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EFFECT OF SEED PRIMING WITH GREEN TEA (*CAMELLIA SINENSIS L.*) AQUEOUS EXTRACT ON GERMINATION AND GROWTH TRAITS OF MUNG BEAN (*VIGNA RADIATA* L.)

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SUMMARY

A laboratory experiment studied the effects of the green tea (*Camellia sinensis* L.) aqueous extract at concentrations of 10, 20, and 30 ppm on the germination and growth traits of the mung bean (*Vigna radiata* L.), carried out in 2021 at the Department of Biology, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, Iraq. The results showed that *Camellia sinensis* green tea extracts played a vital role by significantly boosting all the examined characteristics compared with the control treatment. The aqueous extract of Green tea at concentrations of 10 and 20 ppm gave the best performance in increasing germination rates, germination speed, plant promoter indicator, and seedling strength compared with the control treatment. Meanwhile, the concentration of 30 ppm indicated a significant increase in the shoot length, root system, carbohydrates, proteins, proline, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) compared with other concentrations used and control treatment.

Keywords: Mung bean (*Vigna radiata* L.), green tea (*Camellia sinensis* L.) extract, germination, growth traits, antioxidant enzyme, superoxide dismutase, peroxidase, catalase, carbohydrates, proteins, proline

Key findings: Using natural environmentally friendly materials ,They do not have aharmful effect on humans ,they contain antioxidants.

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INTRODUCTION

The mung bean (*Vigna radiata* L.), also known as the green gram and mash, is a summer herbal plant belonging to the family Fabaceae (Al-Kateb, 1988). Mung bean cultivation mainly occurs in East, Southeast, and South Asia and Arab countries (Hommel, 2020). It serves as an ingredient in both savory and sweet dishes. There are also three subgroups of *Vigna radiata*, including one cultivated (*Vigna radiata subsp. radiata*) and two wild ones (*Vigna radiata* subsp*. Sublobata* and *Vigna radiata* subsp*. glabra*). Mung bean is a short-duration crop with a height of about 15–125 cm (Ali *et al*., 1990; Lambrides and Godwin, 2007).

The mung bean, considered a food crop containing a high amount of protein (25%–28%) and carbohydrates (62%–65%), also serves as animal feed grown in East Asia, India, and the Arab countries, where mung bean cultivation accounts for 9% of all legumes (Al-Fartousi, 2005). It contains vitamins B2, B3, and B6 and Si, Ca, and Omega 3.

Green tea (*Camellia sinensis* L.), dried leaves, and leaf buds are ingredients to make several different teas, including black and oolong teas. Green tea is an aromatic evergreen shrub plant containing fragrant oil of great medical benefit. Widely used in many countries, green tea becomes a stimulant and mental stimulant with ripe leaves picked and dried without fermentation to retain their medical benefits. The newly prepared green tea contains a high percentage of vitamin C with its stimulating tonic, with the tea extract used in various medical and pharmaceutical industries (Tlass, 2008). The caffeine in green tea leaves gets separated from a compound, Tannin Complex, while caffeine is around 5%.

Green tea is unfermented; thus, it can maintain important molecules called polyphenols, which may be responsible for many benefits. Concerning active ingredients in green tea, its leaves contain caffeine by 1%–3.5%, tannins by 10%–24%, and other alkaline substances in different proportions. Minor active substances like theobromine and theophylline also exist, as well as, volatile oil at a ratio of 75:0, which gives a distinctive taste and aroma in tea. Green tea contains vitamins C, B, and B2, including tobacco and bentonic acids. In addition, there are 14 volatile compounds in green tea, including methyl and ethyl alcohol and several aldehydes and sulfur compounds (Al-Hajj, 2000). Green tea helps against high cholesterol and high blood pressure, preventing heart diseases and ovarian cancer, but no good scientific evidence supports most of these uses.

Green tea contains higher antioxidants than black tea because fermenting tea decreases more antioxidants (Bown, 2008). Oxidation is one of the chief life activities of living organisms, as it needs oxygen to complete the oxidation process to obtain energy and produces intermediate compounds called free radicals or reactive oxygen species (ROS); hence, living cells have antioxidant defense mechanisms, which are enzymatic and non-enzymatic (Al-Hayani, 2015). The enzymatic antioxidants are glutathione, glutathione reductase, glutathione peroxidase, catalase, thirodoxine, and superoxide dismutase.

