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ANTIOXIDANT STATUS OF BARLEY GRASS GROWN IN FERTILIZERS FORMULATION OF DIFFERENT TYPES OF FRUIT PEELS

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ABSTRACT

Cereal grass and its ingredients are gaining popularity as functional and nutraceutical foods. Barley grass (BG) juice is known as a super food as it provides dietary supplements to protect age related diseases such as diabetes, cardiovascular diseases, etc. The present study was conducted to evaluate the phytochemical profile of the green grass barley (*Hordeum vulgare L.*) grown in normal soil and soil containing different fruit peel of banana, pomegranate, pineapple and lemon over a period of 13, 15, 17 and 21 days. Quantitative profiling especially the antioxidant profiles such as total phenolic contents (TPC), flavonoids, ascorbic acid and free radical scavenging activity were studied. The results portrayed the antioxidant profile of BG to be different on normal soil and the soil fortified with fruit peels. The highest phenolic values were observed on 17th day in soil with banana peels (0.432±0.01mg TAE/gm) followed by pomegranate (0.312±0.02mg TAE/gm), pineapple (0.306±0.03mg TAE/gm) and lemon peels. The highest FRAP values occurred on day 13 of growth in soil with pineapple peels (4.959±0.07mgAAE/gm) and normal soil whereas in soil with lemon (4.039±0.07mg AAE/gm) and pomegranate (3.94±0.01mg AAE/gm) the highest levels were observed on 15th day.

Keywords: antioxidant, flavonoid, phenolic, ascorbic acid, barley grass

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) belongs to the *Poaceae* family, is the fourth most important cereal crop in the world. Barley grass has been shown to contain high concentrations of amino acids, pigments, antioxidants, and enzymes **[1]**. Barley grass (BG) contains 20 amino acids including gamma-aminobutyric (GABA) and glutamic acid which are involved in energy production, cell building and regeneration which can alleviate oxidative damage of metal toxicities in BG by activating antioxidant defense and reducing the carbonylated proteins **[2]**.

BG is also rich in pigments, which mainly include chlorophyll (Chl) and carotenoids (Car) The Chl and Car pigments are strong natural antioxidants acting as free radical scavengers and the pigments are associated with age-related eye diseases. Chlorophyll derivatives may play a significant role in anticancer activity, because it exhibits a similar antimutagenic effect to 3-methylcholanthrene **[3]**.

BG is a rich source of various phytochemicals such as β-glucan, phenolic acids, flavonoids, lignans, tocols, phytosterols, and folate. Barley plays an important role in reducing the risk of chronic diseases (diabetes, cancer, obesity, cardiovascular disease) as it inhibits both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism, which in turn elevates the SOD and GSH-Px activities **[4]**.

In India, herbal or alternative medicine is gaining popularity and barley grass as a "functional food" is becoming more available and popular. However, the effect of soil enriched with organic manure made up of various fruit peels such as banana, lemon, pomegranate, and pineapple on different germination conditions used for the cultivation of wheatgrass was not studied. The present study assessed the antioxidant potential of barley, during its germination period under different growth conditions.

2. MATERIAL AND METHODS

2.1 Material required-

2.1.1 Plant material

Seeds of barley (*Hordeum vulgare* L.) were collected from local nursery and fruit peels of lemon, banana, pomegranate, and pineapple were collected separately from fruit waste from fruit shops.

2.1.2 Processing of Fruit peels -Peels of banana, pomegranate, pineapple and lemon were sun dried and then dried in a hot air oven at 70 degrees for 3-5 days. The dried fruit peels were powdered and stored at room temperature. 2g Peel powder was added to 100g soil.

2.1.3 Soil preparation

Garden soil was taken and all the unwanted particles were removed. 1kg

soil was distributed in five pots each and 20g different powered fruit peels were mixed respectively in 4 pots and one pot with no added fruit peel was taken as control. These were kept aside for one month, for proper aeration and sunlight with time to time mixing.

2.1.4 Seed sterilization and sowing

Seeds of *Hordeum vulgare L.* were sterilized using 0.2% of Bavistin to avoid fungal infection. The seeds were sown in triplicates in pots having normal soil, soil with pomegranates, lemon, banana and pineapple peels.

2.1.5 Estimation of total phenol content (TPC)

The total phenol content (TPC) was determined by spectrophotometer using tannic acid as standard with some modifications **[5]**. 1.0 ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0 ml of 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of tannic acid equivalent per g dry weight (mg TAE/g).

