination was recorded at pH 9 and germination decreased both with increase or decrease in pH from 9. No germination of prickly sida was recorded at pH 3; germination was similar at pH 7 and 11, but higher than pH 5. Only yellow nutsedge could germinate at pH 3, but germination was significantly lower than other pH values. Highest germination of yellow nutsedge was recorded at pH 7, compared to lower or higher pH, but statistically no difference was observed between pH 5 to 11.

Yousif et al. [22], germination tests were carried out under different pH values in order to determine the influence of pH on Organum compactum seeds germination. At low pH (pH 2), the germination percentage was almost null. When increasing the pH, the germination percentage increased to a maximum value (71% Assara; 61% Darelgaba), but the germination percentage decreased when the pH of the medium exceeded the optimal pH. This reaction of seeds to pH variations could be attributed to the inhibitory effect of acidity and basicity on the catalysts involved in the germination process which inhibits the action of enzymes needed for germination of this species.

Punja and Grojan [23], previous studies on the effects of pH on Sclerotium rolfsii indicate that optimal mycelial growth and sclerotial germination occur at pH (3.0-5.5) and that no growth occurs above pH 8.0 [1,9,24].

Our results confirmed these observations although *M. charantia* in this study did not germinate above pH 10. But show maximum germination at pH (6-7) and minimum germination at pH (9-10).

Susko et al. [25], best described how germination of dame's rocket seeds changed with regard to solution pH. Dame's rocket seeds germinated well (60%) over a wide range of pH (3 to 10; Table 4). High seed germination of dame's rocket over a broad range of pH levels (3 to 10) indicates that pH should not limit germination in most soil types. Germination over a broad range of pH has been reported in other mustard species (pH 4 to 10 in Sisymbrium orientale Torn., Chauhan et al. [26a]; and pH 4 to 10 in Brassica tournefortii Gouan, Chauhan et al. [27b]), as well as numerous other weedy species Oliveira and Norsworthy [28]; Wei et al. [29], but in general, the optimum pH range for seed germination falls within about 5 to 8; germination typically decreased in more acidic (# 4) or alkaline ($ 9) pH conditions. In contrast, seed germination of dame's rocket was high (94%) and did not differ significantly over a pH range of 3 to 8, indicating that seeds should germinate well, even in extremely acidic soils. However, Zibilske et al. [30] reported that the species tends to avoid acidic soils and grows best in very alkaline soils.

The result obtained from our research that the seed germination of *M. charantia* shows maximum germination at approximately in slightly acidic medium (pH-6) but shows minimum germination even in basic medium (pH=9,10), and did not show any germination at higher basic medium(pH=11). As a result from this above discussion the seed shows germination at higher percentage of about 80% in acidic soil rather than basic medium.

According to Hanson and Johnson [31], across all cultivars and germination chamber temperatures, the average first day of germination, the germination percentage, and the Germination Index (GI) were 7.3 d, 38.3% and 28.1, respectively, at pH 6.0. Switchgrass performed well in the pH range from 5-8 where the GI ranged between 17.8 and 21.7. Germination activity dropped off sharply below pH 4.6 and above pH 8. The results obtained from our research show that the okra exhibits high germination rate at the pH of 6.5 while okra shows no germination at the higher pH value.

CONCLUSION

Based on the data from the present study, we concluded that high alkaline pH inhibits germination of *M. charantia* seed. pH is an important factor for plant growth, and the results from this study showed that the pH range 6 to 7 is more suitable for germination of *M. charantia* seed because of the better conditions of the germination process. Highly alkaline pH had a negative effect on the seed germination of *M. charantia* when compared to the control medium without treating pH. In fact, the germination percentage was inversely proportional to the concentration of pH as no germination occurred at pH 11. The seed germination has occurred in a range of pH between 5, 5.5, 6, 7, 9, and 10 but the maximum germination was observed at pH 6 to 7 which was qualified as the optimal pH of germination. Hence, in this study, we have revealed the optimal conditions under which *M. charantia* seeds give a high germination percentage and which will ensure a maximum production.

CONFLICT OF INTEREST

We want to declare that we have no any conflict of interest.

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