The non-enzymatic antioxidants are ubiquinol, uric acid, metalonin, Lipoic acid, ascorbic acid, vitamin E, carotenoid, flavonoids, phenolic compound anthocyanin, zinc, and selenium (Yassin, 2001). Seed priming is one of the most influential enhancers to help them germinate and emerge quickly and to increase the tolerance of seeds to harsh environmental conditions (Heydecker *et al*., 1975; Harris *et al*., 1999). It has shown a positive effect on the germination and emergence of *Zea mays* seedlings (Snap *et al*., 2008). The better and early emergence of the crop increases its competition with the bush and its ability to withstand drought, increase yields, and avoid the costs and time consumed when reseeding (Clark *et al*., 2001). The relevant research aimed to determine the effects of green tea extract containing antioxidants and their role in stimulating the germination of economic plants, including mung bean.

MATERIALS AND METHODS

A laboratory experiment on mung bean (*Vigna radiata* L.) transpired in 2021 at the Department of Biology, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, Iraq. The seeds used underwent washing and sterilization utilizing a 60 mm × 15 mm container. The green tea extract concentrations at 10, 20, and 30 ppm preparations soaked the mung bean seeds of a local cultivar in separate vessels for 12 h with three repetitions per concentration and one each treatment with seed soaking in the distilled water as a control treatment.

Transfer of seeds to sterile Petri dishes with wet filtration papers by distilled water continued, placing 10 seeds in each Petri dish. Each separate treatment had three repetitions per concentration and a control treatment, with all dishes incubated at 25 °C for 10 days (Mahmoud, 2016).

Computations for the various characteristics studied are as follows. The germination ratio (%) and germination speed formulation employed the equations below, with the readings recorded on the second day accordingly (Al-Qaissy *et al*., 2014).

$$\text{Germination ratio\%} = \frac{\text{Number of seeds}}{\text{Total number of seeds}} \times 100$$

$$\text{Germination speed} = \frac{\text{Number of seeds}}{\text{The number of days science germination}} \times 100$$

The promoter indicator's calculation followed the equation by Bouslamo and Schupangh (1984).

The percentage of seeds sowed on the second day × 1 + the percentage of seeds planted on the fourth day × 0.75 + the percentage of seeds planted on the sixth day × 0.5 + the percentage of seeds emerging on the eighth day × 0.25, with the reading recorded on the second day of germination.

The seed vigor measurement used the equation by Ellis and Roberts (1981).

Seed vigor (cm) = Germination (%) × the length of the seedling × 100

The root length measurement used a ruler. Estimating the soluble carbohydrate percentage was according to the method by Herbert *et al*. (1971). In the mung bean seeds, the protein content estimates continued after digesting a known weight of seeds, with the protein proportion calculated according to the following equation:

Protein ratio = Nitrogen % × 6.25
(Bruckner and Morey, 1988)

The proline content estimation also ensued according to the methodology of Bates *et al*. (1973). The assessment of the total effectiveness of the enzymatic antioxidant defense system, including the superoxide dismutase (SOD) enzyme, progressed in the NBT method by measuring riboflavin by the technique of Beyer and Fridovich (1987). The assessment of the total effectiveness of peroxidase (POD) enzyme was according to the method described by Nezih (1985). Assessing the total effectiveness of the catalase (CAT) enzyme employed the method described by Aebi (1974).

Statistical analysis

All the recorded data on various parameters sustained statistical analysis by the SPSS Statistical Program, with the treatment means further compared and separated using the $LSD_{0.05}$ (SAS, 2010).

RESULTS AND DISCUSSION

The findings revealed that the mung bean seeds soaked in green tea extract concentrations at 10 and 20 ppm showed a significant increase in the rate of the indicators, i.e., germination, germination speed, and seed germination promoter with enhanced percentage of 87.6%, 87.2%, and 23.1%, respectively compared with the control treatment (Table 1). It may be due to active substances (amino acids and antioxidants) found in green tea extracts, which stimulate the process of impregnating and encourage germination, eventually facilitating the process of impregnating and tearing the seed's covers, facilitating the emergence of the seed embryo (Yassin, 2001; Azka *et al*., 2021; Ho *et al*., 2023).

The characteristic of the mung bean seed vigor also displayed a significant increase with the green tea extract concentration at 30 ppm, and the increase rate was 274.9% compared with the control treatment (Table 2). The seed vigor characteristic also depended mainly on the germination percentage, which exceeded the control treatment with the green tea extract concentrations at 10 and 20 ppm (Table 1). According to the results, the process of impregnating and mung bean seeds' germination was evident in the different green tea concentrations used, accelerating the seed germination process due to having antioxidants, oils, and amino acids encouraging germination directly compared with the control treatment. Steeping the seeds in green tea stimulates the respiratory enzymes inside the spores; thus, it stimulates the seedling respiration and germination, leading to an increase in growth indicators due to the enhanced growth efficiency and stimulation of the growth of the root and pinnate (Mahmoud, 2016).