2.1.6 Determination of Total flavonoid content

Total flavonoid content was measured by the modified aluminum chloride colorimetric assay **[5]**. The reaction mixture consisted of 1 ml of extract and 4 ml of distilled water taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was added and after 5 minutes, 0.3 ml of 10 % aluminum chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was added and the final volume of the mixture was brought to 10 mL with double-distilled water. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was calculated from the calibration curve and was expressed as mg Ascorbic acid equivalent (AAE) /g of extract.

2.1.7 Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP)

The total antioxidant capacity was determined by spectrophotometry, using ascorbic acid as standard and using the modified FRAP assay **[5]**. 0.1 ml of extract was taken and to it 0.9 ml of ethanol, 5 ml of distilled water, 1.5 ml of HCl, 1.5 ml of potassium ferricyanide, 0.5 ml of 1% SDS and 0.5 ml of 0.2% of ferric chloride was added. This mixture was boiled in a water bath at 50°C for 20 minutes and cooled rapidly.

Spectrophotometrically the absorbance was measured at 750 nm. The antioxidants in samples were derived from a standard curve of ascorbic acid and were expressed as mg ascorbic acid equivalent (AAE)/ g.

2.1.8 Estimation of ascorbic acid. Ascorbic acid was measured spectrophotometrically by 2,4-DNPH method. 0.3 ml of extracts were pipetted out in test tubes **[5]**. To all the test tubes containing extract, distilled water was added to make up to 1.5 ml. To all the test tubes, 0.5 ml of 2, 4- DNPH was added and after proper mixing, test tubes were incubated at 37º C for 3 hours.3.5ml of 80% H_2SO_4 was added to the test tubes to dissolve the orange red osazone crystals formed and absorbance was spectrophotometrically measured at λ 540 nm.

3. STATISTICAL ANALYSIS:

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by one-way ANOVA ($p \leq 0.05$).

4. RESULT AND DISCUSSION

In barley grass grown in normal soil, there was a significant decrease in flavonoid level on the 15th day followed by a significant increase on the 17th day ($p \leq 0.05$) **(Figure 1)**. The flavonoid level decreased significantly from day 13 th to day 15th in barley grass grown in soil with lemon peels. With pomegranate peels, the flavonoid levels remained constant till day 15th followed by significant increase on day 17th. In barley grass with pineapple peels there was a time dependent significant increase in flavonoid levels till day 17th. In all the cases there was a significant decrease in flavonoid levels on day 21st as compared to day 13th, 15th and 17th ($p \leq 0.05$). The highest flavonoid levels were observed in lemon peels (0.414 ± 0.091 mg AAE/gm) on day 13. BG is a rich source of flavonoids which include saponarin, lutonarin, isoorientin, isoscoparine, C-glycosyl flavones, O-glycosyl-C-glycosyl flavones, O-diglycosyl flavones, isoscoparin-7-O-glucoside derivatives, 7-O-[6-acyl]-glucoside, and -7-O-[6-acyl]-glc-4-glucoside of isovitexin **[6]**.

In barley grass grown in normal soil, soil with lemon, banana and pomegranate peels, there was a significant upward trend in levels of phenolic with significant increase in phenolics levels on day 17th and 21st as compared to day 13th ($p \leq 0.05$) **(Figure 2)**. However in barley grown in soil with pineapple peels, there was significant increase in phenolic from (0.14±0.01mgTAE/g) on day 13th to (0.182±0.026mgTAE/g) day 15th to (0.306±0.015mgTAE/g) on day 17th and then reduced significantly on day

21(0.148±0.02mgTAE/g). In all the cases there was significant decrease in phenolic levels on day 21st as compared to day 17th ($p \leq 0.05$) except in normal soil and soil with lemon peels. The major phenolics present in barley grass are Benzoic Acid, Caffeic Acid, Gallic Acid, Syringic Acid, p-Hydroxybenzoic Acid, Ferulic acid. The barley grass has a significantly higher amount of caffeic acid, gallic acid, syringic acid, p-Hydroxybenzoic Acid, Ferulic acid than wheat grass **[6]**. The result of the present study is in accordance with earlier study where nutrient levels in grasses are maximum at jointing stage and decrease with the development of non-phenolic structural components like cellulose and hemicellulose in leaves, leaf veins, stems, and nodes **[7]**. The highest phenolic level was found on the 17th day in barley grass grown on banana peels followed by pomegranate, pineapple and lemon peels. The decrease in levels of phenolic after the 17th day could be due to utilization of phenolic compounds in strengthening plant cell walls by polymerization and cross-linking into lignans and lignins **[8]**.

In barley seeds grown on normal soil, antioxidant levels decreased drastically until it reached its lowest value of (2.04±0.06mgAAE/g) on day 21st **(Figure 3)**. In barley grass grown on soil with lemon peels the antioxidant levels increased significantly from 3.62 ±0.06 mg AAE/g to 4.39±0.07mg AAE/g on day 15th and then decreased significantly from 4.39±0.07 mg AAE/g to 3.24±0.01 mg AAE/g on day 17th and then increased significantly from 3.24±0.01 mg AAE/g to 3.857±0.015 mg AAE/g on day 21st ($p \leq 0.05$). In pomegranate and pineapple, the antioxidant levels remained almost the same from day 13th to day 17th followed by drastically decreased antioxidant activities on day 21st ($p \leq 0.05$). In soil treated with banana peels, the antioxidant levels remain constant on day 13th and 15th followed by a significant increase on day 17th ($p \leq 0.05$). In all the cases there was a drastic dip in antioxidant levels on day 21st. The high antioxidant activity in barley seeds could be due to the presence of flavonoid antioxidants such as flavone-C-glycosides, saponarin, and lutonarin **[9]**. The antioxidant phytonutrients of barley grass include the superoxide dismutase, 2-O-glycosyl isovitexin (2″-*O*-GIV), and protoheme. It was also reported that ascorbic acid has strong antioxidant potential and dynamic changes in antioxidant levels could be due to changes in ascorbic acid during growth of grass **[10]**.

In barley seeds grown on normal soil, the level of the vitamin C decreases drastically until it reaches its lowest value of (0.036±0.06 mg/ml). In soil treated

with banana, pomegranate and pineapple peels, the vitamin c levels remain constant on day 13th, 15th, 17th followed by a significant decrease on day 21. In all the cases there was a drastic dip in vitamin C levels on day 21st. In barley grass grown on soil with lemon peels the vitamin C levels increased significantly from day 13th (0.108±0.02mg/ml) and then increased significantly on day 17th (0.112±0.01mg/ml) and then further decreased on 21st day (0.025±0.03 mg/ml) ($p \leq 0.05$).

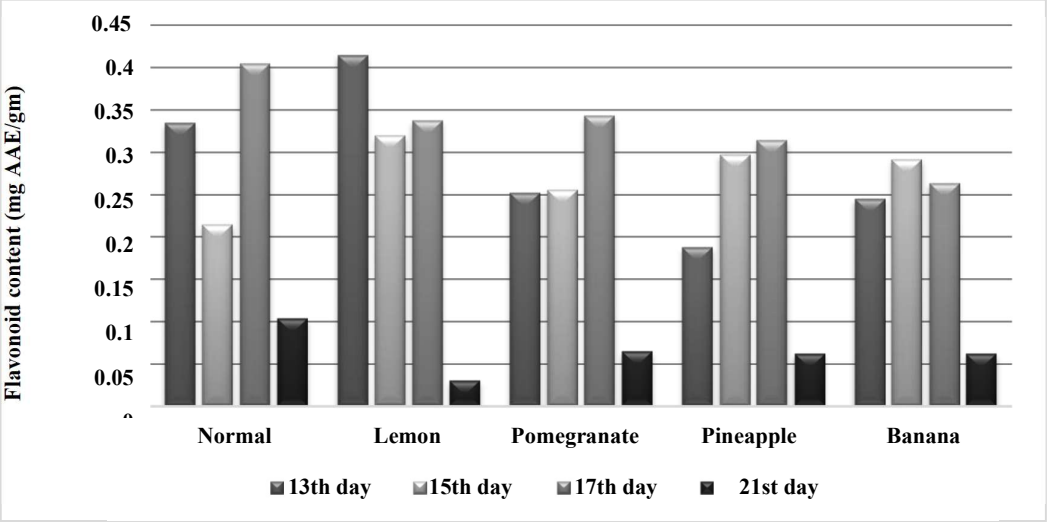

Figure 4.1: Changes of flavonoid activities in barley grass grown in different conditions (normal soil, soil with lemon peels, soil with pomegranate peels, soil with pineapple peels, soil with banana peels) on various days (13 th, 15 th, 17 th and 21st day