In the mung bean seedlings' vegetative and root system, the vegetative system (plant length and height) and root length exhibited a remarkable increase with green tea concentration of 30 ppm versus the control treatment, and, in both traits, the increase was 300% and 100%, respectively (Table 3). After stimulating the germination and the green tea extract entering the seeds, it further accelerates the growth of the root and the plumule because the green tea extract contains a high percentage of minerals, such as, calcium, potassium, ferric, copper, zinc, and all these elements are necessary for the formation of cells and their division and plant growth, in addition to containing natural antioxidants that have a chief role in the disposal of harmful substances. Green tea contains many mineral elements and antioxidants that play a positive role in eliminating free radicals, including theanine, hydroxy anisole, and amino butyric acid (Al-Hajj, 2000; Tlass, 2008).

The green tea extract also contains catechin, polyphenols, and antioxidants, particularly in the ripened leaves, as well as, fructose and sucrose compounds and various amino acids, all of which stimulate the growth of seedlings and increase the cell elongation and their further division. Thus, green tea extracts concentration of 30 ppm bested in the length of the vegetative parts and root system by forming new cells. The antioxidants in green tea extract stimulate respiratory enzymes in fetal tissues, boosting the respiratory processes and carbohydrate consumption. The activity of enzymes helps cells eliminate damage from free radicals by controlling the defense genes of antioxidant enzymes and defense proteins (AL-Hayani, 2015).

The green tea concentration at 30 ppm revealed an increase in the content of carbohydrates, proteins, and proline in mung beans. It is because the green tea extract increased the growth of vegetative parts, thus increasing the efficiency of photosynthesis and the production of carbohydrates, proteins, and proline, which are the prime components of the plants belonging to the family Fabaceae (Table 3). In studying the effects of green tea extract at 10 (GWG1%) and 50 (GWG5%) g/L as the steeping solution on the chemical, nutritional, and microbial quality of wheat grain during 14 days of germination, the said natural growth regularly improved the microbial and nutritional quality of wheat germ (Pakfetrat *et al*., 2020).

Green tea also stimulates the antioxidants found in the plants, with their percentage significantly enhanced (Table 4). The results further authenticated that the green tea extract maintains the cells in their active form, leading to a significant increase in the activities of these enzymes (AL-Hayani, 2015). A past study on tea seed (*Camellia* sp.) concludes the physiological effects on plants, which can increase and decrease growth and yield depending on the applied dose. The growth-enhancing effects could be beneficial commercially to improve crop yield; however, its adverse effects in agriculture and horticulture need consideration before use (Andresen and Cedergreen, 2010).

Table 1. Mung bean seeds' response to soaking with Green tea extract concentrations for germination ratio, speed, and promoting indicator.

Treatments	Germination ratio (%)	Germination speed	Germination promoting indicator
Control	53.3	267	203
10 ppm	100	500	250
20 ppm	100	500	250
30 ppm	73.3	367	223
$LSD_{0.05}$	15.37	77	15

Table 2. Mung bean seeds' response to soaking with Green tea extract concentrations for seed vigor and the length of the vegetative parts and root system.

Treatments	Seed vigor (cm)	Length of vegetative system (cm)	Length of root system (cm)
Control	2267	0.50	1.0
10 ppm	1000	1.23	1.59
20 ppm	1000	1.50	1.75
30 ppm	3667	2.00	2.00
$LSD_{0.05}$	768	0.05	0.12

Table 3. Mung bean seeds' response to soaking with Green tea extract concentrations for the carbohydrates, protein, and proline.

Treatments	Carbohydrate (%)	Protein (%)	Proline (μg g^{-1})
Control	0.5	19.00	7.00
10 ppm	0.7	20.00	9.00
20 ppm	0.8	21.00	10.00
30 ppm	0.9	21.9	11.00
$LSD_{0.05}$	0.14	1.98	1.134

Table 4. Mung bean seeds' response to soaking with Green tea extract concentrations for enzymatic antioxidant defense system, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) enzymes (μg g^{-1} fresh weight of leaf).

Treatments	SOD (μg g^{-1})	POD (μg g^{-1})	CAT (μg g^{-1})
Control	158.78	41.00	8.66
10 ppm	160.22	45.91	10.22
20 ppm	177.00	58.19	11.33
30 ppm	189.11	69.11	15.59
$LSD_{0.05}$	0.809	0.805	1.404

CONCLUSIONS

The green tea concentration at 30 ppm revealed an increase in the content of carbohydrates, proteins, and proline in mung bean (*Vigna radiata* L.). The mung bean seeds soaked with varied concentrations of green tea extracts also showed a significant increase in germination and growth traits of the seedlings because the green tea extract contains different antioxidants.

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