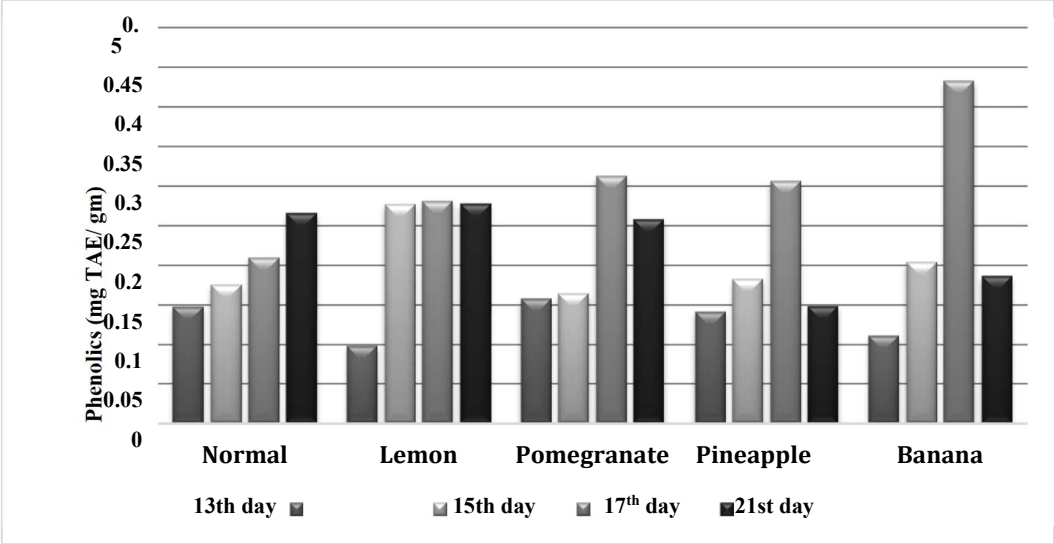

Figure 4.2: Changes of phenolic activities in barley grass grown in different conditions (normal soil, soil with lemon peels, soil with pomegranate peels, soil with pineapple peels, soil with banana peels) on various days (13 th, 15 th, 17 th and 21st days)

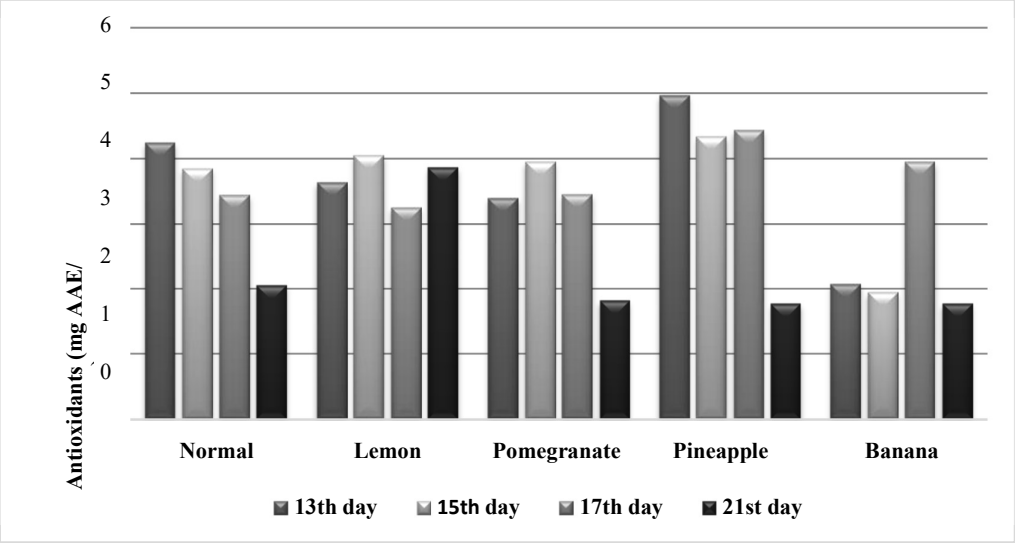

Figure 4.3: Changes of antioxidant activities in barley grass grown in different conditions (normal soil, soil with lemon peels, soil with pomegranate peels, soil with pineapple peels, soil with banana peels) on various days (13 th, 15 th, 17 th and 21st days)

5. CONCLUSION

Barley grass is a natural source of many biologically active compounds such as antioxidants, flavonoids, phenolics etc. which are known to have preventive and therapeutic potential against many known diseases. The results of this study provide information that supplementing the plant with fruit peels enriched soil increases the functional ingredients of young barley leaves. The results also indicate that phytochemicals present in barley grass are strongly dependent on the growth as they tend to lose it as the grass ages or enter later stages of growth as most of these constituents are utilized for plant growth. Regular consumption of barley grass may become a successful and safe strategy to treat chronic disease conditions. Therefore, supplementing the soil with simple organic waste enhances the preventive and therapeutic role of functional ingredients of the young barley grass. Barley grass can be promoted as new nutraceutical, functional food and an alternative to drugs for chronic diseases.

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